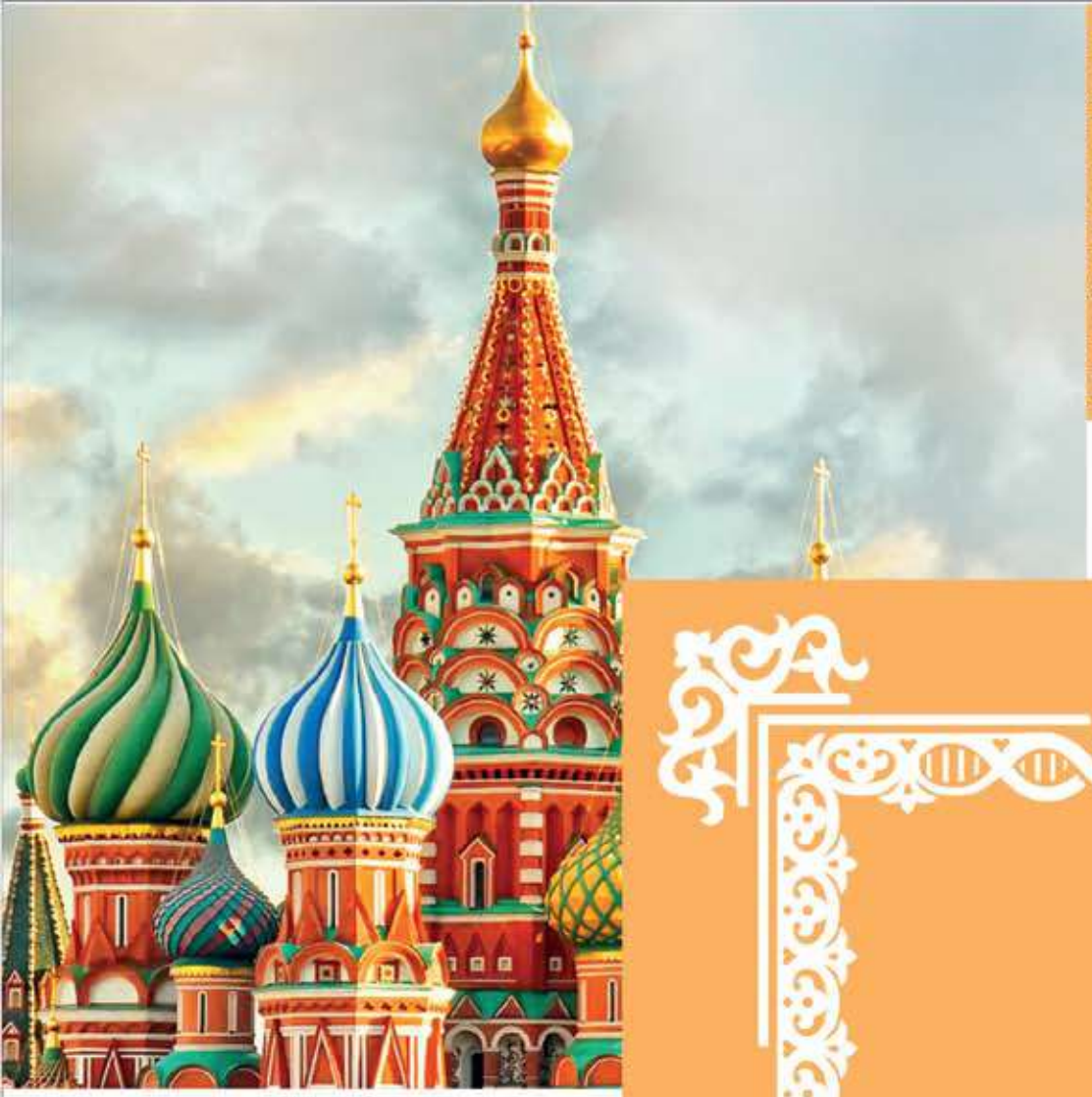


Cathedral of St. Vasily the Blessed, Moscow

The 22nd International Charles Heidelberger Symposium on Cancer Research

Conference Proceedings

Tomsk, Russia, Sept. 17–19, 2018

Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk

National Research Tomsk State University, Tomsk

Tomsk National Research Medical Center of the Russian Academy of Sciences
National Research Tomsk State University

The 22nd International Charles Heidelberger Symposium on Cancer Research

Proceedings of the International Symposium

17–19 September 2018

Publishing house of Tomsk University
2018

Tomsk National Research Medical Center of the Russian Academy of Sciences
National Research Tomsk State University

U.D.C.: 616-006.6
L.L.C: 5

The 22nd International Charles Heidelberger Symposium on Cancer Research: Proceedings of the International Symposium. Tomsk, 17–19 Sep. 2018 [Electronic resource] / Ed. E. L. Choynzonov, E. V. Galazhinskiy, N. V. Cherdyntseva, J.G. Kzhyshkowska; Tomsk National Research Medical Center of the Russian Academy of Sciences, National Research Tomsk State University. – Tomsk: Publishing house of Tomsk University, 2018. URL: http://tnimc.ru/upload/publications/proceedings/2018_22_symposium.pdf
ISBN 978-5-7511-2548-6

The Proceedings include the Abstracts of Scientific papers presented at the 22nd International Charles Heidelberger Symposium on Cancer Research. These Symposiums are devoted to the memory of Charles Heidelberger, who is the famous American scientist in the field of chemical carcinogenesis, tumor biology, and cancer chemotherapy, and they are annually held in different countries all over the world.

This 22nd International Charles Heidelberger Symposium on Cancer Research was held at Tomsk National Research Medical Center and National Research Tomsk State University in Tomsk, Russian Federation in September 17–19, 2018. It was dedicated to the memory of Mrs. Patricia Heidelberger, Major Donor, Co-Founder, President, and Member of the Board of Directors of the Charles and Patricia Heidelberger Foundation for Cancer Research. Mrs. Heidelberger unfortunately passed away in May, 2018, at the very honorable age of 94.

Scientists from the USA, Germany, Chile, France, United Kingdom and Russia attended the 22nd Symposium to present their achievements and discuss the most recent advances in cancer, from basic research to clinical applications.

The main scientific topics were the following:
- Cancer Etiology and Epidemiology
- Molecular Carcinogenesis
- Signal Transduction and Cancer
- Cancer Genetics and Epigenetics
- Tumor microenvironment
- Tumor immunology
- Translational Cancer Medicine.

Keywords: cancer etiology and epidemiology, molecular carcinogenesis,
cancer genetics, epigenetics, tumor microenvironment, tumor immunology, translational cancer medicine.

Reviewers:
Prof. Kadagidze Z.G., Prof. Puzyrev V.P.

ISBN 978-5-7511-2548-6

The Charles and Patricia Heidelberger Foundation for Cancer Research

Dear Colleagues,

It is our great pleasure to invite you to participate in the 22nd International Charles Heidelberger Symposium on Cancer Research, which takes place at Tomsk National Research Medical Center and Tomsk State University in Tomsk, Russian Federation from September 17 to 19, 2018.

Prof. Charles Heidelberger, a famous Researcher in the field of chemical carcinogenesis and cancer chemotherapy, was a member of U. S. National Academy of Sciences. Dr. Charles Heidelberger's laboratory pioneered the synthesis of chemotherapeutic drugs, such as trifluorothymidine and 5-fluorouracil, which are still widely used agents for the treatment of gastric, colon and breast cancers.

The 22nd International Charles Heidelberger Symposium on Cancer Research is dedicated to the memory of Mrs. Patricia Heidelberger, Major Donor, Co-Founder, President, and Member of the Board of Directors of the Charles and Patricia Heidelberger Foundation for Cancer Research. Mrs. Heidelberger unfortunately passed away in May, 2018, at the very honorable age of 94.

The 22nd Symposium brings together scientists from the USA, Germany, Chile, France, United Kingdom and Russia to share with the wider scientific community their high-quality research and discuss the most recent advances in cancer: from basic research to clinical applications.

This symposium is held for the second time in Russia and now is hosted by one of the oldest Siberian cities. Tomsk was founded in 1604 and was a major trading outpost. Much of Tomsk's appeal lies in its well-preserved late-19th- and early-20th-century 'wooden-lace' architecture – carved windows and tracery on old log and timber houses. Early autumn is the best opportunity to appreciate the attractions of Tomsk in all its glory.

We look forward to welcoming you in Tomsk!

INVESTIGATION AND ANALYSIS OF GENE STRUCTURES USING TIME-OF-FLIGHT MASS SPECTROMETRY. PPLICATION OF THE GENOMIC ANALYZER IN SCIENTIFIC AND CLINICAL PRACTICE

A.G. Afanasyev
Biogen-Analitika Ltd, Moscow, Russia

Single polymorphic markers (SNP) are the most common type of genetic polymorphism. Despite the progress in sequencing and postgenomic technologies, point-based genotyping of SNP remains the most popular way, both in human medical genetics and molecular genetics in general. SNP genotyping technologies have evolved over the past three decades from the genotyping of single markers by nucleic acid hybridization, PCR, and the detection of differences in amplicons using various approaches (PCR-RFLP, allele-specific PCR, etc.) to automated multiplex analysis of hundreds of thousands or several million SNP on high-density microarrays of DNA (biochips).

Today the most common method of genotyping SNP in laboratory and research practice is real-time PCR using fluorescently labeled probes (TaqMan-probes). For more extensive studies, genotyping is used on high-density biochips. The first of these approaches allows one to obtain data on single markers in hundreds or thousands of samples, the second - about tens and hundreds of thousands of SNP in units or dozens of samples. At the same time, the niche of intermediate scale, which is most in demand when solving most problems - genotyping tens or hundreds of markers in hundreds of samples - remains poorly filled with modern technological solutions. Approaches based on low-density DNA microarrays or SNaPShot analysis using capillary gel electrophoresis remain relatively inadequate due to limitations on the possible multiplexing and resolution limitations of the separation of amplicons. One of the technologies in the described niche of tasks is the mass spectrometry of biological macromolecules, including DNA. In genetics and molecular biology, mass spectrometry technologies are used for genotyping, analysis of DNA expression and methylation.

Scalability, high accuracy and sensitivity, analysis of a large number of single nucleotide polymorphisms in a multiplex format, has found wide application in cancer research, in particular, in the genotyping of somatic mutations, pharmacogenomics, genotyping of blood groups, and research in the field of agriculture. To develop the locus-specific primer, special software is used that allows analyzing not only quantitative estimation of alleles, but also mini-segregation of gene fragments, 50 bp in size.

The sensitivity rate of mass spectrometry remains high, allowing polymorphism of a segment with a length of 170 bp to be determined. This advantage makes it possible to successfully apply the method of reading SNP for liquid biopsy. Degraded DNA, single tumor cells serve as a material for the detection of tumors at an early stage of tumor development, for which there are a number of panels for the early diagnosis of colorectal cancer and lung cancer. This method is the best for the above analyzes and has found wide application in scientific and clinical laboratories around the world.

Keywords: single polymorphic markers, real-time PCR, mass spectrometry.

ASPARTATE COUPLES ARGININE STARVATION WITH MITOCHONDRIAL DYSFUNCTION IN BREAST CANCER CELLS WITH ASS1 DEFICIENCY

D.K. Ann
Department of Diabetes Complications and Metabolism, Diabetes and Metabolism Research Institute, Beckman Research Institute, City of Hope, Duarte, USA

One of the most common, perhaps under-recognized, metabolic deficiencies (>70%) of cancer cells is the inability to synthesize arginine, due to the suppression of the expression of argininosuccinate synthetase (ASS1), a tumor suppressor. Exploiting this feature, arginine starvation therapy with ADI (arginine deiminase) has entered phase III clinical trials with remarkable tumor specificity

and safety profile. We previously reported that arginine-starved tumor cells were killed by a novel mechanism where nuclear DNA leaked out and was engulfed by giant autophagosome, a form referred to as *chromatophagy (chromatin-autophagy)*. The cell death was *caspase-independent*, but *mitochondria-dependent*. Arginine deprivation rapidly and potently silenced nuclear-encoded mitochondrial genes in a coordinated way and principally by epigenetic means. This resulted in altered mitochondrial dynamics and impaired mitochondrial functions, leading to ROS production and DNA damage. At the same time, the expression of enzymes involved in DNA metabolism were also affected with consequent impaired DNA-repair. We validate that mitochondria are the main target of arginine starvation, and identified an important role of aspartate in maintaining homeostasis of arginine-starved cells. Aspartate is required for NADH and nucleotide production to support the survival of arginine-starved, ASS1-deficiency cells. Thus, the fate of arginine-starved cells isimpacted by the mitochondria quality and the availability of intracellular aspartate. Altogether, this study suggests that dietary arginine restriction and aspartate modulation could be a potential strategy for retarding the progression of ASS1-deficient tumors. In summary, our data uncover an atypical autophagy-related death pathway and suggest that mitochondrial damage is central to linking arginine starvation and chromatophagy in two distinct cellular compartments.

Keywords: breast cancer, argininosuccinate synthetase, metabolic stress, cancer therapy.

MICRORNA EXPRESSION IN TUMOR AND SURROUNDING NON-CANCEROUS MELANOMA TISSUE

M.B. Aksenenko, N.V. Palkina, A.V. Komina, T.G. Ruksha

Federal State Budget Educational Institution of Higher Education «Krasnoyarsk State Medical University named after Professor V.F. Voyno-Yasenetsky» of the Ministry of Health of the Russian Federation, Krasnoyarsk, Russia

MicroRNA as epigenetic regulators has impact on melanoma development and progression. In this study, we aimed to identify the microRNA profile in melanoma and adjacent noncancerous tissue. Skin biopsies (n=21) obtained from melanoma patients were macrodissected. The reverse transcription reaction was performed in a reaction mixture containing 0.1 μl of 5× primers miRNA TaqMan assays specific to the investigated microRNAs and endogenous controls (Cat. № 4427975, Applied Biosystems). Expression levels of miRNAs were determined by real-time PCR using specific primers for miR-363-3p, miR-3591, miR-18a-5p, and miR-146a-5p (Cat. № 4427975, Applied Biosystems). **Results.** miR-146a-5p level was demonstrated as elevated in melanoma cells versus noncancerous adjacent tissue (p=0.002). 38 target genes have been determined for miR-146a-5p of which NRAS gene is known as one of the most frequent mutated in melanoma. Immunohistochemical evaluation of NRAS protein identified its increased level in NRAS-negative tumor tissue as compared to NRAS-mutant tumors (p=0.004). Elucidation of the role of miR-146-a-5p in complex interactions between the tumor and the surrounding cells is necessary for our understanding of the mechanisms of tumor progression and the development of new treatments.

Keywords: Melanoma, non-cancerous melanoma tissue, microRNA.

The development and growth of a malignant tumor depends upon a local interaction between tumor cells and microenvironment. The malignant features of tumor cells cannot be manifested without an important interplay between cancer cells and their local environment [1]. In recent years, a substantial number of reports on individual miRNAs or miRNA patterns have been published providing strong evidence that miRNAs might play an important role in malignant melanoma and help to better understand the molecular mechanisms of melanoma development and progression [2]. In this study, we aimed to identify the microRNA profile in melanoma and adjacent noncancerous tissue with a further assessment of signaling pathways and target genes of microRNAs altered.

Material and Methods

Skin biopsies (n=21) obtained from melanoma patients were macrodissected. RNA was isolated from the patient's tissue, then - microarray with GeneAtlas Microarray System («Affymetrix», California, USA). The reverse transcription reaction was performed in a reaction mixture containing 5× primers miRNA TaqMan assays specific to the investigated microRNAs and endogenous controls

(Cat. № 4427975, Applied Biosystems). Expression levels of miRNAs were determined by real-time PCR using specific 20× primers for miR-363-3p, miR-3591, miR-18a-5p, and miR-146a-5p (Cat. № 4427975, Applied Biosystems). The reaction was performed on a StepOne™ Real-Time PCR System (Applied Biosystems, Singapore, Singapore) with the following temperature cycling protocol: 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min with FAM detection. Bioinformatic analysis was performed using the DIANA-miRPath v.3.0 database. Target genes for miR-146a-5p were determined using three different algorithms: TargetScan 7.0, miRWalk 2.0 and miRTarBase v.4.5, after which only matching genes were selected. The immunohistochemical study was performed according to a standard protocol. The slides were incubated with anti-NRAS (anti-GTPase NRAS Clone AT2G9, Antibodies-online, Atlanta, GA, USA) monoclonal antibodies for 1 h. Negative controls were prepared by performing the same immunohistochemistry protocol without application of primary antibodies. The sequence primers for NRAS gene (exon3) were: forward 5' ATAGCATTGCATTCCCTGTG, reverse 5'-GCGGATATTAACCTCTACAGG and NRAS gene (exon 2) were: forward 5'- GGG GGT TGC TAG AAA ACT A, reverse 5'- ATCCGA CAA GTG AGA GAC A. Cycle conditions were an initial denaturation at 95 °C for 3 min, then 35 cycles of 95 °C for 30 sec, 54 °C for 40 sec., 72 °C for 40 sec, followed by 3 min at 72 °C. The Expression Console and Transcriptome Analysis Console 3.0 (Affymetrix, Santa Clara, CA, USA) software were used for quality control, statistical analysis, and miRNA annotation. The data were automatically statistically analyzed using ANOVA test and FDR corrected values. MicroRNAs expression differences were assessed using a Mann-Whitney U-test and considered significant at $p \leq 0.05$. Mann-Whitney U-test with the Statistica 6.1 software (Stat Soft, Moscow, Russia).

A survey has shown the expression level of hsa-miR-146a-5p to be statistically significantly higher in melanoma cells as compared to the healthy surrounding tissues ($p=0.002$). MiR-146-a-5p this targets included well-known melanoma-associated genes such as NRAS oncogene, microphthalmia-associated transcription factor (MITF), receptor tyrosine kinase c-KIT and AP-2 transcription factors (TFAP2). Previous reports validated NRAS as a confirmed target gene for miR-146a by functional study [3]. It is well established that NRAS is one of the key oncogenes in melanoma development, its activating mutations account for the enhanced melanoma cell proliferation [4]. Immunohistochemical evaluation of NRAS protein identified its increased level in NRAS-negative tumor tissue as compared to NRAS-mutant tumors ($p=0.004$). Interestingly, miR-146a-5p expression levels were more than 4,000-fold higher in NRAS-negative melanomas and 1,600 times higher in NRAS-positive melanomas compared to adjacent noncancerous tissues as it was determined by real-time PCR. It may suggest different effects of miR-146a-5p on melanoma cell biology through its binding to other target genes apart from NRAS. Elucidation of the role of miR-146-a-5p in complex interactions between the tumor and the surrounding cells is necessary for our understanding of the mechanisms of tumor progression and the development of new treatments.

The study was supported by Grant of the Russian Science Foundation (project № 14-15-00074-P).

REFERENCES

1. *Sounni N.E., Noel A.* Targeting the tumor microenvironment for cancer therapy. Clinical Chemistry. 2013 Jan; 59(1): 85-93. doi: 10.1373/clinchem.2012.185363.

2. *Kunz M.* MicroRNAs in melanoma biology. Adv Exp Med Biol. 2013; 774: 103-20. doi: 10.1007/978-94-007-5590-1_6.

3. *Halkein J., Tabruyn S.P., Ricke-Hoch M., Haghikia A., Nguyen N.Q., Scherr M., Castermans K., Malvaux L., Lambert V., Thiry M., Sliwa K., Noel A., Martial J.A., Hilfiker-Kleiner D., Struman I.* MicroRNA-146a is a therapeutic target and biomarker for peripartum cardiomyopathy. J Clin Invest. 2013 May; 123(5): 2143-54. doi: 10.1172/JCI64365.

4. *Mandalà M., Merelli B., Massi D.* NRAS in melanoma: targeting the undruggable target. 2014 Nov; 92(2): 107-22. doi:10.1016/j.critrevonc.2014.05.005.

COMPREHENSIVE ANALYSIS OF *BRCA1* GENE MUTATIONS IN SIBERIAN BREAST CANCER PATIENTS

M.S. Anisimenko[1,2], G.A. Paul[1], N.A. Matyash[1], N.I. Gutkina[2], E.V. Gornostaeva[1,2], D.A. Berdyugina[1,2], A.Yu. Garanin[1,2], A.V. Butorina[1,2], A.E. Kozyakov[3], S.P. Kovalenko[1,2]

BioLink Ltd, Novosibirsk, Russia[1]
Federal Research Center of Fundamental and Translational Medicine, Novosibirsk, Russia[2]
Novosibirsk Regional Oncological Clinic, Novosibirsk, Russia[3]

Mutations in the *BRCA1* gene in patients with breast cancer in the Novosibirsk Region were analyzed in details. The *BRCA1* 5382insC mutation was analyzed among 3850 unselected patients, 138 mutation carriers (3.6%) were identified. 1400 patients were additionally analyzed for carriage of 185delAG, T300G (C61G), and 4153delA. As a result, 1 carrier of the 185delAG mutation (0.07%), 3 carriers of the T300G mutation (0.21%), and 6 carriers of the 4153delA mutation (0.43%) were detected. Analysis of the complete coding sequence of the *BRCA1* gene was conducted for 445 patients with early onset breast cancer. 35 carriers of the 5382insC mutation (7.9%) and 5 carriers of other pathogenic mutations (T300G, 4153delA, E143X, IVS18 + 1G> T and g.41256236_41256237delGG, one case each) were identified in the cohort of 445 patients with early onset breast cancer.

Keywords: *BRCA1* **gene, mutation, breast cancer, hereditary cancer, NGS.**

Introduction

Hereditary factors account for up to 10% of all cases of breast cancer [1]. A significant part of the hereditary forms of breast cancer is caused by mutations in the *BRCA1* and *BRCA2* genes. The likelihood of a malignancy during the lifespan for *BRCA1/2* mutation carriers is very high - up to 90% [2]. The data on the prevalence of specific mutations among patients with unselected breast cancer are limited. In this study, we estimated the prevalence of mutations in the *BRCA1* gene among all breast cancer patients from Novosibirsk with a particular focus on mutation analysis among patients with early onset cancer.

Material and Methods

Blood samples were collected from 3,850 patients diagnosed with breast cancer treated at the Novosibirsk Regional Oncological Clinic from April 2013 to June 2016. An analysis of the mutations of *BRCA1* gene (5382insC, 185delAG, T300G, and 4153delA) was performed using allele-specific real-time PCR. An analysis of the complete coding region of the gene (22 exons, 5592 bp) with the adjacent intron regions (20-50 bp) was performed by targeting sequencing using the Ion PGM instrument (Life Technologies, USA). Bioinformatic analysis of the raw data was based on PRINSEQ technique [3]. The nucleotide sequences obtained in the analysis were compared with the reference sequence of the human genome GRCh37/hg19 using the BWA-MEM software version 0.7.5 [4]. The search for genetic variants was carried out using the SAM tools software version 0.1.19 [5].

Results

3850 unselected DNA samples of breast cancer patients were analyzed for the presence of the common 5382insC mutation in *BRCA1* gene. According to our previous results, the frequency of 5382insC mutation among residents of Novosibirsk city is 0.25% [6]. Analysis of 3850 DNA samples of unselected breast cancer patients revealed 138 heterozygous *BRCA1* 5382insC carriers. Thus, the incidence of 5382insC mutation among breast cancer patients, regardless of family history, age of the disease in the Novosibirsk region, is 3.6%. Of the 3850 DNA samples, 1400 were also analyzed for the presence of 185delAG, T300G (C61G), and 4153delA mutations. As a result, 1 carrier of the 185delAG mutation (frequency 0.07%), 3 carriers of the T300G mutation (frequency 0.21%) and 6 carriers of the 4153delA mutation (frequency 0.43%) were detected.

Analysis of the complete coding sequence of the *BRCA1* gene was conducted for 445 patients with early onset breast cancer (19-40 years). 35 carriers of the 5382insC mutation (7.9%) and 5 carriers of other pathogenic mutations (T300G, 4153delA, E143X, IVS18 + 1G> T and g.41256236_41256237delGG, one case each) were identified in the cohort of 445 patients with early onset breast cancer.

Figure 1 shows the frequency of the 5382insC mutation among unselected breast cancer patients and in the cohort of early onset breast cancer patients in comparison with the frequency of this mutation in general population.

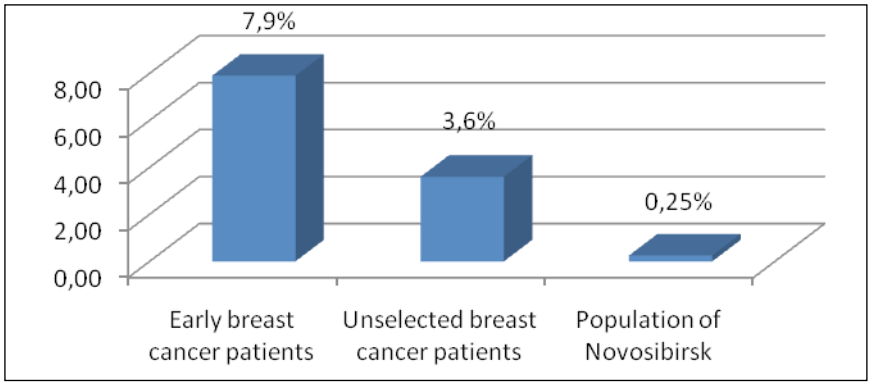


Figure 1. Frequency of the *BRCA1* 5382insC mutation in early onset breast cancer patients, unselected breast cancer patients, and in general population of Novosibirsk city

Conclusions

Data on the frequency of pathogenic mutations in the *BRCA1* gene among unselected breast cancer patients in the Novosibirsk Region were obtained. The prevalence of the mutations among early breast cancer patients was studied. The 5382insC mutation occured 2.2 times more often in early breast cancer patients than among unselected breast cancer patients. Remarkably, the proportion of the 5382insC mutation is 87.5% of all pathogenic mutations in the *BRCA1* gene found in early breast cancer patients.

REFERENCES

1. *Mavaddat N., Antoniou A.C., Easton D.F., Garcia-Closas M.* Genetic susceptibility to breast cancer. Mol Oncol. 2010 Jun; 4(3): 174-91. doi: 10.1016/j.molonc.2010.04.011

2. *Fischer C., Kuchenbäcker K., Engel C., Zachariae S., Rhiem K., Meindl A., Rahner N., Dikow N., Plendl H., Debatin I., Grimm T., Gadzicki D., Flöttmann R., Horvath J., Schröck E., Stock F., Schäfer D., Schwaab I., Kartsonaki C., Mavaddat N., Schlegelberger B., Antoniou A.C., Schmutzler R.* German Consortium for Hereditary Breast and Ovarian Cancer. Evaluating the performance of the breast cancer genetic risk models BOADICEA, IBIS, *BRCA*PRO and Claus for predicting *BRCA1/2* mutation carrier probabilities: a study based on 7352 families from the German Hereditary Breast and Ovarian Cancer Consortium. J Med Genet. 2013 Jun; 50(6): 360–7. doi: 10.1136/jmedgenet-2012-101415.

3. *Schmieder R., Edwards R.* Quality control and preprocessing of metagenomic datasets. Bioinformatics. 2011 Mar 15; 27(6): 863-4. doi: 10.1093/bioinformatics/btr026.

4. *Li H., Durbin R.* Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009 Jul 15; 25(14): 1754-60. doi: 10.1093/bioinformatics/btp324.

5. *Li H., Handsaker B., Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G., Durbin R.; 1000 Genome Project Data Processing Subgroup.* The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009 Aug; 25(16): 2078-9. doi: 10.1093/bioinformatics/btp352.

6. *Anisimenko M.S., Mitrofanov D.V., Chasovnikova O.B., Voevoda M.I., Kovalenko S.P. BRCA1* gene mutations frequency estimation by allele-specific real-time PCR of pooled genomic DNA samples. Breast. 2013 Aug; 22(4): 532-6. doi: 10.1016/j.breast.2012.12.007.

MICRORNA-223 ROLE IN THE PATHOGENESIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

R.R. Basharova[1], O.V. Berezina[1,3], M.L. Perepechaeva[2], A.Yu. Grishanova[2], T.I. Pospelova[1,3], I.B. Kovynev[1,3], M.M. Agakishiev[3], I.N. Nechunaeva[3]

Novosibirsk State Medical University, Novosibirsk, Russia[1]
Institute of Molecular Biology and Biophysics FRC FTM, Novosibirsk, Russia[2]
City Hematological Center of the Municipal Clinical Hospital №2, Novosibirsk, Russia[3]

MicroRNAs are a promising class of molecular markers of tumor growth. The purpose of the study was to determine the the role of miR-223 in the pathogenesis of chronic lymphocytic leukemia (CLL). Real-time PCR was used to study the expression of miR-223 in 35 patients with CLL: in venous blood (plasma, lymphocytes, and extracellular vesicles) and in bone marrow. There were statistically significant differences in the expression of miR-223 among the groups of patients with different prognosis, stages of the disease, and responses to therapy. Progression of CLL was

associated with low expression of miR-223, indicating the involvement of this microRNA in the pathogenesis of the disease.

Keywords: chronic lymphocytic leukemia, microRNA, miR-223.

Importance

At present, microRNAs (miRs, miRNAs) are considered a promising class of molecular markers of tumor growth. Chronic lymphocytic leukemia (CLL) is the first documented example of the involvement of miRNA in cancer progression [1]. It is assumed that many miRNAs, including miR-223, take part in the pathogenesis of CLL and can be regarded as potential molecular markers of the disease. There have been a few publications of studies on the role of miR-223 in CLL, and the results are contradictory.

Purpose of the study: to determine the role of miR-223 in the pathogenesis of chronic lymphocytic leukemia.

Material and Methods

The study population consisted of 35 patients with CLL. The age of the patients was 63.37 ± 8.67 years (mean ± SD); 23 of them were men (66%), and 12 women (34%). Ten patients had stage A of the disease (29%), 19 patients stage B (54%), and six patients stage C (17%) [2]. Based on a molecular genetic test, 11 patients (31%) were classified as an unfavorable prognosis group, and a neutral prognosis was assigned to 24 patients (69%). Indications for treatment were found in 21 patients (60%). Of these, 13 patients (62%) achieved an optimal response (partial or complete remission) to standard polychemotherapy, and eight patients (38%) experienced progression of the disease [3].

Total RNA was isolated from the venous blood (plasma, lymphocytes, and extracellular vesicles) and bone marrow of the patients using the TRIzol RNA extraction kit (Ambion, USA). MiRNA expression was evaluated by real-time PCR according to the TaqMan principle on an iCycler CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, USA). To evaluate the miR-223 expression, the ΔCT method was chosen. As a gene for normalization, small nuclear U6 RNA was selected [4].

Statistical processing of data was carried out by means of a package of applied statistical software tools Statistica 10.0. Statistical significance of differences between the mean values was determined by Student's *t* test and *U* test (Mann-Whitney test). To determine the relation between the levels of miRNA in the groups, the nonparametric Spearman correlation coefficient was calculated. The results were considered statistically significant at $p<0.05$.

Results

The expression of miR-223 in plasma was 83.7 ± 29.1, in lymphocytes 5.1 ± 2.52, in extracellular vesicles 125.14 ± 77.19, and in bone marrow 18.01 ± 4.17 (mean ± SD; arbitrary units). Differences did not reach statistical significance. In the general group of patients, a direct correlation of the mean strength between plasma and lymphocytes was observed ($r=0.63$, $p<0.02$). When CLL in peripheral blood significantly increases the numbers of lymphocytes, there is an increase in the level of miRNA in blood plasma [5].

In the unfavorable-prognosis group, expression of miR-223 in plasma was 1.4 ± 0.62, in lymphocytes 1.5 ± 0.57, in extracellular vesicles 9.65 ± 8.67, and in bone marrow 4.89 ± 3.15, whereas in the neutral prognosis group, these figures were 0.47 ± 0.31, 3.51 ± 1.69, 2.31 ± 1.47, and 4.11 ± 2.83, respectively. The differences were statistically insignificant. In the group of patients with advanced stages of the disease (B and C), which also indicate progression of the disease, a decrease in the expression of miR-223 was observed as compared with the initial stage (A): in plasma, 0.4 ± 0.29 versus 302.19 ± 172.83 ($p\leq0.002$); in lymphocytes, 1.13 ± 0.68 versus 10.21 ± 3.35 ($p\leq0.04$); and in bone marrow, 2.95 ± 0.94 versus 4.17 ± 1.77 ($p\leq0.01$). MiR-223 has not been shown previously to change expression with the progression of CLL, and in some cases, its decline has been documented, as is the case for such B-cell cancers as lymphoma of the mantle zone and lymphoma of the marginal zone, which is associated with epigenetic «silencing» of some genes [6, 7].

The response to treatment is one of the predictors of long-term outcome of a disease. During comparison of the two groups, it was noted that patients who did not achieve the optimal response to treatment had a low level of miR-223 before initiation of treatment as compared to patients who attained partial or complete remission: in plasma, 0.67±0.15 vs. 0.94±0.89 ($p\leq0.04$), in extracellular vesicles 2.3±1.03 vs. 12.11±3.46 ($p\leq0.001$), and in bone marrow 15.06±4.21 vs. 65.16±17.36 ($p\leq0.01$). These data are consistent with the results of some studies [8, 9] showing that a decrease in the level of miR-223 is a marker of unfavorable prognosis and resistance to treatment.

Conclusions

MiR-223 was present in all the analyzed blood components (plasma, lymphocytes, and extracellular vesicles) and bone marrow in patients with CLL. Progression of CLL is associated with low expression of miR-223; this finding points to the participation of this miRNA in the pathogenesis of the disease. Patients with CLL who have a low level of miR-223 in bone marrow, extracellular vesicles, and plasma before the start of chemotherapy statistically significantly do not achieve an optimal response to treatment.

We thank the Proteomic Analysis Center of the Institute of Molecular Biology and Biophysics of the Federal Research Center for Fundamental and Translational Medicine for access to equipment

REFERENCES

1. *Calin G.A., Dumitru C.D., Shimizu M., Bichi R., Zupo S., Noch E., Aldler H., Rattan S., Keating M., Rai K., Rassenti L., Kipps T., Negrini M., Bullrich F., Croce C.M.* Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA. 2002 Nov 26; 99(24): 15524-9.

2. *Binet J.L., Leporrier M., Dighiero G., Charron D., D'Athis P., Vaugier G., Beral H.M., Natali J.C., Raphael M., Nizet B., Follezou J.Y.* A clinical staging system for chronic lymphocytic leukemia: prognostic significance. Cancer. 1977 Aug; 40(2): 855-64.

3. *Hallek M., Cheson B.D., Catovsky D., Caligaris-Cappio F., Dighiero G., Döhner H., Hillmen P., Keating M.J., Montserrat E., Rai K.R., Kipps T.J.; International Workshop on Chronic Lymphocytic Leukemia.* Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008 Jun 15; 111(12): 5446-56. doi: 10.1182/blood-2007-06-093906.

3. *Schwarzenbach H., da Silva A.M., Calin G., Pantel K.* Data Normalization Strategies for MicroRNA Quantification. Clin Chem. 2015 Nov; 61(11): 1333-42. doi: 10.1373/clinchem.2015.239459.

4. *Glinge C., Clauss S., Boddum K., Jabbari R., Jabbari J., Risgaard B., Tomsits P., Hildebrand B., Kääb S., Wakili R., Jespersen T., Tfelt-Hansen J.* Stability of Circulating Blood-Based MicroRNAs - Pre-Analytic Methodological Considerations. PLoS One. 2017 Feb 2; 12(2): e0167969. doi: 10.1371/journal.pone.0167969.

5. *Stamatopoulos B., Meuleman N., Haibe-Kains B., Saussoy P., Van Den Neste E., Michaux L., Heimann P., Martiat P., Bron D., Lagneaux L.* microRNA-29c and microRNA-223 down-regulation has in vivo significance in chronic lymphocytic leukemia and improves disease risk stratification. Blood. 2009 May 21; 113(21): 5237-45. doi: 10.1182/blood-2008-11-189407.

6. *Zhou K., Yi S., Yu Z., Li Z., Wang Y., Zou D., Qi J., Zhao Y., Qiu L.* MicroRNA-223 expression is uniformly down-regulated in B cell lymphoproliferative disorders and is associated with poor survival in patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2012 Jun; 53(6): 1155-61. doi: 10.3109/10428194.2011.642303.

7. *Li S., Moffett H.F., Lu J., Werner L., Zhang H., Ritz J., Neuberg D., Wucherpfennig K.W., Brown J.R., Novina C.D.* PLoS One. 2011 Mar 8; 6(3): e16956. doi: 10.1371/journal.pone.0016956.

8. *Kisilichina D.G., Lugovskaia S.A., Pochtar' M.E., Naumova E.V., Biderman B.V., Sudarikov A.B., Nikitin E.A., Dolgov V.V.* The characteristics of evaluation of expression of ZAP-70 in tumor cells under b-cell chronic lymphatic leukemia using the flow cytofluorometry technique. Klin Lab Diagn. 2012 Aug; (8): 47-52.

POLYMORPHISM OF GENES OF INFLAMMATORY CYTOKINES (IL1B, IL6) AND GENES CONTROLLING CELL CYCLE OR APOPTOSIS (CASP8, TP53) IN GASTRIC CANCER: A PROSPECTIVE «CASE-CONTROL» STUDY IN SIBERIA

A.V. Belkovets[1], S.A. Kurilovich[1,2], Y.I. Ragino[1], V.N. Maksimov[1], L.V. Scherbakova[1], M.I. Voevoda[1]

Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia[1]
Novosibirsk State Medical University, Novosibirsk, Russia[2]

Background

Genetic polymorphism of some inflammatory cytokines (IL1B, IL6) is associated with the risk of developing specific, *H. pylori*-associated diseases, including atrophic gastritis (AG) and gastric cancer (GC). AG is the main precursor lasions of gastric cancer. Noninvasive biomarkers such as pepsinogen I (PG) and PGI/PGII ratio are used for screening AG. Some studies have shown that the polymorphism of genes controlling cell cycle or apoptosis (CASP8, TP53) is associated with GC risk.

Aim: to study the association of *511C/T (rs16944) IL1B* and 174G/C IL6 genes polymorphism, the Arg72Pro 4 polymorphism of the TP53 gene and -652 6N ins/del of the promoter variant (rs3834129) of the CASP8 gene with GC and biomarkers of mucosal atrophy in patients with GC in population-based prospective case-control study in the Siberian population (8 years follow up).

Material and Methods

In the context of international project HAPIEE general population sample was surveyed in Novosibirsk in 2003-2005 (9360 subjects aged 45-69 years), and a base of biomaterials was created. Serum and DNA samples were deeply frozen and stored. In 2012 this database was compared with

the data of the Population Cancer Registry. For each case of GC, an appropriate control case was selected at the ratio 1:2 matching the area of residence, sex and age. Finally 156 serum samples (52 - GC group and 104 – control) were available for the analysis using a panel of serum biomarkers "Gastropanel" (Biohit, Finland) and 146 DNA samples (50 - GC group and 96 - control) were genotyped according to the published method. Serum samples were tested using diagnostic kit for enzyme-linked immunosorbent assays to determine the levels of pepsinogen I (PGI), PGII, PGI/PGII ratio, gastrin-17 and IgG antibodies to *H.pylori*. The standard criteria for the diagnosis of AG were used according to manufacturer's instructions.

Results

The frequency of studied genotypes in the control group did not deviate from Hardy-Weinberg equilibrium. Frequency T/T genotype of the IL1B was found significantly higher in the GC group (16.3%) compared with the control (5.4%) (p=0.03). The T/T genotype was associated with significantly increased risks of GC compared with the C/C genotype (OR=3.4; 95% CI: 1.0-11.0, p=0.03). It was shown that rare T allele carriers have increased risk GC development (OR=1.69; CI: 1.01-2.81, p=0.04) in comparison with wild C allele carriers (OR=0.59; CI: 0.36-0.99, p=0.04). The mean levels of PGI and PGI/II ratio in persons with T/T genotype were significantly lower in GC group than in control (41.3±31.8 µg/l and 4.1±2.9 vs. 131.0±57.2 µg/l and 7.0±2.8; p=0.0001 and p=0.05 respectively). There were no differences in the frequency of IL6 genotypes between the groups «case» and «control». However, the average level of PGI at genotype G/G was aproximately 2 times lower in the case group compared to the control group (52.0±43.7 µg/l vs. 100.4±54.9 µg/l, p=0.008). There was no difference between the frequencies of CASP8 and TP52 genotypes in patients with GC and control group. Mean values of PGI and PGI/PGII ratio were significantly lower in the GC group than in the control group for the ins/del CASP8 genotype (p = 0.036 and p=0.001, respectively).

When conducting multivariate regression analysis with the inclusion of both quantitative and categorical variables in the model, the PGI/PGII ratio (B= -0.726; p<0.001; OR=0.484; 95% CI 0.332-0.706) and T/T genotype of TP53 were significant in the model. The carriers of the genotype T/T of the gene IL1B had increased risk of GC compared to carriers of the genotype C/C (B=3.322; p=0.028; OR=27.704; 95% CI 1.424-539.049). On the contrary, the GC risk was lower in the carriers of pro/pro variant of the TP53 gene than in the carriers of the arg/arg genotype TP53 (B=-3,178; p=0.037; OR=0.042; 95% CI 0.002-0.820).

Conclusion

IL1B polymorphism *(rs16944)* is associated with an increased risk of GC in the population of Western Siberia. The relationship of polymorphisms of 174G/C IL6 gene, -652 6N ins/del of the CASP8 gene and Arg72Pro of TP53 gene with GC risk developing has not been found. However, polymorphism 174G/C of the IL6 gene is associated with atrophic gastritis and the carriers of pro/pro variant of the TP53 gene have lower GC risk (multivariate regression analysis). It is necessary to continue research to confirm their significance.

Keywords: atrophic gastritis, gastric cancer, polymorphism, inflammatory cytokines, genes, IL1B, IL6, apoptosis, TP53, CASP8, pepsinogenes, *H. pylori.*

REFERENCES

1. *Agréus L., Kuipers E.J., Kupcinskas L., Malfertheiner P., Di Mario F., Leja M., Mahachai V., Yaron N., van Oijen M., Perez Perez G., Rugge M., Ronkainen J., Salaspuro M., Sipponen P., Sugano K., Sung J.* Rationale in diagnosis and screening of atrophic gastritis with stomach-specific plasma biomarkers. Scand J Gastroenterol. 2012 Feb; 47(2): 136-47. doi: 10.3109/00365521.2011.645501.

2. *Mocellin S., Verdi D., Pooley K.A., Nitti D.* Genetic variation and gastric cancer risk: a field synopsis and meta-analysis. Gut. 2015 Aug; 64(8): 1209-19. doi: 10.1136/gutjnl-2015-309168.

3. *Chen D., Ma T., Liu X.W., Liu Z.* CASP-8-652 6N ins/del polymorphism and cancer risk: a literature-based systematic HuGE review and meta-analysis. Exp Ther Med. 2012 Oct; 4(4): 762-770.

4. *Liamarkopoulos E., Gazouli M., Aravantinos G., Tzanakis N., Theodoropoulos G., Rizos S., Nikiteas N.* Caspase 8 and caspase 9 gene polymorphisms and susceptibility to gastric cancer. Gastric Cancer. 2011 Oct; 14(4): 317-21. doi: 10.1007/s10120-011-0045-1.

5. *Kamp W.M., Wang P.Y., Hwang P.M.* TP53 mutation, mitochondria and cancer. Curr Opin Genet Dev. 2016 Jun; 38: 16-22. doi: 10.1016/j.gde.2016.02.007.

6. *Xiang B., Mi Y., Li T.F., Liu P.F.* Updated Meta-analysis of the TP53 Arg72Pro Polymorphism and Gastric Cancer Risk. Asian Pac J Cancer Prev. 2012; 13(5): 1787-91.

7. *Belyavskaya V.A., Vardosanidze V.K., Smirnova O.Y., Karakin E.I., Savkin I.V., Gervas P.A., Cherdyntseva N.V., Voevoda M.I.* Genetic status of p53 in stomach cancer: somatic mutations and polymorphism of the 72 codon. Bull Exp Biol Med. 2006 Feb; 141(2): 243-6.

8. *Du Y., Gao L., Zhang K., Wang J.* Association of the IL6 polymorphism rs1800796 with cancer risk: a meta-analysis. Genet Mol Res. 2015 Oct 27; 14(4): 13236-46. doi: 10.4238/2015.October.26.20.

9. *Wang J., He W., Liu J., Nong L., Wei Y., Yang F.* Association of IL-6 polymorphisms with gastric cancer risk: evidences from a meta-analysis. Cytokine. 2012 Jul; 59(1): 176-83. doi: 10.1016/j.cyto.2012.03.032.

10. *El-Omar E.M., Carrington M., Chow W.H., McColl K.E., Bream J.H., Young H.A., Herrera J., Lissowska J., Yuan C.C., Rothman N., Lanyon G., Martin M., Fraumeni J.F.Jr., Rabkin C.S.* Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000. 404: 398–402. doi: 10.1038/35006081.

11. *Machado J.C., Pharoah P., Sousa S., Carvalho R., Oliveira C., Figueiredo C., Amorim A., Seruca R., Caldas C., Carneiro F., Sobrinho-Simões M.* Interleukin-1β and interleukin-1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology. 2001 Oct; 121(4): 823-9.

SALIVARY CYTOKINE LEVELS IN LUNG CANCER DEPENDING ON THE TUMOR SIZE

L.V. Bel'skaya[1], V.K. Kosenok[1,2]

KhimServis LLC, Moscow, Russia[1]
Omsk State Medical University, Omsk, Russia[2]

In this study we have investigated the level of cytokines and acute-phase proteins in saliva of patients with lung cancer in dependence on the tumor size. The case-control study included 121 patients, which were divided into 3 groups: the main group (patients with diagnosed lung cancer, n = 70), the reference group (patients with non-malignant lung pathologies, n = 12) and the control group (conditionally healthy individuals, n = 39). All participants answered the questionnaire, underwent biochemical study of saliva and histological verification of the diagnosis. The content of IL-2 and IL-4 in saliva was reduced in both lung cancer and inflammatory lung diseases, whereas the levels of IL-18, IL-8 and TNF-α were decreasd in lung cancer and increased in non-malignant pathologies. Tumor progression was accompanied by an increase in the level of proinflammatory cytokines (IL-6, IL-8, IL-18, TNF-α), whereas the levels of IL-2, IL-4 and IL-10 were decreased. Although saliva levels of C-reactive protein and tumor markers were increased in lung cancer, they did reach the level of statistical significance as compared with the reference group. Thus, with the only exception of IL-2 and IL-4, the saliva levels of cytokines in lung cancer patients insignificantly differed from the control group.

Keywords: Cytokines, C-reactive protein, Saliva, Lung cancer.

Optimization of methods for diagnostics and prediction of lung cancer, which is the most common malignancy in worldwide, still remains a challenge. It is known that acute-phase proteins (such as C-reactive protein) and cytokines (such as IL-6) can be more easily detected in saliva than in blood serum or plasma. Since cytokines from saliva can accumulate over time, their levels can be detected more effectively than those of cytokines in blood. Saliva is most widely used for the diagnostics of oral cavity diseases including cancer. However, in the case of lung diseases salivary cytokines were used only in diagnostics of tuberculosis. Nevertheless, there is evidence that diagnosis of lung cancer may include the following potentially informative cytokines: IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP). Thus, the aim of this study was to evaluate the level of cytokines and CRP in saliva of patients with lung cancer in dependence of tumor sizes.

Material and Methods

The case-control study included 82 patients from the Omsk Clinical Oncology Center and 39 healthy people selected as the control group. The main group consisted of 70 patients with lung cancer (squamous cell carcinoma – 27, adenocarcinoma – 32, neuroendocrine tumors – 11); the reference group consisted of 12 patients with non-malignant pulmonary pathology (tuberkulema – 4, pnevmofibros – 3, inflammatory pseudotumor – 2, pneumonia – 3). The control group consisted of conditionally healthy patients without lung pathologies. The average age of patients was 59.2 ± 1.1 years for the main group, 56.0 ± 2.1 years for the reference group, and 52.1 ± 2.5 years for the control group. The inclusion criteria were: the age of patients (30-75 years old), no previous treatment including surgery, chemotherapy or radiotherapy at the time of the study, absence of signs of active infection (including purulent processes) and conduction of oral cavity sanation. The exclusion criterion was the absence of histological verification of the diagnosis.

Samples of saliva were collected in the morning after overnight fast by spitting into sterile tubes, followed by centrifugation at 8500 g. The content of salivary cytokines (in pg/mL) (IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, TNF-α) and CRP (in mU/L) was determined by the enzyme-linked immunosorbent assay method. The study was approved at a meeting of the Local Ethics Committee of the Omsk Regional Clinical Hospital «Clinical Oncology Center» on July 21, 2016 (Protocol no. 15).

The statistical analysis was made using Statistica 10.0 (StatSoft, USA) and R (version 3.2.3) software using the nonparametric Wilcoxon test in dependent groups, and the Mann-Whitney U test in independent groups. Results were expressed as the median (Me) and the interquartile range in the form of the 25th and 75th percentile [LQ; UQ]. Differences were considered as statistically significant at $p \leq 0.05$.

Results

The study revealed that lung cancer caused a decrease in the levels of IL-2, IL-4, IL-18, IL-8 and TNF-α, whereas the levels of IL-6 and IL-10 were increased. However, statistically significant differences were found only for IL-4 and TNF-α (Table 1). It should be noted that the increased level of IL-18 in the reference group differed from both the main group (p = 0.0472) and the control group (p = 0.0327).

Table 1

Cytokines level in saliva of patients from investigated groups

Parameter	Control	Lung Cancer	Comparison group
IL-2	1.42 [0.88; 1.88]	1.16 [0.70; 2.36]	0.98 [0.62; 1.85]
IL-4	1.21 [0.43; 1.68]	0.69 [0.23; 1.54]	0.89 [0.31; 1.59]
	-	*p_1=0.0461*	-
IL-6	0.72 [0.55; 1.60]	0.97 [0.49; 2.49]	No data
IL-8	159.42 [83.61; 223.61]	108.00 [60.30; 214.00]	184.42 [156.04; 310.09]
IL-10	1.81 [0.99; 2.31]	2.01 [1.18; 2.90]	1.76 [1.04; 2.46]
IL-18	18.45 [7.40; 36.90]	13.60 [4.79; 56.40]	99.10 [43.80; 176.00]
	-	-	*p_1=0.0327; p_2=0.0472*
TNF-α	2.36 [1.37; 3.09]	0.884 [0.487; 1.880]	2.73 [0.92; 3.25]
	-	*p_1=0.0060*	-
CRP	0.020 [0.015; 0.034]	0.028 [0.015; 0.056]	0.023 [0.013; 0.104]

Note. p_1 – statistically significant differences as compared to the control group, p_2 – differences with the main group

During the next stage of the study, the main group was subdivided according to histological types of lung cancer. For all histological types of lung cancer, the levels IL-4, IL-18, IL-8, and TNF-α were lower, whereas the level of IL-6 was higher than those of the control group. It is interesting to note that the levels of IL-2 and CRP were changed differently for the non-small cell and neuroendocrine lung cancer. An increase in the level of IL-2 was accompanied by a decrease in the CRP concentration in case of neuroendocrine tumors and vice versa for adenocarcinoma and squamous cell lung cancer.

It is interesting to consider the dynamics of concentration of the studied parameters depending on the tumor size (Table 2). In this context cytokines were subdivided into 2 groups according to the nature of concentration changes. During disease progression, the levels of IL-6, IL-8 and Il-18 were increased, while the levels of IL-2, IL-4 and IL-10 were decreased. It should be noted that the detected decrease in the cytokine level was statistically significant in the case of IL-2 (p = 0.0410), IL-4 (p = 0.0193) and IL-10 (p = 0.0485). The CRP level increased with increasing the tumor size (p = 0.0367).

Table 2

Dynamics of cytokines in saliva depending on the size of the tumor

Parameter	$T_1N_{0-3}M_{0-1}$, n=9	$T_2N_{0-3}M_{0-1}$, n=30	$T_3N_{0-3}M_{0-1}$, n=21	$T_4N_{0-3}M_{0-1}$, n=10
IL-2	2.61	1.11	1.14	1.02
	[1.66; 4.94]	[0.64; 2.13]	[0.81; 2.73]	[0.60; 2.52]
	-	*p=0.0069*	*p=0.0404*	*p=0.0410*
IL-4	1.47	1.00	0.53	0.73
	[0.68; 2.00]	[0.16; 1.66]	[0.20; 1.05]	[0.35; 1.16]
	-	-	*p=0.0193*	-
IL-6	0.86	0.75	1.18	4.17
	[0.56; 3.93]	[0.44; 2.34]	[0.33; 1.81]	[4.00; 4.33]
IL-8	68.90	130.00	98.20	151.00
	[54.40; 171.50]	[66.58; 249.00]	[51.70; 160.16]	[117.00; 331.72]
IL-10	2.71	2.01	2.09	1.78
	[1.93; 13.10]	[0.91; 2.65]	[1.29; 3.03]	[1.33; 2.41]
	-	*p=0.0486*	-	p=0.0485

Parameter	$T_1N_{0-3}M_{0-1}$, n=9	$T_2N_{0-3}M_{0-1}$, n=30	$T_3N_{0-3}M_{0-1}$, n=21	$T_4N_{0-3}M_{0-1}$, n=10
IL-18	19.10 [9.68; 60.55]	9.80 [4.28; 68.90]	11.50 [4.59; 33.80]	42.40 [13.60; 170.00]
CRP	0.017 [0.015; 0.020]	0.024 [0.015; 0.041]	0.033 [0.012; 0.121]	0.044 [0.024; 0.056]
	-	-	-	*p=0.0367*
TNF-α	No data	No data	0.88 [0.49; 1.71]	1.26 [0.42; 1.90]

Note. p – statistically significant differences compared to the group $T_1N_{0-3}M_{0-1}$

Conclusions

Our data showed that the cytokine levels in the saliva of lung cancer patients and healthy individuals did not differ statistically. It was demonstrated that the content of IL-2 and IL-4 in saliva was reduced in both lung cancer and inflammatory lung diseases, whereas the levels of IL-18, IL-8 and TNF-α were decreased in lung cancer and were increased in non-tumorous pathologies. Tumor progression caused an increase in the content of proinflammatory cytokines (IL-6, IL-8, IL-18, TNF-α) and decrease in the levels of IL-2, IL-4 and IL-10. The increased levels of C-reactive protein and tumor markers were observed in lung cancer; however, differences in these levels in inflammatory lung diseases were insignificant.

PRECLINICAL STUDIES OF THE DNA VACCINE AGAINST MELANOMA

E.A. Borobova[1,2], D.V. Antonets[1], E.V. Starostina[1], S.F. Oreshkova[1], D.N. Shcherbakov[1], O.Yu. Smirnova[1], S.I. Bazhan[1], A.A. Ilyichev[1], L.I. Karpenko[1], E.D. Danilenko[1], S.G. Gamalei[1], S.V. Usova[1], M.P. Bogryantseva[1]

Siberian Research Center of Virology and Biotechnology «Vector», Novosibirsk, Russia[1]
E.N. Meshalkin National Medical Research Center, Novosibirsk, Russia[2]

Melanoma is the most aggressive and dangerous skin cancer. It is characterized by rapid growth and high incidence of metastasis. Melanoma cells have tumor antigens on their surfaces. Thereby, it is possible to create an effective therapeutic vaccine against melanoma. We created the allele-specific MEL-TCI construct which includes epitopes restricted with HLA-A*02:01. We demonstrated that the designed genetic constructs provided synthesis of respective mRNAs and proteins in a culture of transfected eukaryotic HEK-293T cells. Dendritic cells from HLA-A*02:01+ donors transfected with DNA vaccine constructs induced autologous T-lymphocytes to develop a specific cytotoxic activity to Mel Is melanoma cells in the model of T-cell response induction ex vivo.

Keywords: melanoma, anti-tumor DNA vaccine, T-cell epitopes, artificial polyepitope antigens, cytotoxic response.

Background

Melanoma is the most dangerous malignant skin cancer. It accounts for only 1% of all malignant skin tumors, nevertheless causing 80% fatalities. The standard methods of melanoma treatment achieve positive results, but fail to increase the patients' survival rate. The high mortality and low treatment efficiency necessitate development of new approaches to efficiently control melanoma [1].

High immunogenicity of melanoma cells seems to be quite important. Researchers described a number of highly immunogenic protein antigens (Melan-A/MART-1, gp100, tyrosinase, MAGE-3, and NY-ESO-1) that are specific to melanoma and several other cancer types but are absent (or virtually absent) in normal cells of human organism [2, 3]. High immunogenicity of melanoma cells enables to develop immunotherapeutic strategies to this disease based on advancements in the modern molecular immunology, bioinformatics, and cell technologies. DNA vaccination is one of the most attractive approaches as it induces activation of cytotoxic CD8+ T-lymphocytes that are the main effector cells of antitumor immune response [4].

The aim of the study was to design artificial polyepitope immunogen comprising variety of T-cell epitopes from melanoma antigens, develop a candidate DNA vaccine on their base and evaluate their antitumor response within the system of T-cell response induction *ex vivo*.

Material and Methods

The prediction of T-cell epitopes and design of sequence of an allele-specific T-cell immunogen MEL-TCI were performed using software TEpredict and PolyCTLDesigner [5, 6]. The designed gene was synthesized (Evrogen LLC, Russia) and then cloned in pcDNA3.1 plasmid which is a eukaryotic expression vector. The sequence of cloned gene was verified in sequence analysis.

Evaluating target gene expression in HEK-293T cells transfected with pMEL-TCI was performed by means of three methods: (1) detection of synthesis of specific mRNA in transfected HEK-293T cells; (2) immunochemical staining of transfected cells and (3) immunoblotting using MAb to Gag-epitope.

Analysis of antitumor activity of pMEL-TCI was performed ex vivo using peripheral blood mononuclear cells obtained from HLA-A*0201 positive donors. Immunogenic activity was analyzed using flow cytometry analysis. Cytotoxic activity of effector cells was detected using the colorimetric method of quantitative estimation of the lactate-dehydrogenase content, i.e. cytosolic ferment released from lysed tumor cells.

Results

In the study we approved that the designed DNA vaccine construct MEL-TCI provide synthesis of the target mRNA and proteins in the transfected eukaryotic cell culture HEK 293T. The results of evaluating cytotoxic activity in the designed vaccine - pMEL-TCI - demonstrated its capability to induce lysis of Mel Is melanoma cells that was estimated in the system of T-cell response induction ex vivo. Tumor-specific immune response correlated with capability of vaccine constructs to induce granzyme B synthesis in the co-culture of PBMC and DC. We also carried out pre-clinical studies of the designed DNA vaccine pMEL-TCI in mice and guinea pigs. It was shown the absence of toxic and immunotoxic properties. We approved that there was no any negative influence on reproductive function and mutagenic effect in Eimes test. Studies on rabbits showed that the vaccine pMEL-TCI was not pyrogenic.

Conclusion

Using computer approaches to predict T-cell epitopes and design polyepitope antigens we have been able to develop DNA vaccine - MEL-TCI - capable of inducing synthesis of relevant proteins in human cells and a specific immune response to human melanoma cells. Pre-clinical studies showed that the vaccine pMEL-TCI does not affect negatively the physiological, biochemical and morphological parameters of immunized animals. So the designed DNA vaccine pMEL-TCI is completely safe for using in clinical practice.

REFERENCES

1. *Cancer* Facts & Figures 2018. Atlanta: American Cancer Society. 2018; 76.

2. *Pandolfi F, Cianci R, Lolli S, Dunn I, Newton E, Haggerty T, Boyle L, Kurnick J.* Strategies to overcome obstacles to successful immunotherapy of melanoma. International Journal of Immunopathology and Pharmacology. 2008; 21(3): 493-500. doi:10.1177/039463200802100302.

3. *Parmiani G.* Melanoma antigens and their recognition by T cells. Keio J Med. 2001 Jun; 50(2): 86-90.

4. *Denapoli P.M., Zanetti B.F., dos Santos A.A., de Moraes J.Z., Han S.W.* Preventive DNA vaccination against CEA-expressing tumors with anti-idiotypic scFv6. C4 DNA in CEA-expressing transgenic mice. Cancer Immunol Immunother. 2017 Mar; 66(3): 333-342. doi: 10.1007/s00262-016-1940-4.

5. *Antonets D., Maksyutov A.* TEpredict: software for T-cell epitope prediction. Mol Biol (Mosk). 2010 Jan-Feb; 44(1): 130-9.

6. *Antonets D.V., Bazhan S.I.* PolyCTLDesigner: a computational tool for constructing polyepitope T-cell antigens. BMC Res Notes. 2013 Oct 10; 6: 407. doi: 10.1186/1756-0500-6-407.

CIRCULATING CELL-FREE NUCLEIC ACIDS IN BLOOD PLASMA OF LUNG CANCER PATIENTS EXPOSED TO RADON

O.V. Bulgakova, A.A. Kussainova, A.Zh. Kausbekova, R.I. Bersimbaev

L.N. Gumilyov Eurasian National University, Institute of Cell Biology and Biotechnology, Astana, Kazakhstan

Lung cancer causes aproximately 1.3 million deaths/year worldwide. Radon is one of the most powerful carcinogens especially in terms of lung cancer onset and development. Almost the whole eastern and northern part of Kazakhstan is potentially radon-affected area. Accordingly, the population of Kazakhstan might be exposed to radon. Recently, circulating cell-free nucleic acids

in biological liquids have been considered as potential biomarkers in various diseases including lung cancer. 136 subjects were examined including 49 radon-exposed lung cancer patients, 37 lung cancer patients without radon exposure and 50 age-genders matched healthy controls. Total RNA from blood samples was extracted and used to detect miR-19b-3p and miR-125b-5p expression by quantitative real-time polymerase chain reaction (qRT-PCR). Plasma miR-19b-3p and miR-125b-5p levels were significantly higher in the lung cancer patients' groups compared with healthy control ($P<0.0001$ and $P<0.01$ respectively). No other statistically significant differences were found in the expression level of miRNAs in blood plasma between patients diagnosed with lung cancer exposed to radon and not exposed to radon. Therefore plasma miR-19b-3p and miR-125b-5p levels could be used as noninvasive biomarkers for detection of lung cancer but not for radon impact.

Keywords: circulating cell-free nucleic acids, miRNA, lung cancer, biomarker, miR-19b-3p, miR-125b-5p, radon.

Introduction

Lung cancer is the leading cause of cancer-related mortality in Kazakhstan [1]. According to the World Health Organization [2], epidemiological studies have provided strong evidence for correlations between exposure to indoor radon and lung cancer even at the relatively low levels of radon commonly found in residential buildings. Northern Kazakhstan territory has number of factors that determine natural and man-made manifestations of elevated radioactivity. Granitic rocks, which contain a large amount of uranium, are also active sources of radon in this area [3]. Recently, microRNAs (miRNAs), circulating cell-free nucleic acids in biological liquids, have been considered as potential biomarkers in various diseases. In cells, miRNAs present as short (18-22 nucleotides), non-coding molecules involved in post-transcriptional gene regulation, messanger RNA degradation and/or translation inhibition. Circulating miRNAs, passively leaked or actively transported outside cells, can be stably detected in blood and may be proposed as biomarkers for diagnosis, prognosis or monitoring curative effect in various cancers including lung cancer [4]. The aim of this study is to determine the alterations in circulating cell-free miRNAs in the plasma of lung cancer patients exposed to radon.

Material and Methods

A total of 136 subjects were examined, including, (a) 49 radon-exposed lung cancer patients (RLC); (b) 37 lung cancer patients without radon exposure (LC); (c) 50 healthy controls (C). The study was approved by the Ethical Committee of the Semey State Medical University, Kazakhstan. Total RNA from blood plasma was isolated using the miRCURY RNA Isolation Kit – Biofluids (#300112, EXIQON, Vedbaek, Denmark) in accordance with the protocol. The expression level of miR-19b-3p and miR-125b-5p was determined by evaluating the level of fluorescence emitted by SYBRGREEN tracer using MiRCURY LNATM UNIVERSAL RT microRNA PCR LNATM kit (#204450, #205713, EXIQON, Vedbaek, Denmark). Gene expression was normalized to RNU6B (#203907, EXIQON, Vedbaek, Denmark). All reactions were carried out in triplicate, and the $2^{-\Delta\Delta Ct}$ method ($\Delta CT = CTmiR - CTU6$) was used to quantify the relative miRNA amount [5]. Measurement of radon activity was conducted in accordance with the Rapid Measurement Method of radon and thoron using a Canary 222 Digital Electronic Radon Gas Monitor (LR-03) radiometer (Corentium AS, Norway). The annual effective dose (H) was calculated according to *Quarto et al.* [6]. Data were analyzed by one-way ANOVA with multiple comparison test with 95% confidence intervals using the GraphPad Prism 6 program. (La Jolla, CA, USA).

Results and Discussions

MiR-19b-3p expression level in the plasma of lung cancer patients with and without radon exposure. The total circulating microRNA was isolated from blood plasma using the protocol described above and divided to three groups: patients diagnosed with lung cancer living in areas with a radon concentration below 200 Bq/m^3 for at least 5 years; patients diagnosed with lung cancer who had been exposed to high doses of radon for 5 years and a control group consisting of healthy individuals, who did not have pulmonary pathology and lived in the area with a low levels of radon. miR-19b-3p expression level in patients of RLC group was 6.5 times higher ($P<0.0001$) than in control group. In group of lung cancer patients without radon exposure, the expression level of the same miRNA was 6.9 times higher ($P<0.0001$) compared to miR19b-3p level detected in the blood plasma of healthy individuals.

MiR-125b-5p expression level in the plasma of lung cancer patients with and without radon exposure. The level of miR-125b-5p expression in all three groups is shown in Figure 1.

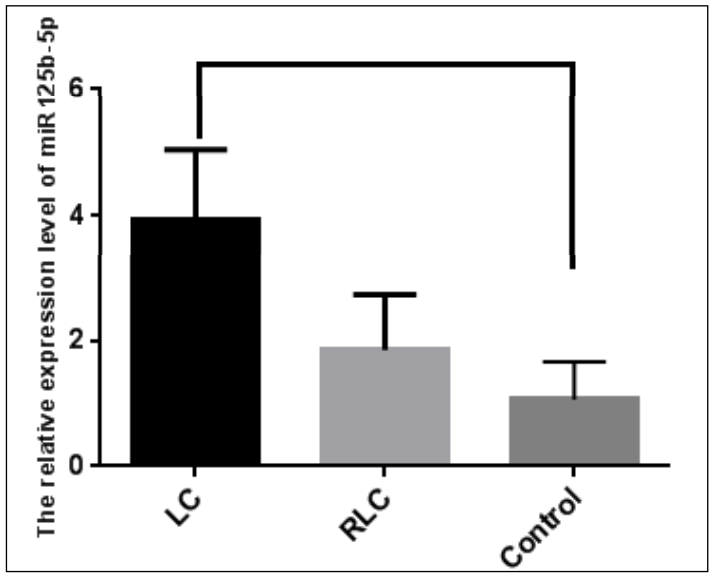


Figure 1. Relative expression level of miR-125b-5p in the plasma of lung cancer patients exposed (RLC) and not exposed (LC) to radon in comparison with the control group (C). *P<0.01vs C

The relative expression level of miR-125b-5p in lung cancer patients was 4 times higher than that in healthy people ($p < 0.01$). However, the comparative analysis of miR-125b-5p blood expression level in radon-exposed lung cancer patients (RLC) and in the control group (Control) revealed no significant difference between the two groups.

Thus, the results obtained suggest that miRNAs miR19b-3p and miR-125b-5p play an important role in cancer development. However, according to the given study, these miRNAs are inappropriate to be utilized as a hallmark of radon impact.

REFERENCES

1. *Bersimbaev R., Bulgakova O.* Residential radon exposure and lung cancer risk in Kazakhstan Radon. InTech, 2017. 93-124. doi: 10.5772/intechopen.71135.

2. *Zeeb H., Shannon F.* WHO Handbook on Indoor Radon: A Public Health Perspective. World Health Organization, Geneva, 2009. 3-21.

3. *Bersimbaev R.I., Bulgakova O.* The health effects of radon and uranium on the population of Kazakhstan. Genes and Environment. 2015 Oct; 37: 18. doi:10.1186/s41021-015-0019-3

4. *Izzotti A., Carozzo S., Pulliero A., Zhabayeva D., Ravetti J.L., Bersimbaev R.* Extracellular MicroRNA in liquid biopsy: applicability in cancer diagnosis and prevention. American Journal of Cancer Research. 2016 Jul; 6(7): 1461-1493.

5. *Livak K.J., Schmittgen T.D.* Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods. 2001 Dec; 25: 402-408.

6. *Quarto M., Pugliese M., La Verde G., Loffredo F., Roca V.* Radon Exposure Assessment and Relative Effective Dose Estimation to Inhabitants of Puglia Region, South Italy. Int J Environ Res Public Health. 2015 Nov 23; 12(11): 14948-57. doi: 10.3390/ijerph121114948.

CARCINOGENICITY OF ENVIRONMENTAL SUBSTANCES AS PARATHION AND MALATHION IN BREAST CARCINOGENESIS

G.M. Calaf

Instituto de Alta Investigación, Universidad de Tarapacá, Arica, Chile and Columbia University Medical Center, NY, USA

Environmental chemicals seem to be involved in the etiology of breast cancers. Studies have addressed the association between human cancer and agricultural pesticide exposure. For example the organophosphorous pesticides have been used extensively to control mosquito plagues. Among them, parathion (P) and malathion(M) have been extensively used. They have many structural similarities with naturally occurring compounds, and their primary target of action in insects is the nervous system; they inhibit the release of the enzyme acetylcholinesterase at the synaptic junction. We have analyzed the effect of these pesticides on phenotypic and molecular characteristics of cells in a) rat mammary gland and lung *in vivo* systems and b) in the normal breast epithelial cell line MCF-10F. There were four experimental groups: control, either M or P, estrogen (E)and M

or P plus E treated for 5 days and sacrificed after 30, 124 and 240 days. Results showed that a) P and M increased cell proliferation of terminal end buds of the 44-day-old mammary gland of rats, followed by formation of mammary carcinomas after about 28 months. Studies showed progressive alterations in a) mammary gland in number of ducts in stage of proliferation (dsp) and secretory lobules (SL) per mm^2; alveoli of lung in stage of proliferation (asp), formation of lymphatic cell aggregates (LCA) (μm^2) and preneoplasias in bronchiolar epithelium and carcinoma *in situ.* M+E showed significantly ($p<0.05$) higher number of dsp, SL, asp, LCA and pre-neoplasias and a significant ($p<0.05$) increase in Rho-A, c-Erb2, c-fos, mp53 and CYP1A2 protein expression after 240 days in treated groups and tumors. *In vitro* studied showed that P, M in combination with E induced malignant transformation of MCF-10F indicated by increased cell proliferation, invasive capabilities and changes in protein expression as shown by increased Rho-A and mutant p53, beta catenin in comparison to control. cDNA human cancer array revealed 17 out of a total of 408 genes affected by pesticides, all involved in the regulation of cell cycle, cell growth, signal transduction pathways. Results indicated that P, M and E treatment increased c-Ha-ras gene expression corroborated by protein expression. A cDNA gene expression array of human drug metabolism included functional genes related with metabolism of drugs, chemical, hormones and micronutrients and showed that MCF-10F cells treated with P and E increased gene expression cytocrome P450 (CYPs), gluthation S-transferase, metallotionein IX, metyltransferase, sulfotransferase in the epithelium of mammary glands well as human breast epithelial cells.

Keywords: gene, malignant transformation, malathion, parathion, breast epithelial cells.

Supported by Ministry of Education (MINEDUC), Universidad de Tarapacá, Arica, Chile

REFERENCES

1. *Cabello G., Valenzuela M., Vilaxa A., Durán V., Rudolph I., Hrepic N., Calaf G.* A rat mammary tumor model induced by the organophosphorous pesticides, parathion and malathion, possibly through acetylcholinesterase inhibition. Environ Health Perspect. 2001 May; 109(5): 471-9.

2. *Calaf G.M., Roy D.* Gene expression signature of parathion-transformed human breast epithelial cells. Int J Mol Med. 2007 May; 19(5): 741-50.

3. *Calaf G.M.***,** *Echiburú-Chau C., Roy D.* Organophosphorous pesticides and estrogen induce transformation of breast cells affecting p53 and c-Ha-ras genes. Int J Oncol. 2009 Nov; 35(5): 1061-8.

4. *Guyton K.Z., Loomis D., Grosse Y., El Ghissassi F., Benbrahim-Tallaa L., Guha N., Scoccianti C., Mattock H., Straif K.; International Agency for Research on Cancer Monograph Working Group, IARC, Lyon, France.* Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 2015 May; 16(5): 490-1. doi: 10.1016/S1470-2045(15)70134-8.

INCREASE IN THE CONCENTRATION OF IL-8 IN LOCAL FLUIDS, AS AN ADDITIONAL CRITERION FOR THE DIAGNOSIS OF CHOROIDAL MELANOMA

M.A. Chernyavskaya, V.V. Atamanov

Federal State Autonomous Institution «S.N. Fedorov National Medical Research Center «MNTK «Eye Microsurgery» of Ministry of Health of the Russian Federation» Novosibirsk branch, Novosibirsk, Russia

Choroidal melanoma is difficult to detect in early stages due to asymptomatic tumor growth in most cases. **The aim of the study** was to determine the concentration of interleukin-8 in the tear fluid of patients with choroidal melanoma. **Material and Methods.** The concentration of interleukin-8(IL-8) in tear fluid was detrmined in 36 patients diagnosed with choroidal melanoma. **Results and discussion**. Choroidal melanoma was diagnosed when the IL-8 concentration in the tear fluid of the affected eye or paired «healthy» eye was above 17.7pg/ml.

Keywords: Choroidal melanoma, interleukin-8, cytokine.

Background

Choroidal melanoma is the most commonly diagnosed primary intraocular malignancy (80–90%) [2, 6]. Choroidal melanoma in early stages is diagnosed infrequently, and systemic metastases appear in 40% of cases within 5 years. Approximately 30–50% of patients die within 10 years of diagnosis and treatment [1]. In the treatment of small choroidal melanoma, the 5-year mortality is 12% [6, 9]. Cancer can metastasize before the onset of eye symptoms (about 2% of cases) [5, 10].

Factors that worsen prognosis are as follows: patient's age, histological type, tumor location and size, tumor spread beyond the primary site of tumor [1, 4].

The aim of the study was to dtermine the concentration of proinflammatory cytokine interleukin-8 in tears of patients with choroidal melanoma.

Material and Methods

The study included 36 patients with choroidal melanoma in the 34-83 age range (mean age, 60 years), who were treated at S.N. Fedorov National Medical Research Center «Eye Microsurgery». The IL-8 content in tear fluid was determined using a solid-phase immunoassay (ELISA) method based on the «sandwich» principle, using the commercial set of the Vector-Best test system (Novosibirsk) according to the protocol recommended by the manufacturer. Twenty healthy volunteers of the same age, who did not have ophthalmologic disease and bacterial and viral infections served as controls. Statistical analysis was carried out using software R [8].

Results and Discussion

Choroidal melanoma was diagnosed when IL-8 concentration in the tear fluid of the affected eye or paired «healthy» eye was above 17.7 pg / ml.

The mean concentrations of IL-8 in the lacrimal fluid in the eye affected by the choroidal melanoma and in the paired «healthy» eye were 19.4 (18.8, 20.7) pg / ml and 19.4 (18.8, 23, 5) pg / ml, respectively. These values were above the threshold concentration of 17.7 pg / ml, thus confirming the presence of choroidal melanoma. The mean concentration of IL-8 in the control group was 10.9 (9.1, 13.7) pg / ml, which was below the threshold diagnostic concentration of 17.7 pg / ml, thereby confirming the absence of choroidal melanoma.

Figure 1 presents the results of the ROC (Receiver Operator Characteristic) analysis of the IL-8 concentration in the lacrimal fluid, where the sensitivity is plotted on the ordinate axis (the probability of truly positive cases with choroidal melanoma, i.e, if IL-8 value is more than 17.7 pg / ml, the patient has a tumor in 100% of cases), and the abscissa presents the specificity of the analysis (the probability of a false positive results, that is, if IL-8 value is less than 17.7 pg / ml, the patient does not have a tumor). The more accurate the test, the closer the area of the ROC curve to 1.0; in this case the area of the ROC curve is 1.

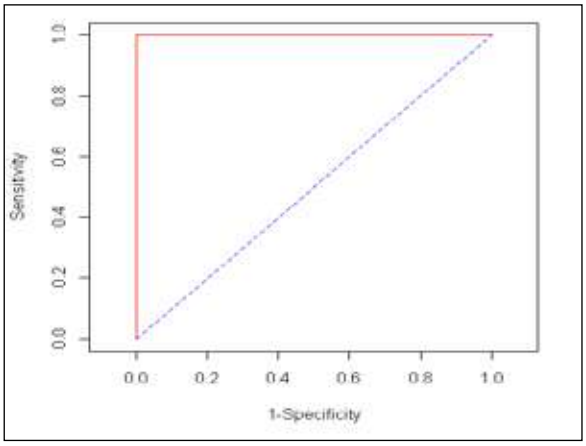


Figure 1. Receiver Operator Characteristic-curve

Conclusion

Thus, the increased level of interleukin-8 (higher than 17.7 pg/ml) in the tear fluid of the eye affected by choroidal melanoma and in the paired «healthy» eye is an additional criterion for the detection of choroidal melanoma. The proposed method is applicable in patients with suspected choroidal melanoma and has a very high diagnostic accuracy.

REFERENCES

1. *Bakalian S., Marshall J.C., Logan P., Faingold D., Maloney S., Di Cesare S., Martins C., Fernandes B.F., Burnier M.N.Jr.* Molecular pathways mediating liver metastasis in patients with uveal melanoma. Clin Cancer Res. 2008 Feb 15; 14(4): 951-6. doi: 10.1158/1078-0432.CCR-06-2630.

2. *Coupland S.E., Dodson A., Liu H., Du M.Q., Angi M., Damato B.E.* Intraocular collision tumour: case report and literature review. Graefes Arch Clin Exp Ophthalmol. 2013 May; 251(5): 1383-8. doi: 10.1007/s00417-012-2216-0.

3. *Damato B.E., Coupland S.E.* Ocular melanoma. Saudi J Ophthalmol. 2012 Apr; 26(2): 137-44. doi: 10.1016/j.sjopt.2012.02.004.

4. *Damato B.E., Heimann H., Kalirai H., Coupland S.E.* Age, survival predictors, and metastatic death in patients with choroidal melanoma: tentative evidence of a therapeutic effect on survival. JAMA Ophthalmol. 2014 May; 132(5): 605-13.

5. *Finger P.T., Kurli M., Shulman J.P.* Treatment of small choroidal melanoma. The Internet Journal of Ophthalmology and Visual Science. 2007; 5(1): 231-240.
6. *Graell X., Caminal J.M., Masuet C., Arias L., Rubio M., Pujol O., Roca G., Arruga J.* Age distribution of uveal melanoma and its relationship to survival. Arch Soc Esp Oftalmol. 2007 Jun; 82(6): 343-7.
7. *Lake S.L., Damato B.E., Kalirai H., Dodson A.R., Taktak A.F., Lloyd B.H., Coupland S.E.* Single nucleotide polymorphism array analysis of uveal melanomas reveals that amplification of CNKSR3 is correlated with improved patient survival. Am J Pathol. 2013 Mar; 182(3): 678-87. doi: 10.1016/j.ajpath.2012.11.036.
8. *Team R.C.* R: A language and environment for statistical computing. 2013; 201.
9. *Margo C.E.* The collaborative ocular melanoma study: an overview. Cancer Control. 2004 Sep-Oct; 11(5): 304-9. doi: 10.1177/107327480401100504.
10. *Finger P.T., Kurli M., Wesley P., Tena L., Kerr K.R., Pavlick A.* Whole body PET/CT imaging for detection of metastatic choroidal melanoma. British journal of ophthalmology. 2004; 88(8): 1095-1097. doi: 10.1136/bjo.2003.039289.

THE ROLE OF KI-67 PROLIFERATIVE INDEX IN THE PROGNOSIS OF LOCALLY ADVANCED CERVICAL CANCER

O.N. Churuksaeva, L.A. Kolomiets, O.V. Savenkova

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia

Immunohistochemical parameters reflecting the activation of oncogenes or tumor suppressor genes, apoptotic and proliferative activity are currently being actively studied as predictive criteria for response to treatment in patients with locally advanced cervical cancer (LACC). The expression level of the *Ki-67 proliferation marker was found to be significantly reduced after* 2 courses of neoadjuvant chemotherapy (NACT), and the initial levels were correlated with the immediate response to treatment.

Keywords: proliferative index Ki-67, cervical cancer, prognosis, overall (OV) and progression-free (PFS) survival.

Objective

Currently, 30 to 45% of patients with LACC died of disease progression within the first five years, therefore, the identification of reliable prognostic criteria is of great importance [1, 2]. The biological behavior of tumors remains sufficiently unpredictable even for patients with the same stage of the disease [3]. Prognostic factors in cancer are often more significant in terms of the disease outcome than the therapeutic effect. Hormone receptors, biochemical markers, expression of oncogenes and antigens associated with proliferation, as well as other molecular markers are widely recognized as prognostically significant [1, 4, 5]. Cell proliferation, which can be estimated using the *mitotic index* (MI) and the *Ki-67* labeling *index* (LI), is one of the most studied parameters of aggressive tumor growth. The Ki-67 antigen is expressed in almost all phases of the mitotic cycle, and, in accordance with this, reflects the proliferative pool of the tumor. The Ki-67 proliferative index is an independent prognostic marker of tumor recurrence, overall and progression-free survival, as well as a predictive factor for response to chemotherapy/radiation therapy [6, 7]. At present, there is no consensus on the impact of Ki-67 on the radiosensitivity of cervical tumors, on the critical prognostic level of proliferative activity of PSM and its relationship with clinical and morphological factors of prognosis and disease outcome [6], therefore further studies are required.

Material and Methods

The Ki-67 level was determined in 25 LACC patients before starting neoadjuvant platinum-based chemotherapy and after its completion. The immunohistochemical study was carried out according to a standard procedure on dewaxed cervical tissue sections obtained from resection or cervical biopsy specimens. Results of staining were assessed using Carl Zeiss Microlmaging light microscope (Germany). For all markers, the localization of staining in the cell (nucleus, cytoplasm, membrane) was evaluated. The results of IGH analysis were evaluated quantitatively and qualitatively by the intensity of cytoplasmic staining. To assess the proliferative activity of the tumor, the number of Ki-67 positive cells per 200-300 tumor cells was calculated. The Ki-67 index was determined by the formula: PA = number Ki-67 positive cells × 100/total number of cells.

Results

The average proliferation index in patients with LACC was 60%. High and very high expressions (more than 50%) were observed in 64% of patients with LACC (Figure 1). A weak level of Ki-67 expression was seen in 16% of LACC patients.

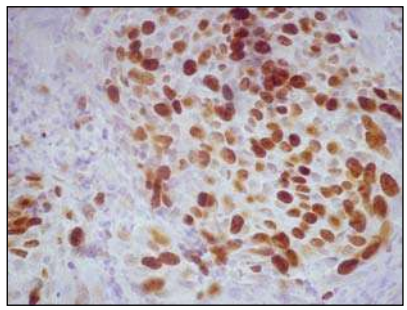


Figure 1. The *Ki-67 labeling index* is higher than 80 % in tumor nests of moderately differentiated squamous-cell cervical carcinoma; magnification ×400

The analysis of immunohistochemical markers before and after 2 courses of NACT in patients with LACC revealed a significant decrease in the expression of the Ki-67 proliferation index after the completion of chemotherapy. It was shown that before starting treatment, the Ki-67 proliferative activity was detected in all LACC patients, while after completion of NACHT, 24% of patients did not express this antigen. The average Ki-67 value decreased from 60% to 42% ($p<0.05$), that is, less than half of tumor cells were in the mitotic phase of the cell cycle. The levels of high and very high expressions of Ki-67 decreased from 64% to 32% compared to the levels observed before treatment. The analysis of the changes in IGH-markers showed that in patients who subsequently developed disease progression, the Ki-67 expression was the highest (85%) before starting antitumor treatment. In patients with complete regression, the Ki-67 level was the lowest (53.9%). In patients with partial tumor regression, the average level of the Ki-67 proliferative activity marker increased slightly after NACT, which apparently caused incomplete tumor regression. The remaining patients showed positive dynamics in decreasing the expression level of the Ki-67 antigen.

The relationship between the Ki-67 index and long-term treatment outcomes in patients with LACC was shown (Figure 2). The 5-year overall survival was significantly higher in LACC patients with the Ki-67 expression of < 50% than in patients with the Ki-67 expression of > 50% (Gehan'sWilcoxon p = 0.01056, Log-RankTest p = 0.00899, Cox-MantelTest p = 0.01011). In patients with the Ki-67 expression of less than 50% and above 50%, the 5-year overall survival rates were 100% and 67%, respectively. The progression-free 5-year survival in patients with LACC, regardless of the level of Ki-67 expression, did not differ significantly.

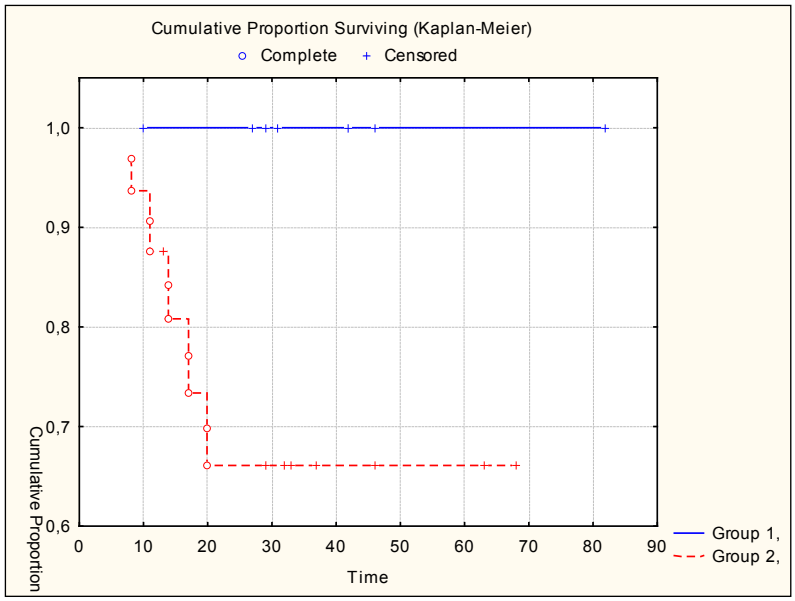


Figure 2. The overall survival of patients with LACC with respect to the initial level of the Ki-67 proliferative activity marker

Conclusion

The Ki-67 expression is determined in all patients with LACC, correlates with clinical response to treatment and long-term treatment outcomes, thus allowing the Ki-67 index to be used as a prognostic factor in LACC.

REFERENCES

1. *Antoneeva I.I., Sidorenko E.G., Abakumova T.V., Pirmamedova S.S.* Expression of proteins associated with tumor progression in cervical cancer. Fundamental study. 2012; 7(2): 269-272. [in Russian]

2. *Kozachenko V.P.* Clinical gynecologic Oncology. A guide for doctors. M.: Medicine; 2005. 376. [in Russian]

3. *Granov A.M., Vinokurov V.L.* Radiation therapy in Oncology and Onco-urology. SPb.: Folio; 2002. 349. [in Russian]

4. *Gaffney D.K., Haslam D., Tsodikov A., Hammond E., Seaman J., Holden J., Lee R.J., Zempolich K., Dodson M.* Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) negatively affect overall survival in carcinoma of the cervix treated with radiotherapy. Int J Radiat Oncol Biol Phys. 2003 Jul 15; 56(4): 922-8.

5. *Lindström A.* Prognostic factors for squamous cell cervical cancer. Tumor markers, hormones, smoking, and S-phase fraction. Umea: Umea university, 2010. 67

6. *Liu S.S., Tsang B.K., Cheung A.N., Xue W.C., Cheng D.K., Ng T.Y., Wong L.C., Ngan H.Y.* Anti-apoptotic proteins, apoptotic and proliferative parameters and their prognostic significance in cervical carcinoma. Eur J Cancer. 2001 Jun; 37(9): 1104-10.

7. *Sultana H., Kigawa J., Kanamori Y., Itamochi H., Oishi T., Sato S., Kamazawa S., Ohwada M., Suzuki M., Terakawa N.* Chemosensitivity and p53-Bax pathway-mediated apoptosis in patients with uterine cervical cancer. Ann. Oncol. 2003 Feb; 14(2): 214-9.

ARSENIC INDUCED POLYADENYLATION OF CANONICAL HISTONE MRNA DISRUPTS CHROMATINHOMEOSTATSIS AND IS HIGHLY CARCINOGENIC

M. Costa, H. Sun, D. Chen, Q. Chen, F.J. Li, Y. Zhu, C. Jin

Dept of Environmental Medicine, NYU School of Medicine, New York, USA

Naturally occurring inorganic arsenic has been identified as the causal agent in lung, bladder, liver, and prostate cancers. Although arsenic has long been known to induce toxicity and carcinogenicity via epigenetic mechanisms, alteration in histone gene expression is scarcely studied. The replication-dependent histone mRNAs end in a conserved 26-nucleotide sequence, and is the binding site for the stem loop binding protein (SLBP). As a critical protein for canonical histone pre-mRNA processing, the loss of SLBP has been shown to induce aberrant polyadenylation of H3.1mRNA, which can lead to cellular transformation. Canonical histone mRNAs are unique in that they are the only mRNAs in multicellular organisms that do not contain poly (A) tail in their 3' ends and thus are unstable. Instead, they contain a stem-loop structure in their 3' ends, which will be bound by Stem-loop binding protein (SLBP). SLBP is essential for processing of the canonical histone pre-mRNA and also controls stability of histone mRNAs. Intriguingly, we found that As exposure induces polyadenylation of canonical histone H3.1 mRNA due to the loss of SLBP, which appeared to be attributable to As-induced degredation of SLBP. Here we report increase in polyadenylated H3.1 mRNA compromises assembly of H3.3 at promoters of 2,000 most active genes. In addition we found that transfection of H3.1 with a poly(A) tail causes a G2/mitotic block in the cell cycle, chromosomal damage, and gamma H2AX phosphorylation. To further distinguish between G2 and M arrest, we determined the cellular level of histone H3S10 phosphorylation, a Mitotic phase specific modification, by flow cytometry assay. The phosphorylation of H3S10 was increased from 4.6% to 11% upon transfection of H3.1 poly (A) as compared with the control we found that transfection of H 3.1 with a poly A coding sequence produced polyadenylation of canonical histone mRNAs and induced a high incidence of cell transformation. To analyze the effect of H3.1 polyadenylation on tumor formation in nude mice, we injected control and H3.1 poly(A) expressing BEAS-2B cells into nude mice to assess tumor formation. Juxtaposing the two groups, mice injected with BEAS-2B/H3.1 poly(A) cells demonstrated 100% tumor formation compared to 33% in the control group. This effect was abrogated by inclusion of a stem loop binding sequence along with the Poly Adenylation sequence. Furthermore As exposure of mice by inhalation or ingestion results in a loss of SLBP in the lung and liver respectively. This *in vivo* data strongly indicate that all the canonical histone mRNAs could be polyadenylated in As-exposed mice given the critical role of SLBP in processing of canonical histone pre-mRNAs, but because of low levels of proliferation of lung and liver polyadenylation of canonical histone mRNA is not observed. However in bone marrow we observe polyadenylation of H3.1 and H4 following exposure of mice to As.

Keywords: Arsenic, Canonical Histone mRNA, Cell Cycle, Epigenetics, Histone.

THE SEARCH FOR MARKERS OF COLLECTIVE CELL INVASION IN BREAST CANCER

E.V. Denisov[1], T.S. Gerashchenko[1], N.M. Novikov[1], L.A. Tashireva[1], N.V. Krakhmal[1], M.V. Zavyalova[1], A.Gautreau[2], N.V. Cherdyntseva[1], V.M. Perelmuter[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Biology Department, Ecole Polytechnique, Paris-Saclay, France[2]

Invasion, or directed migration of tumor cells to adjacent tissues, represents the first step in the process of cancer metastasis. To date, a plethora of data has been accumulated that clarify the mechanisms of cancer cell invasion. In particular, it is known that tumor cells can invade individually or collectively using two main mechanisms of migration – mesenchymal and amoeboid. The movement of tumor cells is observed not only in the invasive front (the interface between tumor and host tissue) but also within the tumor. Cancer cell invasion is initiated and supported by many different molecules: EMT factors, actin cytoskeleton and adhesion proteins, proteases, etc. [1, 2]. Nevertheless, despite a lot of research efforts, there is no clear answer to the key question: how to identify tumor cell invasion in cancer tissue? Simply stated, there are no effective markers or features to distinguish migrating/invading tumor cells from quiescent tumor cells.

Recently, we suggested that intratumor morphological heterogeneity in breast cancer (BC) may be a promising model to identify markers associated with tumor cell invasion. Different morphological structures (tubular, alveolar, solid, trabecular, and discrete), the number of which in tumor varies from case to case, show specific gene expression profiles and signaling pathways associated with EMT and cancer invasion, modulate chemotherapy efficiency and are involved in BC metastasis. In particular, solid and trabecular structures represent variants of collective cell invasion, whereas discrete groups demonstrating the most pronounced mesenchymal phenotype reflect the individual mode of cell movement [3].

Thus, using the data of gene expression profiling of different morphological structures in BC, we chose ten genes that were mentioned previously to be associated with cell migration or whose proteins showed expression or its loss at the invasive front (tips/periphery) of solid and trabecular structures. In accordance with KM Plotter, expression of six of these genes was associated with poor recurrence and distant metastasis-free survival in BC. IHC analysis showed that distant metastasis was significantly associated with heterogeneous expression of these markers in breast tumors, particularly its presence or loss at the edge of solid and trabecular structures. In addition, expression of these invasive markers was also associated with tumor size, grade, and lymph node involvement. Currently, we perform RNA-sequencing to find genes and pathways that are enriched in BC cells expressed invasive markers; the results will be presented in the symposium.

Keywords: cancer, cell migration, collective cell migration, invasion.

The study was supported by the Russian Science Foundation (grant № 14-15-00318)

REFERENCES

1. *Friedl P., Locker J., Sahai E., Segall J.E.* Classifying collective cancer cell invasion. Nat Cell Biol. 2012 Aug; 14(8): 777-83. doi: 10.1038/ncb2548.

2. *Krakhmal N.V., Zavyalova M.V., Denisov E.V., Vtorushin S.V., Perelmuter V.M.* Cancer Invasion: Patterns and Mechanisms. Acta Naturae 2015; 7(2): 17-28.

3. *Denisov E.V., Skryabin N.A., Gerashchenko T.S., Tashireva L.A., Wilhelm J., Buldakov M.A., Sleptcov A.A., Lebedev I.N., Vtorushin S.V., Zavyalova M.V., Cherdyntseva N.V., Perelmuter V.M.* Clinically relevant morphological structures in breast cancer represent transcriptionally distinct tumor cell populations with varied degrees of epithelial-mesenchymal transition and CD44+CD24- stemness. Oncotarget. 2017 May 19; 8(37): 61163-61180. doi: 10.18632/oncotarget.18022.

PROGNOSTIC SIGNIFICANCE OF TGF-B-ASSOCIATED PROTEINS IN BREAST CANCER PATIENTS TREATED WITH ADJUVANT TAMOXIFEN

T.A. Dronova[1,2], N.N. Babyshkina[1,2], S.V. Patalyak[1], N.V. Cherdyntseva[1,2]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University, Tomsk, Russia[2]

We investigated the effect of 3 TGF-β-associated signaling components (TGF-β1, TGF-βR1, TGF-βR2) and ERα on the response to adjuvant tamoxifen treatment in 122 estrogen positive breast cancer patients. Our data suggest that the gene and protein expression of TGF-βR1 as well as *ESR1* rs2228480 and the distribution pattern of ERα expression may play a significant role in the development of tamoxifen resistance/sensitive in breast cancer patients.

Keywords: breast cancer, tamoxifen resistance, estrogen receptor, TGF-β-associated proteins, prognosis markers.

Introduction

Crosstalk between ERα and TGF-β signaling may be critical in the development of tamoxifen resistance/sensitive in breast cancer. It is well known that ERα blocks TGF-β pathway by direct interactions with Smads components [1]. In turn to, TGF-β could act to restrict ERα-mediated proliferation [2]. While evidence is accruing that TGF-β-associated proteins is implicated in the tamoxifen resistance, there have been no integrated studies evaluating their prognostic value in estrogen positive breast cancer patients.

This has prompted us to assess the genetic variation, protein and RNA expressions of the three TGF-β-associated signaling components (TGF-β1, TGF-βR1, TGF-βR2) and ERα in relation to tamoxifen efficacy and survival rate in estrogen positive breast cancer patients.

Material and Methods

This study analyzed clinical data and tissue samples from 122 women with breast cancer who received adjuvant tamoxifen at the Tomsk National Research Medical Center from 2002 to 2014. According to the response to tamoxifen, all patients were divided into tamoxifen resistance (TR) group and tamoxifen sensitive (TS) group. The TGF-βR1rs334354, TGF-βR2rs2228048 and ESR1 (rs2228480, rs2077647, rs1801132, rs3798577) SNPs were analysed using a TaqMan assay. Expression of the TGF-β1, TGF-RI, TGF-RII and ESR1 gene was detected by RT-PCR analysis. Expression of TGF-βRI and the distribution pattern of ERα expression were assessed by immunohistochemistry. Progression-free survival (PFS) was analyzed by Kaplan-Meier curves. SPSS 21.0 (IBM SPSS Statistics, Armonk, NY, USA) was applied for statistical analysis.

Results

When primary tumors from the tamoxifen resistance and tamoxifen sensitive groups were compared, *TGF-βRI* mRNA was significantly overexpressed in the latter group (p=0.041). Furthermore, we demonstrated that TGF-βR1 positive protein expression was more frequent in tamoxifen sensitive tumors as compared to tamoxifen resistance (p=0.030). We found that the wild genotype of the *TGF-βR2*rs2228048 was significantly associated with sensitivity to tamoxifen treatment in breast cancer patients when compared with the mutant and heterozygous genotypes (p=0.045).

We noted a significant association between low mRNA *ESR1* expression and resistance to tamoxifen (p=0.041). Patients carrying a mutant genotype of the *ESR1*rs2228480 had a high of progression risk after tamoxifen treatment than the patients with a wild and heterozygous genotypes (p = 0.013). In addition, we were able to demonstrate that heterogeneous ERa expression was significantly associated with tamoxifen resistance (p = 0.003).

We assessed the association of all studied markers with progression-free survival rate in breast cancer patients. Compared with TGF-βR1-posinive patients, TGF-βR1-negative patients showed significantly poorer outcomes with respect to progression-free survival (p=0.032). Kaplan-Meier analysis showed that there were significant higher death and recurrence risk in patients with heterogeneous ERa expression and mutant genotype of rs2228480 SNP compared with those patients with homogeneous ERa expression pattern and wild and heterozygous genotypes of rs2228480 SNP (p=0.009 and p=0.040, respectively).

Conclusion

Our data suggest that the gene and protein expression of TGF-βR1 as well as *ESR1* rs2228480 and the distribution pattern of ERα expression may play a significant role in the development of tamoxifen resistance/sensitive in breast cancer patients.

The study was supported by the ERA Net RUS Plus S&T CHIT-ALPHA-THER grant, the Russian Foundation for Basic Research (Project № 16-54-76015)

REFERENCES

1. *Band A.M., Laiho M.* Crosstalk of TGF-β and estrogen receptor signaling in breast cancer. J Mammary Gland Biol Neoplasia. 2011 Jun; 16(2): 109-15. doi: 10.1007/s10911-011-9203-7.

2. *Ewan K.B., Oketch-Rabah H.A., Ravani S.A., Shyamala G., Moses H.L., Barcellos-Hoff M.H.* Proliferation of estrogen receptor-alpha-positive mammary epithelial cells is restrained by transforming growth factor-beta1 in adult mice. Am J Pathol. 2005 Aug; 167(2): 409-17.

ANALYSIS OF THE LEVEL OF THE SERUM TUMOR MARKERS AND CHEMICALS IN WATERBASINS OF THE REPUBLIC OF SAKHA (YAKUTIA)

S.D. Efremova[1], A.S. Golderova[1], V.M. Nikolaev[1], E.D. Okhlopkova[1], V.A. Alekseev[1], F.V. Vinokurova[1], E.K. Rumyantsev[1], E.V. Tsypandina[1]

Scientific Center of Complex Medical Problems, Yakutsk, Russia[1]
School of Higher Sportsmanship, Yakutsk, Russia[2]

The concentration of the serum tumor markers in people living in 6 different regions of the Republic of Sakha (Yakutia) was determined by an enzyme immunoassay, and the chemical composition of water used for drinking was evaluated. A total of 675 residents aged 18 to 79 years were examined. Increased concentrations of chemical substances (phosphates, fluorides, manganese, strontium, and lead) in water resulted in increased levels of tumor markers, such as AFP, CEA, PSA and CA-125.

Keywords: tumor markers, chemicals, water bodies, Yakutia.

Relevance

In Yakutia, with a rapid industrial growth, there has been an increase in the cancer incidence. Sewage water pollution cancer increases cancer risk [1, 2].

Material and Methods

A total of 675 residents aged 18 to 79 years living in 6 different regions of the Republic of Sakha (Yakutia), 461 men (mean age 45.01 ± 0.93) and 214 women (mean age 45.54 ± 0.56) were examined. The national composition of the surveyed persons was represented by 246 Yakuts, 194 by indigenous small-numbered peoples of the North (Evenki, Evens and Dolgans), 236 by a Russians, Tatars, etc.

Data on the chemical composition of water taken from water basins for drinking (the Matta River – the Magarass village, Gorny district; near intake the Lena River – the Modut village, Namsky district, «Pool Yasachnaya» – the Neplemennoe village of the Verkhnekolymsky District, the Peleduy River - the Vitim village of the Lensky District, the Aldan River - the Tommot Aldansky District) were provided by the Administration of the Hydrometeorological Service of the Republic of Sakha (Yakutia).

Blood for laboratory tests was taken from the ulnar vein in the morning on an empty stomach. The concentrations of tumor markers: alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), male prostate-specific antigen (PSA) in males, tumor-associated surface antigen (CA-125) in females, were determined by ELISA using Vector Best test kits (Novosibirsk, Russia).

Data of the chemical composition of water were provided by the Administration of the Hydrometeorological Service of the RS (Y).

Results

Of the 15 chemical substances, only 5 elements (phosphates, strontium, fluorides, manganese, calcium) showed reliable correlation with the concentration of tumor markers in the blood of the residents.

The positive correlation between the content of chemical elements in water and the concentration of tumor markers was found: phosphates with CEA (r = 0.599), PSA (r = 0.593), AFP (r = 0.249) and CA-125 (r = 0.280) for p < 0.01; strontium with CEA (r = 0.498), PSA (r = 0.482), AFP (r = 0.222) and CA-125 (r = 0.165), with p <0.01; fluorides with CA-125 (r = 0.476), PSA (r = 0.468), REA (r = 0.436) and AFP (r = 0.257) with p <0.01. There were positive correlations of the level of tumor markers with the content of manganese with AFP (r = 0.274), CEA (r = 0.219), PSA (r = 0.185), with p <0.01, calcium with PSA (r = 0.443), CEA (r = 0.233), AFP (r = 0.128) with p <0.01 and lead with AFP (r = 294) with p <0.01. The relationship between tumor markers expressed by low-differentiated cells and environmental pollution was found.

Conclusion

Increased maximum permissible concentrations in water of chemical substances (phosphates, fluorides, manganese, strontium, and lead) led to the increase in the level of tumor markers: cancer embryonic antigen, alfa-fetoprotein, prostate-specific antigen and tumor-associated surface antigen, thus increasing the risk of developing cancer.

REFERENCES

1. *Ivanov P.M., Tomskiy M.I., Ushnitsky I.D., Kipriyanova N.S., Nikolaeva T.I.* Maxillofacial malignant tumors in Yakutia. Yakut Medical Journal. 2016; 3 (55): 26-29. [in Russia]

2. *Poddubnaya I.V., Axel E.M., Kipriyanova N.S.* Complex analysis of cancer incidence among population of Yakutsk (1990-2003). Siberian Journal of Oncology. 2007; 2 (22): 55-62. [in Russia]

EFFECT OF SHORT-TERM RETINOIC ACID TREATMENT ON ERK1/2 AND AKT ACTIVATION IN LUNG CANCER AND NEUROBLASTOMA CELLS

A.D. Enikeev, A.V. Komelkov, M.E. Akselrod, E.M. Tchevkina

N.N. Blokhin National Medical Research Center of Oncology, Moscow, Russia

Here we studied the non-canonical activity of retinoic acid (RA) with respect to regulation of key signaling pathways in non-small cell lung cancer (NSCLC) and neuroblastoma cells. We found that treatment by all-trans retinoic acid (ATRA) causes short-term activation of Erk1/2 and AKT in both types of cells, although the kinetics of RA-dependent activation of these protein kinases was different. For Erk1/2 we observed rapid (5-15') and late (240') increase in phosphorylation level, that indicate RA-dependent non-genomic and canonical transcriptional activation of Erk1/2 correspondingly. AKT activation was shifted in time without visible break between early and late activation. The obtained results point on diverse mechanisms mediating RA-dependent activation of Erk1/2 and AKT.

Keywords: retinoic acid, ATRA, phosphorylation, Erk1/2 kinase, AKT kinase.

Background

Retinoic acid belongs to the class of retinoids, involved in the regulation of wide spectrum of biological processes including tissue development, immune response, neural function, reproduction, etc. In the majority of cells RA stimulates differentiation, inhibit proliferation and induce apoptosis. According to these activities, ATRA is considered as one of the most promising therapeutic anti-tumor agents [1]. However, the use of RA is strongly restricted by the rapid acquisition of resistance [2], as well as by a number of side effects mediated through RA nuclear receptors. Retinoic acid receptors are ligand inducible transcription factors that regulate activity of huge number of genes involved in cell cycle arrest, cell differentiation and cell death. In addition to the canonical activity, novel nongenomic mechanisms of RA-mediated signal transduction have been described for some cell types [3]. This rapid, nongenomic activity does not rely on gene transcription or protein synthesis, but results in ligand induced modulation of signal transduction molecules such as protein kinase PKCα, extracellular signal regulated kinase 1/2 (ERK1/2) and antiapoptotic kinase Akt [4-7]. The non-genomic RA-dependent activation of signal transduction could represent an additional process contributing to the acquisition of RA-resistance. However, this effect had been shown only for few cell lines and the mediators of non-transcriptional activity of atRA as well as mechanisms of this phenomenon remain poorly understood. Importantly, the RA-dependent non-transcriptional activation of the above-mentioned targets in the same cells has

not yet been studied. Therefore, it remains unclear, whether the mechanisms of RA-dependent activation of Erk1/2 and Akt are the same or different.

Material and Methods

Here we studied the effect of short-term ATRA treatment on the activity of Akt and ERK1/2 kinases in NSCLC and neuroblastoma cells.

NSCLC cells (lines A549, H460) and neuroblastoma cells (lines SK-N-AS, SH-SY-5Y) were incubated in DMEM in the presence of ATRA at final concentration 5 μM and 1 μM correspondingly. After the fixed periods of treatment (5, 15, 30, 60 and 240 min) cells were detached and 10 mkg of protein were subjected to SDS-PAGE followed by immunoblotting. Phosphorylation of pAkt and pERK1/2 was detected using anti-pAkt (S473) and anti-pERK-1/2(T202/Y204) (Cell Signalling) antibodies correspondingly.

Results

We found RA-dependent activation of both protein kinases in all studied cell lines, but the kinetics differed drastically. ERK-1/2 demonstrates two well-defined peaks of phosphorylation in all cells: the first one corresponds to 5-15' of ATRA treatment (for SH-SY-5Y cells – 5-30'), the second occurs after 240' of incubation. Notably, in intermediate period (60') the crucial decrease of ERK-1/2 activity (to the level lower than in control cells) was observed. Such a delineation of peaks corresponds probably to the rapid non-genomic and long-term transcriptional activation of Erk1/2 correspondingly. The activation of Akt was observed from 5' of ATRA treatment with the maximum at 30' (with an exception of SH-SY-5Y cells, where the peaks of P-AKT were observed at 5-15' and 60-240' while the pronounced decrease was detected at 30'). Noteworthy, in contrast to P-Erk1/2, the level of P-Akt does not fall to the level of control. We have found that the activation of Erk1/2 and Akt does not coincide in time and even shows the opposite distribution in the same cells at the same time point.

Conclusion

We have found that ATRA causes rapid activation of Erk1/2 and Akt in both types of cells. The different kinetics of RA-dependent activation of these kinases evidences on diverse mechanisms mediating RA activity with respect to these targets.

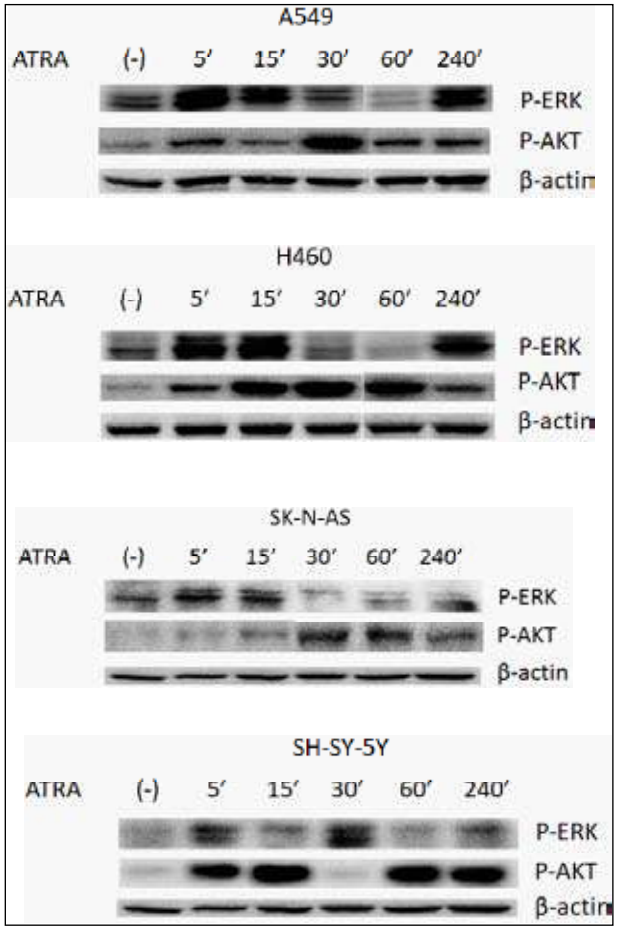


Figure 1. Analysis of ERK1/2 (pThr202/Tyr204) and AKT pSer473 levels after ATRA treatment

REFERENCES

1. *Bushue N., Wan Y-JY.* Retinoid pathway and cancer therapeutics. Adv Drug Deliv Rev. 2010 Oct 30; 62(13): 1285-98. doi: 10.1016/j.addr.2010.07.003.

2. *Freemantle S.J., Spinella M.J., Dmitrovsky E.* Retinoids in cancer therapy and chemoprevention: promise meets resistance. Oncogene. 2003 Oct 20; 22(47): 7305-15.

3. *Masia S., Alvarez S., de Lera A.R., Barettino D.* Rapid, nongenomic actions of retinoic acid on phosphatidylinositol-3-kinase signaling pathway mediated by the retinoic acid receptor. Mol Endocrinol. 2007 Oct; 21(10): 2391-402.

4. *Ochoa W.F., Torrecillas A., Fita I., Verdaguer N., Corbalán-García S., Gomez-Fernandez J.C.* Retinoic acid binds to the C2-domain of protein kinase C(alpha). Biochemistry. 2003; 42(29): 8774-8779. doi:10.1021/bi034713g

5. *García-Regalado A., Vargas M., García-Carrancá A., Aréchaga-Ocampo E., González-De la Rosa C.H.* Activation of Akt pathway by transcription-independent mechanisms of retinoic acid promotes survival and invasion in lung cancer cells. Mol Cancer. 2013; 12: 44. doi:10.1186/1476-4598-12-44

6. *Quintero Barceinas R.S., García-Regalado A., Aréchaga-Ocampo E., Villegas-Sepúlveda N., González-De la Rosa C.H.* All-Trans Retinoic Acid Induces Proliferation, Survival, and Migration in A549 Lung Cancer Cells by Activating the ERK Signaling Pathway through a Transcription-Independent Mechanism. Biomed Res Int. 2015; 2015: 404368. doi: 10.1155/2015/404368.

7. *Persaud S.D., Lin Y.W., Wu C.Y., Kagechika H., Wei L.N.* Cellular retinoic acid binding protein I mediates rapid non-canonical activation of ERK1/2 by all-trans retinoic acid. Cell Signal. 2013 Jan; 25(1): 19-25. doi: 10.1016/j.cellsig.2012.09.002.

THE NOVEL SMALL MOLECULE CBL0137 (CURAXIN) EXHIBITS ANTILEUKEMIC ACTIVITY

T.I. Fetisov[1], K.A. Kuzin[1], R.G. Zenkov[1], A. Safina[2], E.A. Lesovaya[1,3], L.S. Trukhanova[1], E.E. Antoshina[1], T.G. Gorkova[1], K. Gurova[2], M.G. Yakubovskaya[1], K.I. Kirsanov[1,4]

Blokhin NMRCO, Department of Chemical Carcinogenesis, Moscow, Russia[1]
Roswell Park Cancer Institute, Department of Cell Stress Biology, Buffalo, USA[2]
Ryazan State Medical University, Ryazan, Russia[3]
RUDN University, Moscow, Russia[4]

Curaxin CBL0137 is a new promising antitumor drug, which was selected as NfkB-signaling inhibitor and p53 activator in different cancer cell lines. To investigate whether Curaxin may be applied for acute leukemia therapy, we used three models: human leukemia cell lines, blood samples of leukemia patients and murine leukemia models in vivo. CBL0137 decreased leukemic cells viability, reduced expression of WNT target genes in vitro and ex vivo and demonstrated antileukemic activity in vivo.

Keywords: curaxin, WNT signaling pathway, Myeloid, Lymphoid, Leukemia.

Introduction

Curaxin CBL0137 is a low molecular weight carbazole derivative that demonstrated antitumor activity [1]. Inducing chromatin remodeling and c-trapping of the facilitating chromatin transcription complex, Curaxin influences NF-kB and p53 in the directions desired for cancer therapy [2, 3]. Destabilizing nucleosomes via disruption of histone/DNA interactions, Curaxin induces interferon response [4]. Previously we found that Curaxin inhibited Wnt/β-catenin signaling pathway. This effect was demonstrated for many cancer cell lines. The Wnt-signaling pathway is a key mediator of patterning decisions during embryonic development and homeostatic self-renewal of adult tissues, but its persistent activation is necessary in pathogenesis of different diseases, including malignancies. Recently, it has been demonstrated that Wnt/β-catenin signaling dysregulation is associated with the development of hematological malignancies and nowadays the components of this signaling pathway are considered to be promising targets for the therapy of myeloid and lymphoid leukemia. In this study, we analyzed the biological effects of Curaxin on different types of leukemia.

Material and Methods

We used K562 (human Chronic Myeloid Leukemia), KG1 and THP-1 (human Acute Myeloid Leukemia), Wehi-3 (Murine Acute Myeloid Leukemia), P388 (Murine Acute Lymphoblastic Leukemia), CCRF Cem and CCRF SB (human T- and B-cells Acute Myeloid Leukemia), RPMI 8226 and H929 (human Multiple Myeloma). Leukemia cells from patient blood samples were obtained by means of density-gradient centrifugation method. Cytotoxicity of CBL0137 against different leukemic cells was measured by MTT. Gene expression analysis was performed using Real-Time PCR and Western Blotting. Flow cytometry was used in cell cycle and apoptosis assays. Double

staining for Annexin V-FITC and propidium iodide was performed to estimate the apoptotic rate. Analysis of antileukemic activity of Curaxins in vivo was performed on two murine models: myelomonocytic leukemia WEHI-3 and the lymphocytic leukemia P388 [4].

Results

We demonstrated that CBL0137 revealed cytotoxicity against cultured cell lines and leukemic cells, obtained from the patients of Blokhin NMRCO. Moreover, Curaxin increased apoptosis level in leukemic cell lines.

It was found that expression of WNT-target genes (c-Myc, CCND1, Lef1, Survivin) was significantly reduced by Curaxin treatment in cell lines as well as in blood samples.

On the murine model of lymphocytic leukemia P388, we showed that intravenous injections of CBL0137 delayed appearance of the first nodules at the site of transplantation, inhibited tumor growth and increased lifespan of experimental mice compared to control ones.

Using the murine model of myelomonocytic leukemia WEHI-3, we observed the effects of intravenous injections of CBL0137: reduced weight of liver, thymus and spleen of experimental mice respectively to the corresponding controls from the mice, which were injected with saline solutions. Furthermore, the histopathological examination of the livers of animals with leukemia revealed a lot of hepatic lesions and the neoplastic cell nests in the sinusoids. Curaxin treatment reduced liver infiltration by leukemic cells.

The neoplastic cells in the leukemic spleen presented large irregular nuclei with clumped chromatin and the red pulp fading away. Antileukemic activity of Curaxin was estimated on WEHI-3 and P388 murine models in vivo. Mice treated with CBL0137 had spleen with clear red pulp and few less numbers of neoplastic cells respectively to the control group.

Conclusion

Our results demonstrate that CBL0137 inhibits activity of WNT/β-catenin signaling and reveals cytotoxic activity against leukemic cells both in vitro and ex vivo and possesses antileukemic activity against murine transplantable leukemia in vivo.

This work was supported by Russian Scientific Foundation (№ 17-75-20124).

REFERENCES

1. *Safina A., Cheney P., Pal M., Brodsky L., Ivanov A., Kirsanov K., Lesovaya E., Naberezhnov D., Nesher E., Koman I., Wang D., Wang J., Yakubovskaya M., Winkler D., Gurova K.* FACT is a sensor of DNA torsional stress in eukaryotic cells. Nucleic Acids Res. 2017 Feb 28; 45(4): 1925-1945. doi: 10.1093/nar/gkw1366.

2. *Leonova K., Safina A., Nesher E., Sandlesh P., Pratt R., Burkhart C., Lipchick B., Gitlin I., Frangou C., Koman I., Wang J., Kirsanov K., Yakubovskaya M.G., Gudkov A.V., Gurova K.* TRAIN (Transcription of Repeats Activates INterferon) in response to chromatin destabilization induced by small molecules in mammalian cells. Elife. 2018 Feb 5; 7. pii: e30842. doi: 10.7554/eLife.30842

3. *Gasparian A.V., Burkhart C.A., Purmal A.A., Brodsky L., Pal M., Saranadasa M., Bosykh D.A., Commane M., Guryanova O.A., Pal S., Safina A., Sviridov S., Koman I.E., Veith J., Komar A.A., Gudkov A.V., Gurova K.V.* Curaxins: anticancer compounds that simultaneously suppress NF-κB and activate p53 by targeting FACT. Sci Transl Med. 2011 Aug 10; 3(95): 95ra74. doi: 10.1126/scitranslmed.3002530.

4. *Fetisov T.I., Tilova L.R., Lesovaya E.A., Antoshina E.E., Gor'kova T.G., Trukhanova L.S., Belitskiy G.A.* Antitumor effect of the curaxin CBL0137 on the models of colon cancer. Advances in molecular oncology. 2016; 3(3): 67-72.

THE BRANCHED ACTIN NETWORKS THAT DRIVE CELL MIGRATION DETERMINE PATIENT PROGNOSIS IN BREAST CANCER

A. Gautreau

Ecole Polytechnique, Université Paris-Saclay, France

During cell migration, branched actin networks provide the force that projects the plasma membrane. Actin is polymerized in a branched manner by a unique molecular machine, called the Arp2/3 complex. In addition to the leading edge of migrating cells, the Arp2/3 complex is activated at many subcellular locations, where the force it provides allows fission of transport intermediates in intracellular trafficking. This is the role of different activators to activate the Arp2/3 complex at different locations [1]. The WAVE molecule activates the Arp2/3 complex at the leading edge of migrating cells. WAVE is embedded in a multiprotein complex that regulates its activity. Our group identified an Arp2/3 inhibitory protein, which we named Arpin. Arpin specifically antagonizes WAVE at the leading edge of migrating cells [2]. When the expression of WAVE complex subunits and of Arpin was examined in mammary carcinomas from a large retrospective cohort of patients, half of tumors were found to either overexpress a limiting subunit of the WAVE complex or to down-

regulate the expression of Arpin. In multivariate models, the patients displaying an upregulation of the activator or a downregulation of the inhibitor have poor prognosis of metastasis-free survival [3]. Recently we analysed the combinatorial complexity of assembling WAVE and Arp2/3 complexes, because many subunits of these complexes are encoded by paralogous genes. The genes that were the most significantly associated with metastasis-free survival of breast cancer patients were also the most efficient at regulating cell migration. Statistical models of retrospective cohorts were even an efficient way to discover specific paralogous genes encoding subunits conferring singular behaviors to the complexes thatthey assemble.

Keywords: actin polymerization, Arp2/3 complex, nucleation promoting factors, molecular machines.

REFERENCES

1. *Molinie N, Gautreau A.* The Arp2/3 Regulatory System and Its Deregulation in Cancer. Physiol Rev. 2018; 98: 215–38.
2. *Dang I., Gorelik R., Sousa-Blin C., Derivery E., Guérin C., Linkner J., Nemethova M., Dumortier J.G., Giger F.A., Chipysheva T.A., Ermilova V.D., Vacher S., Campanacci V., Herrada I., Planson A.G., Fetics S., Henriot V., David V., Oguievetskaia K., Lakisic G., Pierre F., Steffen A., Boyreau A., Peyriéras N., Rottner K., Zinn-Justin S., Cherfils J., Bièche I., Alexandrova A.Y., David N.B., Small J.V., Faix J., Blanchoin L., Gautreau A.* Inhibitory signalling to the Arp2/3 complex steers cell migration. Nature. 2013 Nov 14; 503(7475): 281-4. doi: 10.1038/nature12611.
3. *Lomakina M.E., Lallemand F., Vacher S., Molinie N., Dang I., Cacheux W., Chipysheva T.A., Ermilova V.D., de Koning L., Dubois T., Bièche I., Alexandrova A.Y., Gautreau A.* Arpin downregulation in breast cancer is associated with poor prognosis. Br J Cancer. 2016 Mar 1; 114(5): 545-53. doi: 10.1038/bjc.2016.18.

RNA SEQUENCING OF SINGLE CELLS OBTAINED FROM IMMUNOLABELED TUMOR SECTIONS: THE FIRST EXPERIENCE

T.S. Gerashchenko[1,2], E.V. Denisov[1,2], A.M. Kiselev[3], N.M. Novikov[1,2], L.A. Tashireva[1], D.V. Pyankov[4], N.V. Cherdyntseva[1,2], V.M. Perelmuter[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University, Tomsk, Russia[2]
Almazov National Medical Research Centre, Saint-Petersburg, Russia[3]
Genomed Ltd, Moscow, Russia[4]

We performed laser microdissection-assisted RNA sequencing of single cells of breast tumor sections immunostained according the homemade protocol of maintaining RNA integrity.

Keywords: single-cell RNA sequencing, immunofluorescence, tumor heterogeneity.

Introduction

RNA sequencing (RNA-seq) is a highly informative method for characterizing transcriptional activity in different tissues and for studying molecular interactions between cells [1]. In cancer research, tumor complexity and heterogeneity challenge RNA-seq of tumor bulk that provides information regarding the average transcriptome and necessitate RNA-seq from single cells [2]. To date, single-cell RNA-seq became a powerful tool to profile cell-to-cell variability on a genomic scale [3]. This approach is critically important for the identification of tumor cell populations that are involved in cancer progression and sensitivity to treatment and of transcripts/genes associating with the realization of the metastatic cascade [2, 4]. In particular, the recent studies showed that single-cell transcriptome profiling can identify and characterize clinically important subpopulations of tumor cells to develop successful targeted treatments [5-7].

At present, single-cell RNA-seq is successfully applied to analyze transcriptomes of tumor cells obtained from hematoxylin and eosin stained sections using laser microdissection or from tumor bulk using fluorescence-activated cell sorting (FACS) [8, 9]. However, there are no any data regarding RNA-seq of tumor cells obtained from immunolabeled sections using laser microdissection. It is probable related to the problems of isolation of high-quality RNA samples from immunostained sections. Nevertheless, this approach would allow to assess transcriptome of tumor cells located in different geographic and microenvironmental regions of the tumor and to prevent the isolation of stromal and inflammatory cells that share tumor markers in FASC analysis.

In this study, we share the experience of RNA sequencing of single cells obtained from immunostained sections of frozen breast tumor samples using laser microdissection.

Method and Results

Frozen breast tumor samples were cryosectioned and used for immunofluorescence staining with antibodies to CK7 (sc-23876, 1:50, Santa Cruz), KIF14 (HPA038061, 1:500, Sigma), and WAVE-2 (1:500, [10]) proteins. We modified the protocol of immunostaining to avoid RNA degradation. In particular, we reduced time of incubation with antibodies and used PBS buffer with RNAlater (Thermo Fisher Scientific). Paired tumor sections were immunostained. First of them was used to estimate quality of RNA samples isolated using RNeasy Plus Micro Kit (Qiagen). RNA integrity number (RIN) varied from 5.6 to 6.3 (2200 Tape Station, Agilent). Second sections were used to isolate CK7+KIF14+, CK7+KIF14−, CK7+WAVE-2+ and CK7+WAVE-2− single cells (the number varied from 1 to 50) using laser microdissection guided under fluorescence (PALM, Carl Zeiss). Microdissected samples were used to generate cDNA libraries (SMARTer Stranded Total RNA-Seq Kit v2, Takara, USA). The quality of cDNA libraries was assessed using HS D1000 ScreenTape (2200 Tape Station, Agilent), the concentration – dsDNA HS kit (Qubit 4.0, Thermo Fisher Scientific). Library concentration varied from 0.694 to 92.2 ng/ul, fragment size ranged from 200 to 800 bp (average size of 359 bp). Libraries were pooled according Illumina recommendations and sequenced as single-end 75 bp reads on a NextSeq500 (Illumina). The total number of reads was ~450 mln for 19 samples pooled. At present, bioinformatic processing is performing; the detailed information will be presented in the presentation.

Conclusion

We developed and implemented the protocol of RNA sequencing of single cells obtained from immunolabeled tumor sections using laser microdissection. This approach represents an effective tool for the assessment of transcriptome of different tumor cell populations, especially in context of spatial intratumor heterogeneity.

The study was supported by the Russian Scientific Foundation (grant № 14-15-00318).

REFERENCES

1. *Costa-Silva J., Domingues D., Lopes F.M.* RNA-Seq differential expression analysis: An extended review and a software tool. PLOS One. 2017; 12(12): e0190152. doi:10.1371/journal.pone.0190152.

2. *Wang Y., Navin E.N.* Advances and applications of single cell sequencing technologies. Mol Cell. 2015 May 21; 58(4): 598-609. doi: 10.1016/j.molcel.2015.05.005.

3. *Saliba A-E., Westermann A.J., Gorski S.A., Vogel J.* Single-cell RNA-seq: advances and future challenges. Nucleic Acids Res. 2014 Aug; 42(14): 8845-60. doi: 10.1093/nar/gku555.

4. *Wang Z., Gerstein M., Snyder M.* RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 2009 Jan; 10(1): 57-63. doi: 10.1038/nrg2484.

5. *Chung W., Eum H.H., Lee H.O., Lee K.M., Lee H.B., Kim K.T., Ryu H.S., Kim S., Lee J.E., Park Y.H., Kan Z., Han W., Park W.Y.* Single-cell RNA-seq enables comprehensive tumour and immune cell profiling in primary breast cancer. Nat Commun. 2017 May 5; 8: 15081. doi: 10.1038/ncomms15081.

6. *Miyamoto D.T., Zheng Y., Wittner B.S., Lee R.J., Zhu H., Broderick K.T., Desai R., Fox D.B., Brannigan B.W., Trautwein J., Arora K.S., Desai N., Dahl D.M., Sequist L.V., Smith M.R., Kapur R., Wu C.L., Shioda T., Ramaswamy S., Ting D.T., Toner M., Maheswaran S., Haber D.A.* RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. Science. 2015 Sep 18; 349(6254): 1351-6. doi: 10.1126/science.aab0917.

7. *Tirosh I., Izar B., Prakadan S.M., Wadsworth M.H., Treacy D., Trombetta J.J., Rotem A., Rodman C., Lian C., Murphy G., Fallahi-Sichani M., Dutton-Regester K., Lin J.R., Cohen O., Shah P., Lu D., Genshaft A.S., Hughes T.K., Ziegler C.G., Kazer S.W., Gaillard A., Kolb K.E., Villani A.C., Johannessen C.M., Andreev A.Y., Van Allen E.M., Bertagnolli M., Sorger P.K., Sullivan R.J., Flaherty K.T., Frederick D.T., Jané-Valbuena J., Yoon C.H., Rozenblatt-Rosen O., Shalek A.K., Regev A., Garraway L.A.* Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science. 2016 Apr 8; 352(6282): 189-96. doi: 10.1126/science.aad0501.

8. *Ståhl P.L., Salmén F., Vickovic S., Lundmark A., Navarro J.F., Magnusson J., Giacomello S., Asp M., Westholm J.O., Huss M., Mollbrink A., Linnarsson S., Codeluppi S., Borg Å., Pontén F., Costea P., Sahlén P., Mulder J., Bergmann O., Lundeberg J., Frisén J.* Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science. 2016 Jul 1; 353(6294): 78-82. doi: 10.1126/science.aaf2403.

9. *Villani A.C., Satija R., Reynolds G., Sarkizova S., Shekhar K., Fletcher J., Griesbeck M., Butler A., Zheng S., Lazo S., Jardine L., Dixon D., Stephenson E., Nilsson E., Grundberg I., McDonald D., Filby A., Li W., De Jager P.L., Rozenblatt-Rosen O., Lane A.A., Haniffa M., Regev A., Hacohen N.* Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science. 2017 Apr 21; 356(6335). pii: eaah4573. doi: 10.1126/science.aah4573.

10. *Gautreau A., Ho H-Y H., Li J., Steen H., Gygi S.P., Kikschner M.W.* Purification and architecture of the ubiquitous Wave complex. Proc Natl Acad Sci. U S A. 2004 Mar 30; 101(13): 4379–4383. doi: 10.1073/pnas.0400628101.

ONCOLOGY DISEASE IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION WITH NONOBSTRUCTIVE CORONARY ATHEROSCLEROSIS

S.B. Gomboeva[1], V.V. Ryabov[1,2,3], S.V. Demyanov[1]

Cardiology Research Institute, Tomsk National Research Medical Centre, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University, Tomsk, Russia[2]
Siberian State Medical University, Tomsk, Russia[3]

The aim of this study was to evaluate the cancer incidence among patients with myocardial infarction with nonobstructive coronary atherosclerosis (MINOCA). **Material and Methods.** The non-randomized controlled study included patients with acute coronary syndrome (ACS) treated between 2010 and 2015. Inclusion criteria comprised NOCA (normal coronary arteries / plaques <50%) confirmed by invasive coronary angiography (CAG) and age over 18 years at the time of randomization. The exclusion criterion was revascularization of the coronary arteries. We compared the study population with patients from POLINCOR study (NCT03122340), which included patients with ACS with obstructive CAD confirmed by invasive CAG, who were admitted to the department of emergency cardiology in 2015-2016 [5]. **Results.** Among 5794 patients, who were hospitalized with ACS to the Department of Emergency Cardiology in 2010-2016, 2.8% (161) patients demonstrated nonobstructive coronary atherosclerosis confirmed with CAG. One (0.6%) patient died. There were 94 (58%) cases with acute myocardial infarction, 27 (17%) with unstable angina, 15(9%) with pseudo-coronary scenario of myocarditis, 7 (4%) with cardiomyopathy, and 16 (10%) with arrhythmias, congenital heart defects, aortic dissection. Among the patients with MINOCA, there were 30 (19%) patients with malignant and non-malignant tumors with cancer, including 7 (23%) patients with cancer of kidneys, sigmoid colon and cervix. Bladder cancer was diagnosed in 1 (0.6%) case before admission to the hospital, and lung cancer was identified in 1 (0.6%) case at autopsy. The cancer incidence rate in patients with MINOCA was higher than in patients with obstructive CAD. Conclusion. The proportion of patients with MINOCA among patients with ACS in 2015-2016 was 2.8 % (161). MINOCA enconpasses a heterogeneous group of diseases, including AMI, unstable angina, myocarditis, cardiomyopathy, arrithmia, aortic dissection and others. Hospital mortality was 0.6%. The cancer incidence in patients with MINOCA was higher than in patients with obstructive CAD.

Keywords: myocardial infarction, nonobstructive coronary artery, MINOCA, oncology disease, cancer.

Introduction

Myocardial infarction with myocardial infarction with nonobstructive coronary atherosclerosis (MINOCA) represents a heterogeneous group of diseases. According to previous studies, the incidence of MINOCA is 1-14% [1]. One of the causes of MINOCA is coagulation disorders associated with tumor [1] and endothelial dysfunction after chemo- and radiotherapy which can lead to coronary vasospasm and thrombosis [2]. It is known [3] that the risk of cancer is 46% higher in patients with MI than in patients without MI within 6 months after MI diagnosis. Most studies were focused on the risk of cancer in patients with obstructive coronary artery disease (CAD). Little data is available on increased risk of cancer in patients with MINOCA. According to the SWEDHEART registry [4], cancer is an independent predictor of mortality in MINOCA. Further research is important to understand underlying mechanisms of MINOCA.

The aim of the study was to evaluate the cancer incidence among patients with MINOCA.

Material and Methods

Non-randomized open controlled study included patients, who were admitted to the department of emergency cardiology due to acute coronary syndrome (ACS) in 2016 and analyzed medical histories of ACS patients treated between 2010 and 2015. Inclusion criteria comprised NOCA (normal coronary arteries / plaques <50%) confirmed by invasive coronary angiography (CAG), patients' age over 18 years at the time of randomization. The exclusion criterion was revascularization of the coronary arteries.

We compared the study population with patients from POLINCOR study (NCT03122340) which included patients with ACS with obstructive CAD, confirmed by invasive CAG [5].

Results

Among 5794 people who were hospitalized with acute coronary syndrome to the Department of Emergency Cardiology in 2010-2016, 2.8% (161) patients demonstrated nonobstructive coronary atherosclerosis confirmed by CAG. One (0.6%) patient died. There were 94 (58%) cases with acute myocardial infarction, 27 (17%) with unstable angina, 15(9%) with pseudo-coronary scenario of myocarditis, 7 (4%) with cardiomyopathy, and 16 (10%) with arrhythmias, congenital heart defects, aortic dissection. The average age of the patients was 57 years, the proportion of men was 66%. The prevalence of dyslipidemia, peripheral atherosclerosis and family history are more common in patients with MINOCA than in patients with obstructive CAD (Table 1).

Among the patients with MINOCA, there were 30 (19%) patients with malignant and non-malignant tumors, including 7 (23%) patients with cancer of kidneys, sigmoid colon and cervix. Bladder cancer was confirmed in 1 (0.6%) case before admission to the hospital, and lung cancer was identified in 1 (0.6%) case at autopsy. The incidence of oncology diseases in patients with MINOCA was higher than in patients with obstructive CAD (Table 1).

Table 1

Clinical and anamnestic characteristics of the patients

	MINOCA	**MI CAD
N	161	57
Man, n (%)	107(66%)	42(74%)
Average age, Me (Q1;Q3)	57(50;61)	58(51;64)
Arterial hypertension, n (%)	122(76%)	45(79%)
Dyslipidemia, n (%)	128(80%)*	36(63%)*
Body mass index, Me(Q1;Q3), (kg/m^2)	28(24;32)	27(24;29)
Family history, n (%)	59(37%)*	13(23%)*
Smoking, n (%)	95(59%)	34(60%)
Diabetes mellitus	24(15%)	9(16%)
Chronic Kidney Disease, n (%)	4(2,5%)	2(4%)
Glomerular filtrate rate, Me(Q1;Q3), (ml/min/1,73 м^2)	73(60;82)	72(60;86)
Peripheral atherosclerosis, n (%)	108(67%)*	30(53%)*
Stroke, n (%)	12(7%)	3(5%)
Previous myocardial infarction, n (%)	9(6%)	4(7%)
Heart Valve Diseases, n (%)	3(2%)	0
Nonmalignant/malignant tumors, n (%)	30 (19%)*	0*

*p<0,05 **- data from NCT03122340

Conclusion

The proportion of patients with MINOCA among the patients with ACS in 2015-2016 was 2.8 % (161). These results were consistent with other recent studies INOCA presents heterogeneous group of diseases, such as AMI, unstable angina, myocarditis, cardiomyopathies, arrhythmias, aortic dissection and other noncardiac diseases. Hospital mortality was 0,6 %. Incidence of oncology diseases in the patients with MINOCA was higher than in the patients with obstructive CAD.

REFERENCES

1. *Pasupathy S., Air T., Dreyer R.P., Tavella R., Beltrame J.F.* Systematic review of patients presenting with suspected myocardial infarction and nonobstructive coronary arteries. Circulation. 2015 Mar 10; 131(10): 861-70. doi: 10.1161/CIRCULATIONAHA.114.011201.

2. *Letsas K.P., Korantzopoulos P., Evangelou D., Pappas L.K., Kardaras F.* Acute Myocardial Infarction with Normal Coronary Arteries in a Patient with Hodgkin's Disease A Late Complication of Irradiation and Chemotherapy. Tex Heart Inst J. 2006; 33(4): 512-4.

3. *Rinde L.B., Småbrekke B., Hald E.M., Brodin E.E., Njølstad I., Mathiesen E.B., Løchen M.L., Wilsgaard T., Brækkan S.K., Vik A., Hansen J.B.* Myocardial infarction and future risk of cancer in the general population-the Tromsø Study. Eur J Epidemiol. 2017 Mar; 32(3): 193-201. doi: 10.1007/s10654-017-0231-5.

4. *Nordenskjöld A.M., Baron T., Eggers K.M., Jernberg T., Lindahl B.* Predictors of adverse outcome in patients with myocardial infarction with non-obstructive coronary artery (MINOCA) disease. Int J Cardiol. 2018 Jun 15; 261: 18-23. doi: 10.1016/j.ijcard.2018.03.056.

5. *Vyshlov E.V., Tsoi E.I., Sultanov V.S., Trusov V.B., Ryabov V.V.* Hypolipidemic and hepatoprotective effect of polyprenol-containing drug in patients with acute coronary syndrome. Bulletin of Experimental Biology and Medicine. 2018; 165(3): 289-292. [in Russian]

DRUGS TO CONTROL HAZARDOUS VIRUSES INCLUDING SOME INVOLVED IN HUMAN MALIGNANCIES

E. Huberman
NovaDrug and University of Illinois, Chicago, USA

Hepatitis C virus (HCV) and human immunodeficiency virus (HIV) are implicated in human cancers. Drugs controlling these viruses may impede HCV/HIV-induced malignancies.

Using a bacterial construct and mammalian cells, we identified three distinct classes of small molecules with anti-HCV activity. These compounds also inhibited unrelated RNA viruses such as HIV, corona, and Ebola viruses.

NovaDrug's compounds do not display genotoxicity in the mouse micronuclei test nor in the Ames or cell culture mutagenesis assays.

To investigate their mode of action, we used the *in vitro* HIV/peripheral blood cell model, which has useful tools for locating the viral life cycle phase where the drug displays its inhibition. For this study, we tested the ability of the drugs to affect virus cell entry, reverse transcription, genomic integration, translation, and viral release. The results indicated that NovaDrug's compounds operate after virus entry but prior to reverse transcription. As such, they act early in the HIV life cycle.

Representatives of the three drug classes failed to consistently affect the production of 30 different cytokines. Nor did they regularly inhibit the catalytic activity of 359 known protein kinases. An exception was a subgroup of one of the drug classes. This subgroup consistently inhibited the catalytic activity of a protein kinase (PK), termed LK. Moreover, LK specific siRNAs restrained HCV replication, thus implicating LK in HCV life cycle. Studies are ongoing to pinpoint the relevant targets of NovaDrug's antivirals.

Currently, there are a limited number of clinical drugs with a wide range of antiviral activity; NovaDrug's compounds display such a range.

Keywords: human immunodeficiency virus, hepatitis C virus, human malignancies, therapy.

HPV-NEGATIVE CERVICAL CANCER: A DISTINCT TYPE OF THE UTERINE CERVIX WITH POOR PROGNOSIS

M.K. Ibragimova[1,2], M.M. Tsyganov[1], I.V. Deryusheva[1], L.A. Kolomiets[1], O.N. Churuksaeva[1], N.V. Litviakov[1,2]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University, Tomsk, Russia[2]

The real-time PCR method was used to study cervical scrapings from 116 patients with stage I–IV primary cervical cancer. Decreased survival and poor prognosis were found in patients with HPV-negative cervical cancer.

Keywords: cervical cancer, human papillomavirus.

Introduction

Nowadays, human papillomavirus (HPV) is considered to be the main causative agent of cervical cancer progression [1][Bosch, 2011 #133][Bosch, 2011 #60]. According to various studies, up to 80–90% of patients diagnosed with cervical cancer (CC) are also carriers of high-risk HPV [2]. A small number of cervical cancers are not caused by HPV or the virus can be eliminated during oncogenesis, i.e. such cancers are HPV-negative. Some researchers claim that such CC subtype may be encountered only due to lab test errors [3]. However, there is another view of the phenomenon. Along with HPV-associated CC, there is HPV-negative tumors, which belong to a more aggressive tumor class and whose oncogenesis essentially differs from that of HPV-associated tumors [4]. The **aim of the study was** to evaluate relapse-free and overall survival for HPV+ and HPV- patients diagnosed with primary CC.

Material and Methods

The research included 116 patients aged from 24 to 79 years and diagnosed with stage I–IV primary CC. The diagnosis was histologically verified; tumors were defined according to the FIGO classification. Complex examination included pelvic examination, colposcopy, cytological and histological tests. Scraping samples from the cervical canal and the outer part of the cervix served as study materials. Detection and genotyping of HPV DNA was carried out via the real-time PCR method using a RotorGene 6000 (Corbett Research, Australia) and Amplisens® reagent sets (Amplisens® HPV HCR-screen; Amplisens® HPV HCR-genotype (Moscow, Russia). The Fisher's ratio test was applied to assess statistical relevance of differences in the qualitative attributes occurrence distribution between the groups. Survival rates were evaluated according to the Kaplan-Meier method.

Results and discussion

The presence of HPV+ CC associated with one or several types of HPV was verified in 84 patients (72.4%), while 32 patients (27.6%) were not diagnosed with HPV. HPV- patients underwent second biopsy, DNA purification, detection and genotyping of HPV DNA in examined samples. Absence of the viruses was verified as a result. The patients were divided into 2 groups depending on the HPV contamination: group 1 – HPV+ patients (n=84) with the average age of 42.1±1.7 years; group 2 – HPV- patients (n=32) with the average age of 45.5±1.6 year old. The groups did not differ in terms of general clinical and pathologic findings: tumor size, lymphatic cancer spread and histotype. Genotyping of HPV+ samples showed that HPV genotype 16 was present in 67.8% of cases. These results were consistent with other recent studies [5, 6]. HPV types 33 and 31 were ranked as the 2nd and 3d most common, respectively (22.6% and 20.2%), whereas some authors reported that HPV 18 was the 2nd most common HPV type [5]. A low viral load (<3 lg HPV DNA/10^5 cells) had 22.6%, who were considered to have low risk of CC [7]. High viral load (>3 lg HPV DNA/10^5 cells) was observed in 77.4% of patients, who were considered at have high risk for CC. Decrease in both the overall and disease-free survival rates was observed in HPV- patients diagnosed with CC. Figure 1 A, B demonstrate disease-free and overall survival of patients with CC: disease-free survival for HPV+ and HPV- patient groups was 102 and 68 months, respectively; overall survival was 52 and 83 months, respectively. The 2-year disease-free survival rate in both groups was 92.0% and 73.0%, respectively. The 2-year overall survival rate in HPV+ and HPV- patients was 86.0% and 65.0%, respectively. Decreased survival may be caused by different oncogenic mechanisms of CC, which may explain the differences in the disease outcome. Harima et al. reported that HPV-cancer is a separate group and this particular subtype responds poorly to radiotherapy [8]. The data on the matter dates back to 2006: HPV viral load in cervical scrapings of patients diagnosed with CC was examined before treatment. The results showed that the hierarchy of CC prognoses depending on its severity was as follows: HPV+ tumors with high viral load, HPV+ tumors with low viral load and the worst prognoses occurred for HPV- tumors [9].

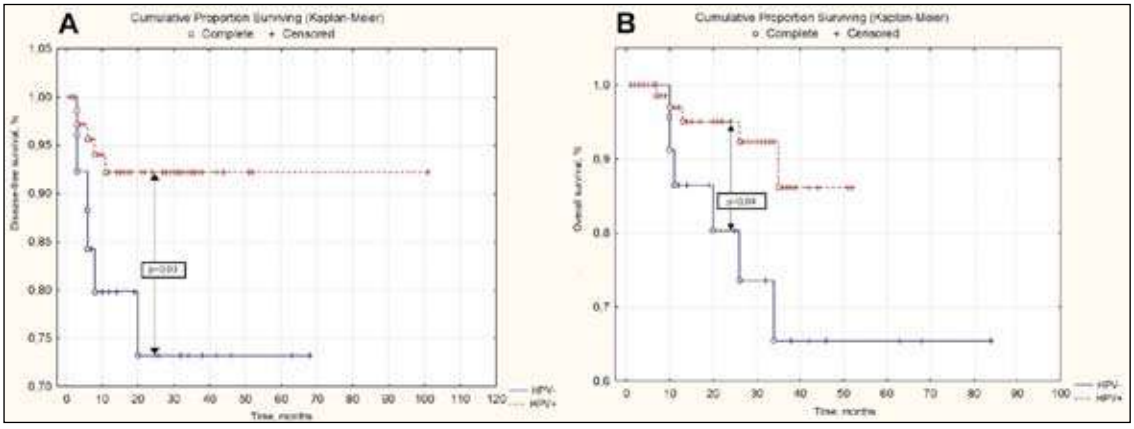


Figure 1. Relapse-free survival (A) and overall survival (B) of patients diagnosed with cervical cancer

Conclusions

The current research has focused on the determinants of infection with oncogenic HPV types, the assessment of prophylactic and therapeutic vaccines, and the development of screening strategies incorporating HPV testing and other methods as adjunct to cytology. These are fundamental stepping stones for the implementation of effective public health programs aimed at the control of cervical cancer.

HPV- cervical tumors are 2.5 times less frequently found than HPV+, but they have significantly worse prognoses. The presence or absence of HPV DNA in a tumor may become a new independent prognostic factor for cervical cancer. HPV- cervical cancer pathogenesis is understudied and requires further and detailed research.

The reported research was funded by Russian Foundation for Basic Research and the government of the region of the Russian Federation, grant № 18-44-703004.

REFERENCES

1. *Bosch F.X.* Human papillomavirus: science and technologies for the elimination of cervical cancer. Expert opinion on pharmacotherapy. 2011; 12(14): 2189-2204. doi: 10.1517/14656566.2011.596527.
2. *Nazarova N.M., Prilepskaya V.N., Sulamanidze L.A., Mzarelua G.M., Bestaeva N.V.* Papillomavirus infection: prevalence, diagnosis and treatment (literature review). Lechashhij vrach. 2013; 11: 15-19.
3. *Böhmer G., van den Brule A.J., Brummer O., Meijer C.L., Petry K.U.* No confirmed case of human papillomavirus DNA-negative cervical intraepithelial neoplasia grade 3 or invasive primary cancer of the uterine cervix among 511 patients. American journal of obstetrics and gynecology. 2003; 189(1): 118-120.
4. *Komarova L.E.* Current aspects of cytological screening for cancer of the cervix uteri: a review. Tumors of female reproductive system. 2009; 3: 78-83. [in Russian]
5. *Muñoz N., Bosch F.X., de Sanjosé S., Herrero R., Castellsagué X., Shah K.V., Snijders P.J., Meijer C.J.; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group.* Epidemiologic classification of human papillomavirus types associated with cervical cancer. New England J. of Medicine 2003; 348: 518-527. doi: 10.1056/NEJMoa021641.
6. *Kolomiets L.A., Churuksaeva O.N., Shpileva O.V., Urazova L.N., Rodicheva N.S.* Prevalence of various human papillomavirus (HPV) types in patients with cervical intraepithelial neoplasia (cin) and cervical cancer in tomsk region. Siberian Journal of Oncology. 2012; 3: 41-45. [in Russian]
7. *Sasieni P., Castanon A., Cuzick J.* Effectiveness of cervical screening with age: population based case-control study of prospectively recorded data. BMJ. 2009 Jul 28; 339: b2968. doi: 10.1136/bmj.b2968.
8. *Harima Y., Sawada S., Nagata K., Sougawa M., Ohnishi T.* Human papilloma virus (HPV) DNA associated with prognosis of cervical cancer after radiotherapy. International J. of Radiation Oncology. 2002; 52: 1345-1351.
9. *Datta N.R., Kumar P., Singh S., Gupta D., Srivastava A., Dhole T.N.* Does pretreatment human papillomavirus (HPV) titers predict radiation response and survival outcomes in cancer cervix? a pilot study. Gynecologic oncology. 2006; 103: 100-105. doi: 10.1016/j.ygyno.2006.01.058.

RELATIONSHIP BETWEEN THE LEVEL DIFFERENT POPULATIONS OF CIRCULATING TUMOR CELLS BEFORE TREATMENT AND EFFEC TO NEOADJUVANT CHEMOTHERAPY IN PATIENTS WITH BREAST CANCER

E.V. Kaigorodova[1,2], N.A. Tarabanovskaya[1], E.I. Simolina[1], V.M. Perelmuter[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Siberian State Medical University, Tomsk, Russia[2]

The relationship between the level of different populations of circulating tumor cells before the treatment (before biopsy) in patients with breast cancer with the effect of neoadjuvant chemotherapy was evaluated. The study showed that patients with a complete and / or partial tumor regression after NACT level CTC with sign EMT in the blood before biopsy was significantly lower than in patients who have no observed response to treatment (stabilization and / or progression) (p= 0.02).

Keywords: heterogeneity of circulating tumor cells; EMT (epithelial-mesenchymal transition), neoadjuvant chemotherapy (NACT), breast cancer.

Introduction

CTCs are a heterogeneous population. Some cells are cancer stem cells, other cells are in an EMT (epithelial-mesenchymal transition) state, and most of the cells do not have EMT and stemness properties [1, 2]. It was shown that neoadjuvant chemotherapy (NACT) on breast cancer does not act on CTCs in the EMT state [3]. The biological properties of circulating tumor cells (CTCs), and their dynamics during neoadjuvant chemotherapy are important, both for disease progression prediction and therapeutic target determination, with the aim of preventing disease progression. It has been observed that significant changes in the quantity of the different subsets of circulating tumor cells in patients' blood were observed after carrying out the 3rd course of NACT. NACT causes significant changes in the quantity of six CTC subsets, with various combinations of stemness and epithelial-mesenchymal transition (EMT) properties [4]. The aim of our study was to

assess the relationship between the level of different populations of circulating tumor cells before treatment (before biopsy) with the effect of NACT.

Material and Methods

The prospective study includes 30 patients with invasive breast cancer T2-4N0-3M0 aged 32 to 60 years admitted for treatment in Cancer Research Institute, Tomsk National Research Medical Center. Neoadjuvant chemotherapy was carried out for 14 patients. 16 patients were in the group without NACT. Venous blood samples taken before and after biopsy Venous blood samples (5 mL) were collected in tubes containing heparin and used for examination within 2 h.

The various pools of circulating tumor cells were determined using monoclonal antibodies to EpCam, CD44, CD45, CD24, N-cadherin, labeled with different fluorochromes on flow cytometry BD FACSCanto ™ II.

Evaluating the effectiveness of neoadjuvant chemotherapy was carried out on a scale RECIST (Response Evaluation Criteriain Solid Tumors from 2000 YG) 14 days after the second and fourth courses of chemotherapy on the basis of clinical examination, ultrasound examination of the breasts and mammography.

The obtained data were processed using variation statistics. Assessment of the normal distribution of the results was performed using the Kolmogorov-Smirnov test. The significance of differences was assessed using the nonparametric Mann-Whitney test.

Results

The study showed that patients with a complete and / or partial tumor regression after NACT level CTC with sign EMT in the blood before biopsy was significantly lower than in patients who have no observed response to treatment (stabilization and / or progression) (p= 0.02).

Table

Different populations of circulating tumor cells (CTCs) in the blood of breast cancer patients before biopsy in groups with and without the effect of neoadjuvant chemotherapy, Me (Q1–Q3), cells per μL

	CTC	Presence of a response to NACT (N=6)	No response to NACT (N=8)
1	CTC-1 without stemness and EMT properties (EpCam+CD45-CD44-CD24-N-cadherin-)	0,0 (0,0-0,0)	0,62 (0,18-1,92) p=0,06
2	CTC-2 without stemness and with EMT properties (EpCam+CD45-CD44-CD24-N-cadherin+)	0,06 (0,02-0,09)	0,67 (0,23-1,22) p=0,02
3	CTC-3 with stemness and without EMT properties (EpCam+CD45-CD44+CD24-N-cadherin-)	0,0 (0,00-0,24)	0,00 (0,00-0,02) p=0,85
4	CTC-4 with stemness and EMT properties (EpCam+CD45-CD44+CD24-N-cadherin+)	0,51 (0,21-0,73)	0,63 (0,02-0,73) p=0,56
5	CTC-5 with stemness, without EMT properties and without EpCAM membrane expression (EpCam-CD45-CD44+CD24-N-cadherin-)	2,37 (0,00-5,84)	0,23 (0,00-1,52) p=0,92
6	CTC-6 with stemness and EMT properties and without EpCAM membrane expression (EpCam-CD45-CD44+CD24-N-cadherin+)	0,49 (0,06-2,35)	0,57 (0,22-3,31) p=0,46

Conclusion

Thus, the level of CTC with a sign of EMT before treatment is associated with the effect of NACT. The level of CTC with stemness (without and in combination of EMT) before treatment is not associated with the effect of NACT. The results are useful for determining tactics and creating a personalized approach to the treatment of patients with invasive breast carcinoma.

The study was conducted with financial support from the Council for Grants of the President of the Russian Federation MD-544.2018.7

REFERENCES

1. *Kaigorodova E.V.* Circulating tumor cells: clinical significance in breast cancer (Review). Annals of the Russian academy of medical sciences. 2017; 72(6): 450-457. doi: 10.15690/vramn833. [in Russian]

2. *Kaigorodova E.V., Tarabanovskaya N.A., Staheeva M.N., Savelieva O.E., Tashireva L.A., Denisov E.V., Perelmuter V.M.* Effect of minor and major surgical injury on the level of different populations of circulating tumor cells in the blood of breast cancer patients. Neoplasma. 2017; 64(3): 437-443. doi: 10.4149/neo_2017_315.

3. *Mego M., Mani S.A., Lee B.N., Li C., Evans K.W., Cohen E.N., Gao H., Jackson S.A., Giordano A., Hortobagyi G.N., Cristofanilli M., Lucci A., Reuben J.M.* Expression of epithelial-mesenchymal transition-inducing transcription factors in primary breast cancer: The effect of neoadjuvant therapy. Int J Cancer. 2012 Feb 15; 130(4): 808-16. doi: 10.1002/ijc.26037.

4. *Kaigorodova E.V., Savelieva O.E., Tashireva L.A., Tarabanovskaya N.A., Simolina E.I., Denisov E.V., Slonimskaya E.M., Choynzonov E.L., Perelmuter V.M.* Heterogeneity of Circulating Tumor Cells in Neoadjuvant Chemotherapy of Breast Cancer. Molecules. 2018 Mar 22; 23(4). pii: E727. doi: 10.3390/olecules23040727.

MRNA EXPRESSION OF CELLULAR MOVEMENT PROTEINS AND CALPAINS 1 AND 2 IN METASTASIS OF HEAD AND NECK SQUAMOUS CELL CARCINOMA AND NON-SMALL CELL LUNG CANCER

G.V. Kakurina[1], E.S. Kolegova[1], I.V. Kondakova[1], A.Yu. Dobrodeev[1], D.N. Kostromitsky[2], O.V. Cheremisina[1], E.L. Choinzonov[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk Regional Oncology Center, Tomsk, Russia[2]

The paper presents the results of the expression change in mRNA of actin binding proteins (ABPs): adenylyl-cyclase associated protein 1 and cofilin 1 in metastasis of non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinoma (HNSCC). The expression of calpain 1 and 2 was also studied as possible modulators of the ABPs. We showed the dependence of the expression level of ABPs and calpaines 1 and 2 mRNA on the presence of metastases in patients with HNSCC and NSCLC. The data indicated the involvement of ABPs and calpain system in tumor progression in NSCLC and HNSCC.

Keywords: non-small cell lung cancer, head and neck squamous cell carcinoma, metastases, prognosis.

Background

Non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinoma (HNSCC) are the most common types of cancer [1, 2]. These cancers are usually asymptomatic during their early stages [3]. Metastases are very common in the late stages of cancer. Molecular mechanisms of metastasis formation have not been extensively studied. Actin binding proteins (ABPs) play an important role in the mechanisms of metastasis because they participate in the migration and invasion of tumor cells [4-6]. Such ABPs as little-studied adenylyl-cicalase associated protein 1 (CAP1) and its partner-cofilin play a fundamental role in the work of the cytoskeleton [5, 6]. Therefore, the study of the CAP1 and cofilin role in the metastasis of NSCLC and LSCC is of great importance. It is known that ABPs are a substrate for the calpain proteolytic system [7]. Therefore, the study included calpaines 1 and 2, as possible modulators of the ABPs system.

Material and Methods

We evaluated the expression of ABPs (CFL1, CAP1) and calpaines (CAPN1, CAPN2) in paired tissue samples of NSCLC patients (T2-3N0M0, n = 15, T2-3N1-2M0, n = 10) and HNSCC patients (T2-3N0M0, n = 14; T2-4N1-2M0, n = 9) using SYBR Green I RT-PCR in real time with reference to normal tissue. As the referee gene the «housekeeping» gene of the GAPDH enzyme (glyceraldehydes-3-phosphate dehydrogenase) was used. Statistical analysis of the results was performed using Statistica 6.0 software.

Results

It was shown that the mRNA expression of CAP1 in tumor tissue of HNSCC and NSCLC patients with regional metastases was higher than that in patients without metastases. The expression of CFL1 mRNA was higher only in patients with NSCLC with metastases to the regional lymph nodes (Table 1). In general the results do not contradict the literature data [3, 6].

Table 1

The expression of mRNA of CAP1 and cofilin1 in tumor tissue depending on presence of metastases in regional lymph nodes (N)

Marker	NSCLC		HNSCC	
	$T_{2-3}N_0M_0$	$T_{2-3}N_{1-2}M_0$	$T_{2-3}N_0M_0$	$T_{2-3}N_{1-2}M_0$
CAP1	1.70±0,28	3,40±0,40*	7,17±6,78	13,43±4,50*
CFL1	2,00±0,05	5,40±0,98*	12,85±23,6	8,26±14,08

Note: * - differences were significant compared to $T_{2-3}N_0M_0$, $p < 0.05$.

In patients with HNSCC, the mRNA expression of CAPN1 and CAPN2 was higher in patients with regional metastases than in patients without regional metastases. On contrary, in patients

with NSCLC, the mRNA expression of CAPN1 and CAPN2 was lower in patients with regional metastases than in patients having no metastases (Table 2). A multidirectional change in activity of proteolitic systems in primary tumors depending on the presence of lymphogenous metastases was previously shown [7, 8].

Table 2

mRNA expression of CAPN1 and CAPN2 in tumor tissue depending on presence of metastases in regional lymph nodes (N)

Marker	NSCLC		HNSCC	
	$T_{2-3}N_0M_0$	$T_{2-3}N_{1-2}M_0$	$T_{2-3}N_0M_0$	$T_{2-3}N_{1-2}M_0$
CAPN1	56,70±10,53	36,78±11,50*	6,96±13,55	10,37±9,54
CAPN2	37,79±9,22	4,80±0,80*	2,69±0,58	8,39±3,07*

Note: * - differences were significant compared to $T_{2-3}N_0M_0$, $p < 0.05$

Conclusion

The dependence of mRNA expression level of ABPs and calpaines on the presence of metastases in patients with HNSCC and NSCLC was shown. The data obtained indicated the involvement of ABPs and the calpain system in progression of NSCLC and HNSCC. The paper shows the feasibility of using CAP1, cofilin1 and calpain 1 and 2 for predicting metastases in patients with NSCLC and HNSCC.

This work was supported by the Russian Foundation for Basic Research (grant number 17–04–00198 A and 18-415-703003 r_mol_a).

REFERENCES

1. *Arantes L.M.R.B., De Carvalho A.C., Melendez M.E., Lopes Carvalho A.* Serum, plasma and saliva biomarkers for head and neck cancer. Expert Rev Mol Diagn. 2018 Jan; 18(1): 85-112. doi: 10.1080/14737159.2017.1404906.

2. *Kakurina G.V., Kondakova I.V., Choinzonov E.L.* Prognosing head and neck squamous cell carcinoma metastases. Vopr. Onkol. 2012; 58(1): 26-32. [in Russia]

3. *Kakurina G.V., Kolegova E.S., Cheremisina O.V., Zavyalov A.A., Shishkin D.A., Kondakova I.V., Choinzonov E.L.* Adenylyl Cyclase-Associated Protein 1 in Metastasis of Squamous Cell Carcinoma of the Head and Neck and Non-Small Cell Lung Cancer. AIP Conference Proceedings. 2016; 1760. 020023.

4. *Kondakova I.V., Yunusova N.V., Spirina L.V., Kolomiets L.A., Villert A.B.* Association between Intracellular Proteinase Activities and the Content of Locomotor Proteins in Tissues of Primary Tumors and Metastases of Ovarian Cancer. Russ J Bioorgan Chem. 2014. 40(6); 681–687/

5. *Kakurina G.V., Kolegova E.S., Kondakova I.V.* Adenylyl Cyclase-Associated Protein 1: Structure, Regulation, and Participation in Cellular Processes. Biochemistry (Mosc). 2018 Jan; 83(1): 45-53. doi: 10.1134/S0006297918010066.

6. *Paavilainen V.O., Bertling E., Falck S., Lappalainen P.* Regulation of cytoskeletal dynamics by actin-monomer-binding proteins. Trends Cell Biol. 2004 Jul; 14(7): 386-94.

7. *Shashova E.E., Kolegova E.S., Zav'yalov A.A., Slonimskaya E.M., Kondakova I.V.* Changes in the Activity of Proteasomes and Calpains in Metastases of Human Lung Cancer and Breast Cancer. Bull Exp Biol Med. 2017 Aug; 163(4): 486-489. doi: 10.1007/s10517-017-3834-7.

8. *Kakurina G.V., Cheremisina O.V., Choinzonov E.L., Kondakova I.V.* Circulating Proteasomes in the Pathogenesis of Head and Neck Squamous Cell Carcinoma. Bull Exp Biol Med. 2017 May; 163(1): 92-94. doi: 10.1007/s10517-017-3745-7.

INFLUENCE OF METHORMIN ON METABOLIC ACTIVITY LIKE THE ANTI-CANCER DRUG

D.S. Kaplun[1,2], O.P. Shatova[3], I.I. Zinkovych[1]

Donetskiy National Medical University named after M. Gorky, Donetsk, Ukraine[1]
Federal Research Center «Fundamental Foundations of Biotechnology» of the Russian Academy of Sciences, Moscow, Russia[2]
Russian National Research Medical University named after N.I. Pirogov, Moscow, Russia[3]

The effect of the antidiabetic drug metformin drug on the metabolic activity of Caki1 and HEK293 was studied. It was found that the incubation of cells with metformin leads to a decrease in their metabolic activity. The effect of metformin, discovered by us, has a linear dose-dependent character.

Keywords: metformin, proliferation, MTT, Caki1, HEK293.

The anticancer effect of metformin has been demonstrated in several types of cancer; however, the mechanisms involved are incompletely understood. Metformin exerts its anti-tumor effects

mainly through the AMPK/LKB1/TORC1 signaling pathway, thereby causing apoptosis in cancerous cells [1]. Metformin can reduce proliferation of cancer cells [2], angiogenesis, levels of insulin and insulin-like growth factors [3]. Other targets for anti-tumor effect of metformin could be the antagonization of obesity, anti-inflammatory effects and killing of cancer stem cells [4]. In addition, the effect of metformin on the metabolism in tumor tissues was shown [5, 6]. We investigated the cell line of kidney cancer (Caki1), which is notable for containing a large number of mitochondria, in order to analyze the effect of metformin on metabolism. To assess the specific effects of metformin on cancer cells, human embryonic kidney cells (HEK 293) were used as a comparison. Evaluation of metabolic activity was carried out with a tetrazolium dye test - MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide). Restoration of MTT depends on the cellular metabolic activity: the activity of NADPH dehydrogenases and the concentration of NADPH.

Material and Methods

Cells were maintained in medium (DMEM) (Thermo Fisher Scientific, USA), supplemented with 10% inactivated fetal bovine serum (FBS) (Thermo Fisher Scientific, USA), 100 IU / ml penicillin and 100 ng / ml streptomycin (PanEco, Moscow). The cells were cultured at 37 °C in a 90% humidified incubator with 5% CO_2. ThenAn 8-channel multipipette was used, 100 µl DMEM medium was added to each well of a 96-well plate. The control wells were loaded only with DMEM medium, and in experimental wells DMEM containing 10 mM metformin. A certain number of cells were addedintroduced into each well of a 96-well plate. Preliminary cell counting was performed on a Countess instrument (Thermo Fisher Scientific, USA). 100 µl of 5×10 5 cells / ml of the solution for the first well was added and the contents were pipetted. Thus, the first well had the maximum number of cells, which was 2.5×105. Accordingly, serial dilutions of 1.25×105 and 6.25×104 were made further, and so on. Those. 100 µl from the first well was taken and added to the second well while stirring constantly. Then repeated for the next wells. From the last well that contained the minimal number of cells was withdrawn and discarded. The cells were then incubated for 24 hours in a CO_2 incubator at 37 °C. After 24 hours, 0.5 mg / ml of MTT dissolved in DMEM + / + medium was added to each well, incubated for 3 hours at 37 ° C in an atmosphere of 5% CO_2. A medium was taken and 100 µl of dimethylsulfoxide (DMSO) was added, thereby dissolving the formazan crystals. The optical density was spectrophotometrically measured (iMark Microplate Absorbance Reader, Bio-Rad USA) at a wavelength of 540 nm versus MTT solution in DMSO.

Results

When evaluating the effect of metformin on the cancer cell line Caki1, we detect that metformin reduced metabolism at $p \leq 0.01$ (Figure 1) in samples with a cell concentration of 2.5 * 105, 1.25 * 105, 0.6125 * 105. Decreased proliferation was observed approximately in 50% samples, compared with the control group (without metformin).

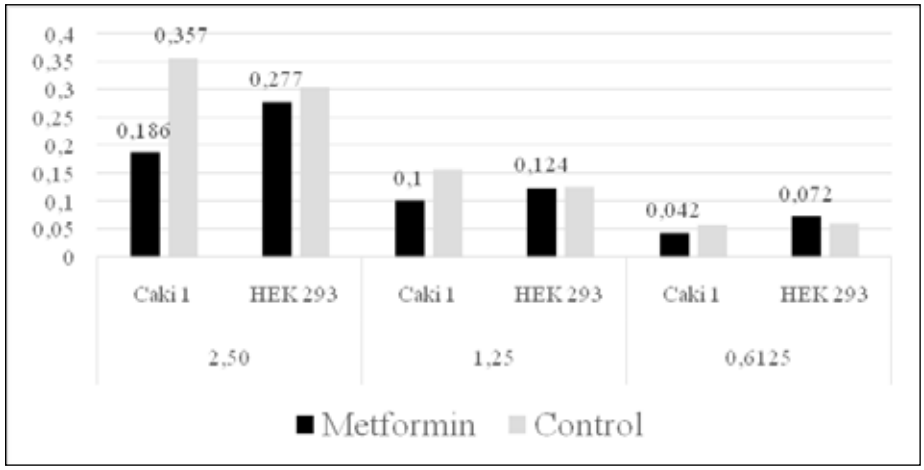


Figure 1. Effect of metformin on Caki1 and HEK293 (axis y – the optical density).

For comparison, we studied the effect of metformin on HEK293. It was established that for untransformed embryonic kidney cells there were no statistically significant differences in metabolism after adding of metformin compared to control samples (Figure 1). Despite the fact that there are no statistically significant differences at a given concentration of metformin, at a cell concentration of 2.5×105 we see a tendency to decrease metabolic activity. Perhaps this indicates that embryonic kidney cells are less sensitive to this drug.

We analyzed the effect of various concentrations of metformin on the Caki1 cell line. Thus, when metformin 5mM was added to the medium, the metabolic activity of the cells was reduced by 16.8% ($p = 0.11$), which had no statistically significant difference with the control. But with the addition of 10 mM metformin, there was a two-fold decrease in proliferation as compared to control samples ($p = 0.001$) (Figure 2).

Further, an increase in the concentration of metformin to 20 mM and 40 mM led to a statistically significant decrease in metabolic activity up to 53% and 63%, respectively.

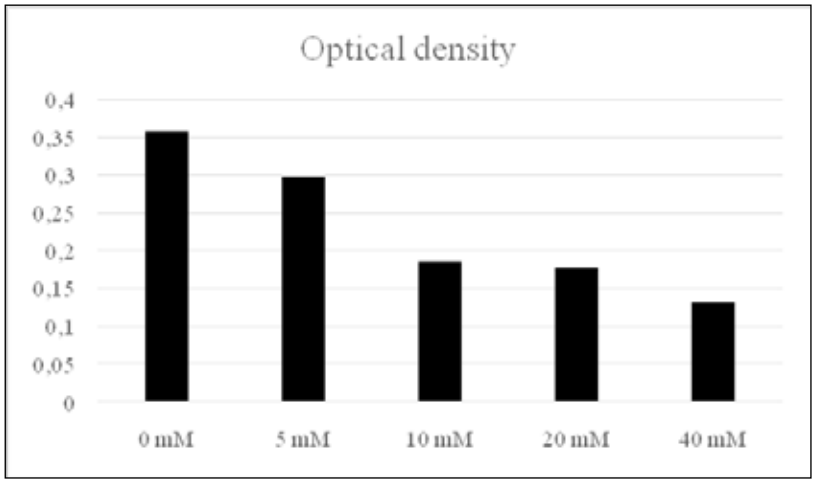


Figure 2. The dose-dependent effect of metformin on the metabolic activity of Caki1.

A significant difference in the optical density values was also obtained between samples of 10 mM and 40 mM ($p = 0.001$).

Conclusions

It was found that metformin has an inhibitory effect on the metabolism of Caki1 and HEK 293. The maximum expressed inhibitory effect of metformin was observed at a concentration of Caki1 cells of 2.5×10^5 cells. When analyzing dose-response effects of metformin on Caki1, it was found that the higher the concentration of metformin, the more pronounced the decrease in metabolism, namely the work of NADPH-dehydrogenases.

REFERENCES

1. *Koo S.H., Flechner L., Qi L., Zhang X., Screaton R.A., Jeffries S., Hedrick S., Xu W., Boussouar F., Brindle P., Takemori H., Montminy M.* The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature. 2005; 437: 1109–1111. doi: 10.1038/nature03967.

2. *Pereira F.V., Melo A.C.L., Low J.S., de Castro İ.A., Braga T.T., Almeida D.C., Batista de Lima A.G.U., Hiyane M.I., Correa-Costa M., Andrade-Oliveira V., Origassa C.S.T., Pereira R.M., Kaech S.M., Rodrigues E.G., Câmara N.O.S.* Metformin exerts antitumor activity via induction of multiple death pathways in tumor cells and activation of a protective immune response. Oncotarget. 2018 May 25; 9(40): 25808-25825. doi: 10.18632/oncotarget.25380.

3. *Denley A., Carroll J.M., Brierley G.V., Cosgrove L., Wallace J., Forbes B., Roberts C.T.Jr.* Differential activation of insulin receptor substrates 1 and 2 by insulin-like growth factor-activated insulin receptors. Mol Cell Biol. 2007; 27: 3569–3577. doi: 10.1128/MCB.01447-06.

4. *Vallianou N.G., Evangelopoulos A., Kazazis C.* Metformin and Cancer. Rev Diabet Stud. 2013 Winter; 10(4): 228-35. doi: 10.1900/RDS.2013.10.228.

5. Shatova O.P., Butenko E.V., Khomutov E.V., Kaplun D.S., Sedakov I.E., Zinkovich I.I. Metformin impact on purine metabolism in breast cancer. Biomeditsinskaya Khimiya. 2016; 62(3): 302-305. [in Russian].

6. *Shatova O.P., Butenko E.V., Khomutov E.V., Kaplun D.S., Sedakov I.E., Zinkovych I.I.* Metformin impact on purine metabolism in breast cancer. Biomed Khim. 2016 Mar; 62(3): 302-5. doi: 10.18097/PBMC20166203302.

INFLUENCE OF METFORMIN ON ERYTHROCYTE

D.S. Kaplun[1,2], O.P. Shatova[1], I.I. Zinkovych[1]

Donetsk National Medical University of Maxim Gorky, Donetsk, DPR[1]
Federal Research Centre “Fundamentals of Biotechnology” of the Russian Academy of Sciences, Moscow, Russia[2]

The aim of our study was to investigate the effect of metformin on the cell membrane.

Metformin, a drug with diabetes with a well-established side effect and safety profiles, is widely studied for its anti-tumor activity in a number of cancers. But its mechanism of action in the clinical arena remains unclear. It is known that the short-term administration of metformin has

an antitumor effect that significantly affects the indirect, insulin-dependent pathway. The role of the direct path remains to be determined [1, 2].

Materials and Methods

Whole blood on oxalate in a volume of 0.5 ml was incubated for 60 minutes in a thermostat at 37 °C. In the first experimental series, 0.25 ml of a 30 mM metformin solution prepared in physiological saline was added to the whole blood. In the second control series, an appropriate volume of physiological sodium chloride solution was added. After incubation, 0.1 ml of blood samples from both series were used for the determination of the erythrocyte osmotic fragility by the method of L.I. Idelson [3] evaluated by the degree of hemolysis of erythrocytes in a hypotonic solution of 0.4 % sodium chloride, using photoelectrocolorimetry at a wavelength of 540 nm. To assess the effect of metformin on the antioxidant protection of erythrocytes spectrophotometrically (412 nm), the level of reduced glutathione was determined. The activity of glucose-6-phosphate dehydrogenase was determined by a standard kit of Sentinel Diagnostics

Statistical analysis was performed using the license package StatSoft Statistica 10.0. The results were presented as the arithmetic mean and its standard deviation ($M \pm \sigma$). Differences were assessed by the Student's test, considering them to be significant at $p < 0.05$.

Results

It was found that incubation of whole blood with metformin at a final concentration of 10 mM significantly affects the stability of erythrocyte membranes to hypotonic solutions. In the presence of metformin, the degree of hemolysis was statistically significant, 1.12 times higher than in the control series of experiments, respectively 63.0 ± 13.7% and 51.4 ± 10.0% ($p = 0.008$).

The observed effects of metformin on the stability of plasma membranes of erythrocytes in relation to hypotonic solutions can be explained by several mechanisms, for example, the effect of metformin on the changes in cell membranes [4]. It is impossible to exclude the direct modification of metformin by cellular mechanisms of transmembrane transport of ions and water [5, 6]. The effects of metformin on the erythrocyte osmotic fragility can be mediated. It is known that metformin is able to influence the reactions of carbohydrate metabolism [4]. This version is supported by the results obtained on the change in the pool of reduced glutathione in erythrocytes after their treatment with metformin (figure 1).

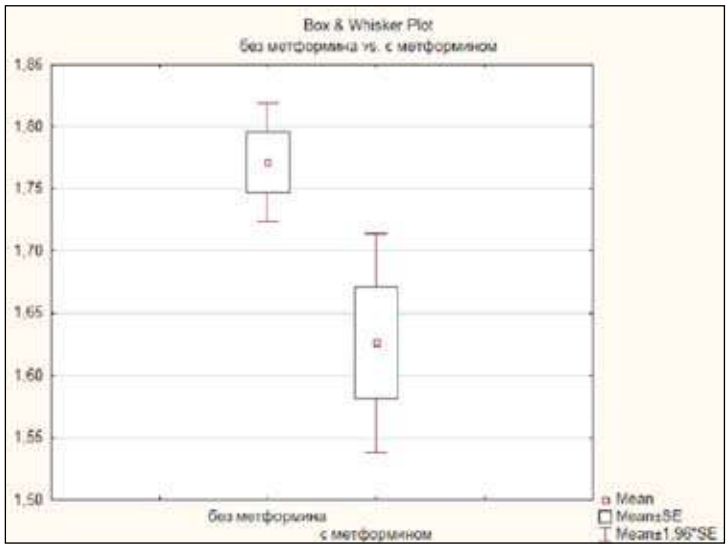


Figure 1. Influence of metformin (30 mM) on the concentration of reduced glutathione in erythrocytes at a preliminary incubation of whole blood.

The main role of glucose-6-phosphate dehydrogenase is the synthesis of NADPH. One of the main roles of NADPH in erythrocytes is the restoration of glutathione. The lack of reduced glutathione and the action of drugs, such as Pamakhin, cause changes in the surface of red blood cells, which increases their destruction. The lack of glutathione is simultaneously accompanied by an increase in the formation of toxic peroxides, which also has a negative effect on the state of the cell membrane. Thus, the insufficiency of glucose-6-phosphate dehydrogenase is the cause of drug hemolytic anemia [4].

Based on the results obtained, we decided to look at the activity of glucose-6-phosphate dehydrogenase in erythrocytes during incubation with metformin (10 mM). We found a decrease in the activity of the enzyme under the influence of metformin (figure 2).

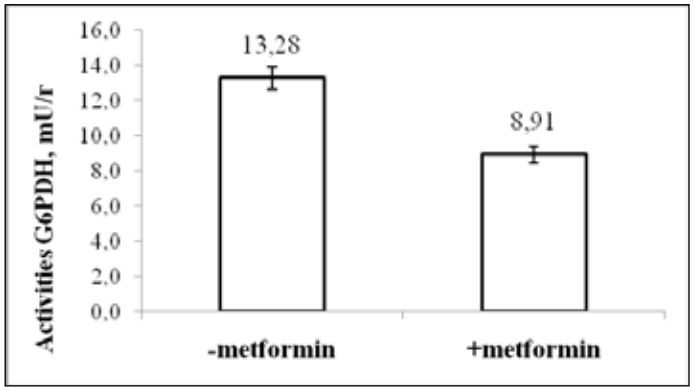


Figure 2 Changes in the activity of glucose-6-phosphate dehydrogenase by metformin.

Conclusion

We found that metformin reduced the activity of glucose-6-phosphate dehydrogenase that resulted in a decrease in glutathione, thus leading to the destruction of erythrocyte membranes, i.e. to a decrease in the osmotic resistance of erythrocytes.

REFERENCES

1. *Maniar K., Singh V., Chakrabarti A., Bhattacharyya R., Banerjee D.* High dose targeted delivery on cancer sites and the importance of short-chain fatty acids for metformin's action: Two crucial aspects of the wonder drug. Regul Toxicol Pharmacol. 2018 Aug; 97: 1516. doi: 10.1016/j.yrtph.2018.05.013.

2. *Camacho L., Dasgupta A., Jiralerspong S.* Metformin in breast cancer an evolving mystery. Breast Cancer Res. 2015 Jun 26; 17: 88. doi: 10.1186/s13058-015-0598-8.

3. *Medvedev B.I., Pomaskin I.N., Nikolaeva I.S. Markina O.K.* Adrenoreactivity erythrocytes in dynamics of uncomplicated pregnancy and the treatment of preterm labor. Bulletin of South Ural State University. 2009; 39(172): 125. [in Russian]

4. *Smirnova O.M.* Place of metformin in the modern treatment of prevention of type 2 diabetes. Diabetes. 2010; 3: 83. [in Russian]

5. *Krysova A.V., Kunshin A.A., Tsirkin V.I.* Sexual peculiarities of osmotic resistance of human erythrocytes, detected with an exposure of erythrocytes in distilled water. Physiology Herald of Nizhnegorodsky University of N.I. Lobachevsky. 2011; 2(2): 266272. [in Russian]

6. *Gritti M., Würth R., Angelini M., Barbieri F., Peretti M., Pizzi E., Pattarozzi A., Carra E., Sirito R., Daga A., Curmi P.M., Mazzanti M., Florio T.* Metformin repositioning as antitumoral agent: selective antiproliferative effects in human glioblastoma stem cells, via inhibition of CLIC1-mediated ion current. Oncotarget. 2014 Nov 30; 5(22): 1125268.

INTEGRATION MOLECULAR TESTING IN DIAGNOSIS, TREATMENT AND PREVENTION OF MEDULLAR THYROID CANCER IN CHILDREN

T.P. Kazubskaya[1], V.M. Kozlova[1], T.T. Kondrat'eva[1], R.B. Shishkov[1], Yu.I. Trofomov[2], F.A. Amosenko[1], V.J. Brjezovsky[1], V.G. Polaykov[1], L.N. Lubchenko[1]

N.N. Blokhin National Medical Research Center of Oncology, Moscow, Russia[1]
Research and Clinical Center of Otorhinolaryngology FMBA, Moscow, Russian[2]

Modern molecular genetic technologies in cancer study promotes their introduction into clinical practice, opening up new opportunities in diagnosis, choice of tactics for management and prevention of hereditary forms of malignant tumors in children. Medullar thyroid cancer (MTC) in children occurs either in or non-heritable form, the outcome of disease depends on early diagnosis.

The aim is to evaluate the possibilities of practical application of molecular diagnostic in the medical genetic counseling of the medullar thyroid cancer.

Design/Methods

Information and follow up date from 33 patients with MTC and 47 their family members were collected and studied in NN Blokhin Research Cancer Center. The age of the patients was from 2.5 to 21 years. A total of patients were examined between 1994 and 2016 years. The genetic tests for *RET* mutations were analyzed on peripheral blood samples from 33 patients and 47 their relatives, and their clinical significance was studied. In this study, six separate exons 10, 11 and 13-16 of the RET gene were analyzed using polymerase chain reaction-single-stranded conformational polymorphism (PCR-SSCP) analyses.

Results

Hereditary MTC detected in patients was associated with rare endocrinopathies included endocrine neoplasia (MEN) type 2 A (MEN 2A), type B (MEN 2B) and familial MTC (FMTC) as a variant of MEN2A. About 25% of patients with MTC have one of these familial syndromes with autosomal dominant type of inheritance. Germline mutations of *RET* proto-oncogene (chromosome 10 (10q11-2) is the cause of these syndromes. Testing the gene in the families of patients with MTC has made possible to identify a number of characteristic "RET-dependent" phenotypes. Of the 17 patients with MEN2A syndrome, 14 had a genotype in exon 11, substitution C634R (Cys→Arg), C634S (Cys→Ser) codon 634 and one of them- del632-636ins6 (exon 11). It is interesting that one of the patients had two mutations in exon 14 (I852M) and the second - in exon 13 (Y791F). In families of the carriers of the genotype (C634R) MTC appeared at different ages. Moreover, in these families pheochromocytomas affected 50% of the relatives, and some of them had this tumor before MTC. The earliest manifestation MTC in patients with the genotype (C634R) was the child 3.5 years old. This child underwent prophylactic thyroidectomy. A postoperative histological examination in the removed gland showed microcarcinomas. In the carrier of the mutation in exon 11 (C634S) MTC appeared at 11 years old. His mother with the same mutation has been diagnosed with MTC in her 23s, after that she had pheochromocytoma in 34 years. In this family many other relatives on the mother's side had MTC diagnosed in the several generations. In a child 2.5 years with the genotype (del632-636ins6) exon 11 preventive thyroidectomy revealed C-cell hyperplasia. In his family, MTC in combination with a pheochromocytoma were observed after 28 years in several generations. Identified genotypes were associated with high penetrance for MTC and pheochromocytoma. An interesting family is child of 4 years old with the *RET* mutations (I852M) inherited from the mother and the mutation (Y791F) inherited from the father. His mother was cure with thyroidectomy for MTC in her 24s and two size adrenalectomy for pheochromocytoma in 25 years old. She also had two mutations (C634R and I852M), one of which (C634R) was inherited from her father with the same diseases, and the other mutation (I852M) that origin remained unknown. The father of this child had the mutation (Y791F) and at 28 years of age with no signs of disease. The presence of two mutations in the child and beginning increased of calcitonin level became the cause of preventive thyroidectomy, which did revealed C-cell hyperplasia. The specific in an amino acid change in carriers of different mutations in this family correlated with the different course of the disease, showing incomplete penetrance of (Y791F). mutation. In MEN2A families were indicate the *RET* mutations (C634R), (C634S) and (del632-636ins6) that predisposes to extremely risk of medullary thyroid cancer associated with a life-long risk of phaeochromocytoma. In 12 children, MTC (with regional and distant metastases) is diagnosed at the age of 6 to 17 years. Children have marfanoid habitus and mucosal neuromas (mucous cheeks and lips), which allowed identification of MEN 2B syndromes. Patients with MEN 2B typically have a point mutation in the intracellular kinase catalytic domain. All children have the same mutation in exon 16 (M918T), codon 918. The fathers of these children were clinically healthy. Their mothers did not have a mutation, except one of them with MEN 2B. She died from the progression of pheochromocytoma. Probably in 11 children with MEN 2B the mutations aroused «de novo». Familial forms MTC was found in 4 families. Patients with FMTC have incidence only MTC that aroused in relatives over the age of 28 during the management and there was no metastasis. The *RET* mutations in these families were found in codons 620 and 804.

A total of 10 children carriers of *RET* mutations underwent prophylactic thyroid surgery. MTC was found in six of 10 children (60 per cent) aged 2,5 years or more. Of four children 2 had foci C-cell hyperplasia. For two of them thyroid surgery was prophylactic. The clinical course of disease (familial MEN) for the carrier of *RET* mutation in codons 620 и 804 was more favorable. According to our observations, the carrier of *RET* mutation in codon 634 recommended prophylactic thyroid surgery at the age before 5 years, regardless of the calcitonin level. Antenatal diagnosis of the disease with MEN-2A and familial MEN syndromes were carried out.

Conclusion

Genetic testing of *RET* mutations helps the early diagnosis and stratifies of the risk developing MTC. The efficacy and safe surgery treatment of MTC depends of the type of mutation. Diagnosis of *RET* mutations allows genetically dividing the disease in depending of the type of mutation that determines the severity of the course of the disease and the management of patients There was an opportunity to prevent the development of the disease, give a more accurate prognosis of the disease course, informs the timing of thyroidectomy and possibility for patients to plan a family.

Keywords: Multiple endocrine neoplasia type 2, germline *RET* mutations.

CLINICAL AND MORPHOLOGICAL FEATURES OF ENDOMETRIAL CANCER WITH DIFFERENT STRUCTURAL VARIANTS OF METABOLIC SYNDROME

A.Yu. Kishkina[1], L.A. Kolomiets[1,2], N. V. Yunusova[1,2]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Siberian State Medical Univercity, Tomsk, Russia[2]

Over the past decades, endometrial cancer incidence has been significantly increased [1]. The *obesity epidemic* has had a dramatic impact on *endometrial cancer* [2]. In accordance with the international recommendations, there are 3, 4 and 5 components of metabolic syndrome (MS). However, the frequency of occurrence of these components of MS in patients with endometrial cancer has not been studied yet. The goal of the study was to evaluate clinical and morphological features of endometrial cancer: clinical course, depth of invasion, metastatic lymph nodes, morphological variants depending on structural variants of MS. **Material and Methods.** The study included the results of retro-and prospective analysis of clinical data of 238 endometrial cancer patients, who underwent surgical treatment at the clinic of Oncology research Institute of Tomsk NRMC from 2013 to 2017. **Results.** According to the criteria of the IDF, out of the 238 patients with endometrial cancer, 135 (56.7%) had metabolic syndrome. Assessment of the frequency of occurrence of 3, 4 and 5 components of MS were analyzed in 31 patients. It was revealed that 3 components of MS were found in 6 (19.3%) patients, 4 components in 16 (51%) patients and 5 components in 8 (25.8%) patients. **Conclusions.** The characteristics of the course of endometrial cancer on a background of metabolic syndrome were the depth of invasion up to ½ of the myometrium – 65,3%, moderate degree of differentiation is 66,1%.

Keywords: endometrial cancer, obesity, metabolic syndrome.

Relevance

Over the past decades, the incidence of endometrial cancer has been significantly increased [1] The *obesity epidemic* has had a dramatic impact on *endometrial cancer* [2]. Obesity leads to increased estrogen level produced by fat tissue, which is one of the major risk factors of endometrial cancer. Other factors that increase the risk of endometrial cancer, regardless of obesity are diabetes and hypertension. [5]. Obesity is not always unambiguous in its manifestations and consequences, justifying in the first place, the distinction between phenotypes and emphasizing the need for comparative study in cancer patients, including patients with endometrial cancer [4]. In accordance with international recommendations [DF, 2005, ESH-ESC, 2007], there are three -, four -, and five-components of MS. The proportion of patients with three-component variant of MS is much higher than with four and five components [6]. The most common ternary form of MS is a combination of abdominal obesity, hypertension and lower HDL cholesterol. The leading components of MS are abdominal obesity and hypertension. For example, in St. Petersburg, the prevalence of all five components of MS (that is its full form) in the 30-55 year-old individuals with abdominal obesity was small and amounted for 12.3 % [IDF 2005] and 11.2 % [ESH-ESC, 2007]. The prevalence of MS varies in different regions of Russia and it depends not only on racial difference but also on traditions, lifestyle, physical activity, nutritional behavior (consumption of high-calorie, rich in animal fats), education, gender, genetic predisposition, ethnicity and many other factors [7].

Objective of the study: to evaluate clinical and morphological features of endometrial cancer: clinical course, depth of invasion, metastatic lesion of lymph nodes, morphological variants depending on structural variants of MS.

Material and Methods

The study included the results of retro-and prospective analysis of the clinical data of 238 patients with endometrial cancer, who received surgical treatment at the clinic of Oncology research Institute of Tomsk NRMC from 2013 to 2017. Stage of endometrial cancer was determined in accordance with international FIGO classification (2009) and TNM (UICC, 7th revision, 2010). For the detection of MS, we used criteria of the International diabetes Federation (IDF 2005). Among endometrial cancer patients, 135 (56.7%) patients were diagnosed with MS.

Results

According to the criteria of the International diabetes Federation (IDF 2005), out of the 238 patients with endometrial cancer, there were 135 (56.7%) with metabolic syndrome. Stage I was observed in 127 (94%) patients, stage II in 4 (2.9%) patients and stage III in 4 patients (2.9%). All patients were diagnosed with endometrioid adenocarcinoma. When assessing the depth of invasion, it was found that the tumor within the mucosa was observed in 18 (14.1%) patients, invasion depth of less than ½ of the myometrium in 83(65.3%) patients, and invasion depth of more than ½ of the myometrium in 26 (20.4%) patients. High-grade tumor was found in 21 (16.5%) patients, intermediate grade tumor in 84 (66.1%) patients, and low-grade tumor was observed in 22 (17.3%) patients. In the assessment of reproductive function, it was noted that 108 (85%) patients were in menopause, and 19 (14.9%) patients were of reproductive age. The patients' mean age was 54.3 years. Regarding postoperative complications, there were: pelvic hematoma, subacute ischemic stroke in posterior cerebral artery on the left and thrombophlebitis of the GSV (popliteal segment) and tibial veins on the right. The frequency of 3- 4 - and 5-component MS was analyzed in 31 patients. It was revealed that the 3-component MS was found in 6 (19.3%) patients, 4-component MS in 16 (51%) patients and 5 component MS in 8 (25.8%) patients. The 4-component MS was the most common. The most common 4-component MS combinations were as follows: abdominal obesity, elevated blood pressure, reduced HDL cholesterol and elevated glucose level (31.2%) followed by abdominal obesity, elevated blood pressure, reduced HDL cholesterol and elevated triglycerides (31.2%). The most common 3-component MS combinations were: abdominal obesity, elevated blood pressure level with a violation of carbohydrate and lipid metabolism (50%)

Conclusions

The proportion of endometrial cancer with metabolic syndrome were the depth of invasion of less than ½ of the myometrium (65.3%) and intermediate grade tumor (66.1%). The 4-component MS was the most common combination of MS.

REFERENCES

1. *Sheikh M.A., Althouse A.D., Freese K.E., Soisson S., Edwards R.P., Welburn S., Sukumvanich P., Comerci J., Kelley J., LaPorte R.E., Linkov F.* USA endometrial cancer projections to 2030: should we be concerned? Future Oncol. 2014 Dec; 10(16): 2561-8. doi: 10.2217/fon.14.192.

2. *Fader A.N., Arriba L.N., Frasure H.E., von Gruenigen V.E.* Endometrial cancer and obesity: epidemiology, biomarkers, prevention and survivorship. Gynecol Oncol. 2009 Jul; 114(1): 121-7. doi: 10.1016/j.ygyno.2009.03.039.

3. *Nevadunsky N.S., Van Arsdale A., Strickler H.D., Moadel A., Kaur G., Levitt J., Girda E., Goldfinger M., Goldberg G.L., Einstein M.H.* Obesity and age at diagnosis of endometrial cancer. Obstet Gynecol. 2014 Aug; 124(2 Pt 1): 300-6. doi: 10.1097/AOG.0000000000000381.

4. *Berstein L.M., Poroshina T.E., Turkevich E.A., Vasilyev D.A., Baltrukova A.N., Kovalenko I.M., Berlev I.V.* Features of endometrial cancer in patients with 'metabolically healthy' versus 'standard' obesity: the decreasing frequency of metabolically healthy obesity. Future Sci OA. 2015 Nov 1; 1(4): FSO68. doi: 10.4155/fso.15.68.

5. *IDF* Clinical Guidelines Task Force. Global guideline for type 2 diabetes. International Diabetes Federation, Brussels. 2005; 79.

6. *Simonova G.I., Mustafina S.V., Pechenkina E.A., Rymar O.D., Nikitin Yu.P.* Epidemiologic background for the control of carbohydrate metabolic imbalance. Aktual'nye voprosy boleznei serdtsa i sosudov. 2009; 2: 18-23. [in Russian]

7. *Capewell S., Ford E.S., Croft J.B., Critchley J.A., Greenlund K.J., Labarthe D.R.* Ciovascular risk factor trends and potential for reducing coronary heart disease mortality in the United States of America. Bull World Health Organ. 2010 Feb; 88(2): 120-30. doi: 10.2471/BLT.08.057885.

PARAFFIN EMBEDDED CANCER TISSUE 2D TERAHERTZ IMAGING AND MACHINE LEARNING ANALYSIS

Yu.V. Kistenev[1,2], A.V. Borisov[1,2], A.I. Knyazkova[1,2], O.A. Zakharova[1], E.A. Sandykova[1,2], L.V.Spirina[2,3], A.K.Gorbunov[3]

Tomsk State University, Tomsk, Russia[1]
Siberian State Medical University, Tomsk, Russia[2]
Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[3]

Absorption spectra of paraffin-embedded prostate and adenocarcinoma cancer tissues and healthy tissues have been measured in the 0.3-3 THz range. The Principal Component Analysis was applied to separate informative features in measured THz spectra. The Support Vector Machine (SVM) classifier was created which allows to distinguish the tumor tissues from healthy tissues, including classification of prostate cancer tissue stage according to the Gleason scale.

Keywords: THz spectroscopy, paraffin-embedded samples, adenocarcinoma-affected tissues, principal component analysis, support vector machine.

Introduction

Formalin-fixed paraffin-embedded (FFPE) tissue samples are widely used worldwide in diagnosis and research. The protocol for FFPE tissue samples sectioning has been developed to prevent cross-contamination and distributed between participating centers [1]. An international panel of pathologists developed a consensus histopathology evaluation form, as a result, the histopathology evaluation of the cases assured the presence of the targeted tissue, identified the presence of other tissues that could disturb the molecular diagnosis and allowed the assessment of tissue quality. Thus, the protocol allows processing correctly FFPE tissue samples for study.

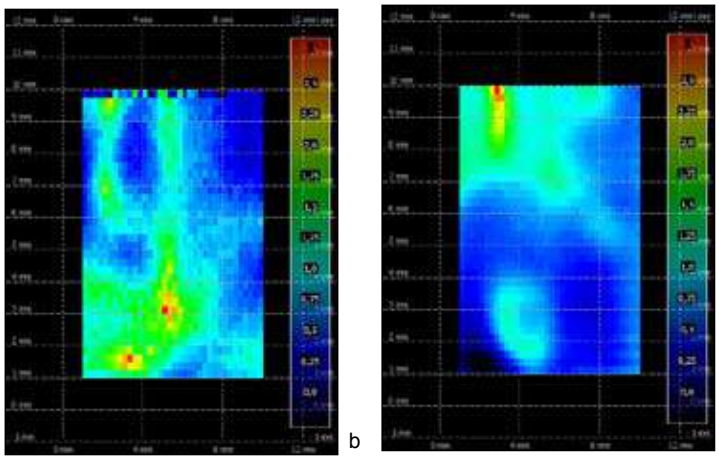
The cancer diagnostics is known to be one of the main problems in medicine. A challenge in the cancer diagnosis and surgery is operative tumor localization. The investigation of paraffin tissue blocks could provide useful information to determine the position and extent of the embedded tissue prior to the histopathology sectioning [2].

Difference in THz absorbance between malignant tumor and normal tissues can be useful for instrumental diagnosis. Recently, the THz imaging has been used to identify skin tumors [3], breast cancer [4], colon cancer [5], malignant tumor in liver [6], in brain [7]. To realize potential of THz imaging, efficient methods of automatic image content analysis should be created.

The results of application of Machine Learning approach in paraffin embedded cancer tissue terahertz 2d imaging analysis will be discussed in the report.

Results

Absorption spectra of paraffin blocks were measured using of Time-domain THz spectrometer (EKSPLA, Estonia) with tuning range 0.3-3 THz. A spatial 2D absorption spectra scanning with averaging over scans at every spatial point (1024 scans) was carried out. The spatial step of scanning was varied from 0.1 mm to 1.0 mm. The examples of THz images are presented in the Figure 1. In total, 100 scans for cancer and healthy tissues were analyzed.



a b

Figure 1. 2D image of prostate cancer metastasis in lymph node (a) and healthy node (b), 0.4 THz

The results of SVM classification of lymph node metastasis and healthy node THz images are presented in the Table 1.

Table 1

SVM classification of lymph node metastasis and healthy node THz images

Binary classification	Sensitivity		Specificity	
	Mean	Standard deviation	Mean	Standard deviation
Cancer tissue / Healthy tissue	0.987	0.0002	0.983	0.0002

The work was carried out under partial financial support of the Russian Fund of the Fundamental Research (grant № 17-00-00186 and № 18-42-703012).

REFERENCES

1. *Mena M., Lloveras B., Tous S., Bogers J., Maffini F., Gangane N., Kumar R.V., Somanathan T., Lucas E., Anantharaman D., Gheit T., Castellsagué X., Pawlita M., de Sanjosé S., Alemany L., Tommasino M.; HPV-AHEAD study group*. Development and validation of a protocol for optimizing the use of paraffin blocks in molecular epidemiological studies: The example from the HPV-AHEAD study. PLoS One. 2017 Oct 16; 12(10): e0184520. doi: 10.1371/journal.pone.0184520.

2. *Bowman T., Wu Y., Gauch J., Campbell L.K., El-Shenawee M.* Terahertz imaging of three-dimensional dehydrated breast cancer tumors. J Infrared Millim Terahertz Waves. 2017; 38(6): 766-86. doi: 10.1007/s10762-017-0377-y.
3. *Joseph C.S., Yaroslavsky A.N., Neel V.A., Goyette T.M., Giles R.H.* Continuous wave terahertz transmission imaging of non-melanoma skin cancers. Lasers Surg Med. 2011 Aug; 43(6): 457-62. doi: 10.1002/lsm.21078.
4. *Chen H., Lee W.J., Huang H.Y., Chiu C.M., Tsai Y.F., Tseng T.F., Lu J.T., Lai W.L., Sun C.K.* Performance of THz fiber-scanning near-field microscopy to diagnose breast tumors. Opt Express. 2011 Sep 26; 19(20): 19523-31. doi: 10.1364/OE.19.019523.
5. *Reid C.B., Fitzgerald A., Reese G., Goldin R., Tekkis P., O'Kelly P.S., Pickwell-MacPherson E., Gibson A.P., Wallace V.P.* Terahertz pulsed imaging of freshly excised human colonic tissues. Phys Med Biol. 2011 Jul 21; 56(14): 4333-53. doi: 10.1088/0031-9155/56/14/008.
6. *Miura Y., Kamataki A., Uzuki M., Sasaki T., Nishizawa J., Sawai T.* Terahertz-wave spectroscopy for precise histopathological imaging of tumor and non-tumor lesions in paraffin sections. Tohoku J Exp Med. 2011; 223(4): 291-6. doi: 10.1620/tjem.223.291.
7. *Oh S.J., Kim S.H., Ji Y.B., Jeong K., Park Y., Yang J., Park D.W., Noh S.K., Kang S.G., Huh Y.M., Son J.H., Suh J.S.* Study of freshly excised brain tissues using terahertz imaging. Biomed Opt Express. 2014 Jul 29; 5(8): 2837-42. doi: 10.1364/BOE.5.002837.

CELLULAR MOTILITY PROTEINS AS NEW MOLECULAR MARKERS FOR EARLY DIAGNOSIS OF CANCER

I.V. Kondakova, N.V. Yunusova, L.V. Spirina, G.V. Kakurina, O.V. Cheremisina, L.A. Kolomiets, E.L. Choinzonov

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia

In the process of neoplastic transformation epithelial cells acquire the property of motility. In the present work, the contents of actin-binding proteins and calpain activity in malignant tumors and precancer tissues were studied for estimation of the involvement of locomototor proteins in the process of carcinogenesis. The contents of actin-binding proteins were evaluated by flow cytofluorimetry, Western blotting and by the ELISA. In patients with dysplastic changes in the epithelium of the larynx, the content of CAP1 was decreased compared to the group of patients with laryngeal cancer, stage I. An increase in arp3, gelsolin, cofilin-1, thymosin-β4 in endometrial cancer was shown in comparison to hyperplasia. Single-factor logistic regression analysis showed that the activity of calpaines in hyperplastic tissue and the levels of thymosinβ-4and cofilin can be an independent predictor of the development of cancer.

Keywords: cancer, precancer, cellular motility, actin-binding proteins, calpains.

Introduction

Neoplastic transformation and following cancer progression are associated with the basic cancer properties as disorders of the cell locomotion. The acquirement of the malignant phenotype leads to changes in cell cytoskeleton, that are important for epithelial cancer cell proliferation, migration and epithelial-mesenchymal transition [1, 2]. The remodeling of actin cytoskeleton plays a central role in generating force to drive cell locomotion, which is regulated by a plethora of actin binding proteins (ABPs). These ABPs perform the different functions: bind actin monomers (thymosin β-4); depolymerize and sever filaments (cofilin-1, gelsolin) and facilitate the formation of filament bundles (Arp2/3) [3]. Recently, a new ABP - cyclase-associated protein (CAP 1) has been discovered, which cuts actin filaments [4]. Cancer cell movement is linked to calcium-dependent intracellular nonlysosomal cysteine proteases calpains which affect cancer cell motility through many pathways, such as the Wnt- and the NF-κB- signaling pathways [8, 9]. ABPs and calpains play an important role in pathogenesis of cancer [5, 6, 7, 10]. **The aim of the study** was to compare the content of ABPs and calpain activity in cancerous and precancerous tissues.

Material and Methods

The material for the study was the samples of cancerous and precancerous tissues. The study included 32 primary patients with histologically verified cancer of the larynx and laryngopharynx (T1-4N0-3M0), 12 patients with chronic inflammatory laryngeal and laryngopharyngeal gadiseases associated with dysplastic changes in the mucous membrane (DI-III). Also the study included 31 postmenopausal patients with stage I-II endometrioid EC and 40 patients with typical and atypical endometrial hyperplasia.

Tissue homogenates of cancerous and precancerous tissues of the larynx and laryngopharynx were analyzed using the Human Adenylyl cyclase-associated protein ELISA Kit (Cusabio) on an Anthos Reader 2020 (Biochrom) microplate reader. The content of Arp3, gelsolin, cofillin and thymosin β-4 levels was determined by Flow cytometry and Western blotting. The total calpain

activity was estimated by fluorimetric method. Statistical analysis was performed using Statistica 10.0 software.

Results

Analysis of the CAP1 content in tissue samples showed that the amount of protein in laryngeal epithelium of patients with dysplastic changes (DI-III) was significantly higher compared the cancer tissues with stage T1N0M0 (41.00 (25.00; 76.00) v.s. 25.00 (25.00; 25.00), $p < 0.05$).

We have revealed the increased levels of cofilin-1, thymosin β-4 and decreased expression of Arp3 in cancer tissues in comparison to endometrial hyperplasia.

The results on the total activity of calpains in hyperplastic and malignant tissues of endometrium are presented in Table 2. The activity of calpains in the ER tissue was 7.4 times higher than for endometrial hyperplasia ($p = 0.000$). Also in laryngeal cancer tissue, the activity of calpaines in tumor tissue was higher than that observed in dysplastic tissues (Table 2). Single-factor logistic regression analysis showed that the levels of thymosinβ-4, cofilin and total calpain activity were independent cancer risk factors in patients with endometrial hyperplasia.

Table 2

Total activity of calpaines in hyperplastic and malignant tissues of the endometrium.

Samples	N	Me (Q1;Q3)	P (U-test)
Endometrial hyperplasia	12	23 (10,4;28,8)	
Endometrium carcinoma	10	171,5 (77,5-333,0)	P_1=0,0000
Dysplasia of larynx and laryngopharynx	11	28,6 (13,5-39,4)	
Cancer of larynx and laryngopharynx	30	125,7 (67,0-256,0)	P_2=0.0000

Note: P_1 – a significant difference between endometrial hyperplasia and endometrium carcinoma, P_2 - between dysplasia of the larynx and laryngopharynx and cancer of the larynx and laryngopharynx.

Conclusions

The results showed the involvement of actin-binding proteins and calpains in carcinogenesis. Levels of thymosinβ-4, cofilin and activity of calpaines in hyperplastic tissue can be an independent predictor of the development of cancer.

This work was supported by the Russian Foundation for Basic Research (grant № 17–04–00198 A).

REFERENCES

1. *Kalluri R., Weinberg R.* The basics of epithelial-mesenchymal transition. J Clin Invest. 2009 Jun; 119(6): 1420-8. doi: 10.1172/JCI39104.

2. *Liu P., Wang C., Ma C., Wu Q., Zhang W., Lao G.* MicroRNA-23a regulates epithelial-to-mesenchymal transition in endometrial endometrioid adenocarcinoma by targeting SMAD3. Cancer Cell Int. 2016 Sep 5; 16(1): 67. doi: 10.1186/s12935-016-0342-1.

3. *dos Remedios C.G., Chhabra D., Kekic M., Dedova I.V., Tsubakihara M., Berry D.A., Nosworthy N.J.* Actin binding proteins: regulation of cytoskeletal microfilaments. Physiol Rev. 2003; 83: 433-473.

4. *Kakurina G.V.; Kolegova E.S.; Kondakova I.V.* Adenylyl Cyclase-Associated Protein 1: Structure, Regulation, and Participation in Cellular Processes. Biochemistry (Mosc). 2018 Jan; 83(1): 45-53. doi: 10.1134/S0006297918010066.

5. *Kazazian K., Go C., Wu H., Brashavitskaya O., Xu R., Dennis J.W., Gingras A.C., Swallow C.J.* Plk4 Promotes Cancer Invasion and Metastasis through Arp2/3 Complex Regulation of the Actin Cytoskeleton. Cancer Res. 2017 Jan 15; 77(2): 434-447. doi: 10.1158/0008-5472.CAN-16-2060.

6. *Kakurina G.V., Kondakova I.V., Cheremisina O.V., Shishkin D.A., Choinzonov E.L. Adenylyl Cyclase-Associated Protein 1 in the Development of Head and Neck Squamous Cell Carcinomas.* Bull Exp Biol Med. 2016 Mar; 160(5): 695-7. doi: 10.1007/s10517-016-3252-2.

7. *Kondakova I.V., Yunusova N.V., Spirina L.V., Kolomiets L.A., Villert A.B.* Association between Intracellular Proteinase Activities and the Content of Locomotor Proteins in Tissues of Primary Tumors and Metastases of Ovarian Cancer. Bioorg Khim. 2014 Nov-Dec; 40(6): 735-42.

8. *Wang E., Wang D., Li B., Ma H., Wang C., Guan L., Zhang H., Yi L., Li S.* Capn4 promotes epithelial-mesenchymal transition in human melanoma cells through activation of the Wnt/β-catenin pathway. Oncol Rep. 2017 Jan; 37(1): 379-387. doi: 10.3892/or.2016.5247.

9. *Hoskin V., Szeto A., Ghaffari A., Greer P.A., Côté G.P., Elliott B.E.* Ezrin regulates focal adhesion and invadopodia dynamics by altering calpain activity to promote breast cancer cell invasion. Mol Biol Cell. 2015 Oct 1; 26(19): 3464-79. doi: 10.1091/mbc.E14-12-1584.

10. *Shashova E.E., Kolegova E.S., Zav'yalov A.A., Slonimskaya E.M., Kondakova I.V. Changes in the Activity of Proteasomes and Calpains in Metastases of Human Lung Cancer and Breast Cancer.* Bull Exp Biol Med. 2017 Aug; 163(4): 486-489. doi: 10.1007/s10517-017-3834-7.

BIODISTRIBUTION OF CHLORINE PHOTOSENSITIZER IN TUMOR MICROENVIRONMENT AND MORPHOLOGICAL CHANGES IN HUMAN SKIN CANCER AFTER PHOTODYNAMIC THERAPY

K.S. Korchagina[1], S.V. Gamayunov[1], V.V. Yuzhakov[1], A.D. Kaprin[1], S.A. Ivanov[1], Yu.S. Romanko[1], V.V. Dudenkova[2], E.A. Sergeeva[3], N.M. Shakhova[3], N.K. Fomina[1]

A. Tsyb Medical Radiological Research Center – branch of the National Medical Research Radiological Center of the Ministry of Health of the Russian Federation, Obninsk, Russia[1]
Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia[2]
Institute of Applied Physics RAS, Nizhny Novgorod, Russia[3]

Photodynamic therapy (PDT) has become a well-studied therapy for malignant tumors. At different stages PDT requires imaging to plan, evaluate and monitor the treatment. The contribution of different types modes of imaging in this context is important and continues to increase. Distribution of Photodithazine, as chlorin e6-based photosensitizer (PS), in human skin carcinoma samples was studied ex vivo using laser scanning microscopy (LSM) prior to exposure. Morphological changes in tumors after PDT were evaluated by two independent experts using histological staining study (H&E). The morphological changes and the data from laser scanning microscopy demonstrate that the disorganization of tumour vascular bed is a key event in PDT with Photodithazine, which is probably due to the predominant accumulation of the drug in the tumour stroma.

Keywords: photodynamic therapy, Photodithazine, photosensitizer biodistribution, human skin carcinoma, laser scanning microscopy, pathomorphosis.

Introduction

PDT has become a well-studied therapy for cancer. PS, proper laser irradiation, and oxygen are essential components for effective PDT in clinical cancer treatment [1]. At different stages PDT requires visualization to plan, evaluate and monitor treatment. The contribution of different types of imaging in this context is important and continues to increase [2].

The aim of the research was to study morphological changes after PDT in the human skin tumor and to show the possibility of LSM to observe the biodistribution of chlorin e6-based photosensitizer.

Material and Methods

The protocol of the study was approved by the local Ethics Committee of the Nizhny Novgorod State Medical Academy. The study involved 32 patients. PDT was performed with a morphologically verified basal cell carcinoma T1-2N0M0 with the following laser radiation parameters: wavelength 662 nm, power density of 0.30 W/cm^2, energy density of 150-250 J/cm^2. As a photosensitizer, we used Photodithazine in a dose of 1.0 mg/kg. To study the development of morphological changes, biopsy was performed at definite time intervals: 2 hours after PS introduction, immediately after PDT, 2 hours after PDT, 1, 4, 6, 12 days after PDT. Study of PS distribution in human skin carcinoma samples was conducted using 510 META laser scanning microscope ex vivo prior to laser exposure. Morphological changes in tumours after PDT were evaluated by two independent experts using histological staining study (H&E).

Results

LSM imaging of samples prior to laser exposure showed that PS concentration in the perivascular space and extracellular matrix of the tumor microenvironment are higher than the concentration in the tumor cells (Figure 1, *a-c*). Fluorescence of PS was proved by spectral analysis. Immediately after PDT and after 2 hours no signs of pathomorphosis were found while there was a slight oedema of tissues. In the early period after PDT (1-2 day. Figure 1. *e, f*) the main morphological changes were haemorrhages, plethora of blood vessels, thrombi formation, stasis and the sludge phenomenon. In the late period (4-6 days), necrotic changes of tumour prevailed, and repair processes were observed by the 12th day (Figure 1, *g*).

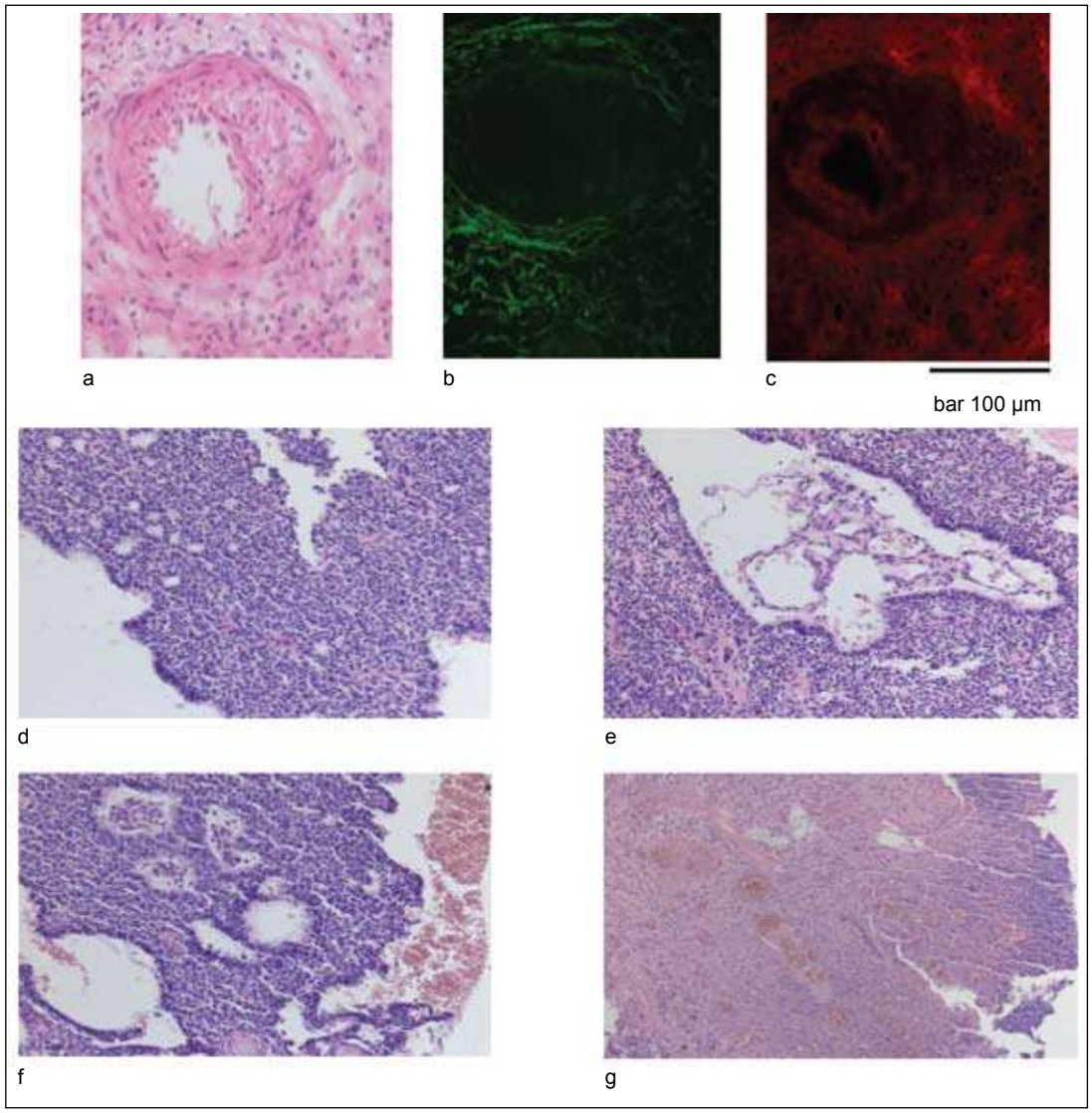


Figure 1. Images of postoperative artery sample, 120 minutes after the intravenous injection of Photodithazine (*a-c*). Images of biopsy samples of skin basal cell carcinoma before PDT (*d),* 1 day after PDT (*e*), 2 day after PDT (*f*), and 12 day after PDT (*g*); *a, d-g* - H&E stained sample; *b*, *c* - fluorescence image, non-stained sample, photo done with LSM; *b* - excitation - 800 nm, detection - 400 nm (detection of collagen); *c* - excitation – 632 nm, detection – 655-710 nm (fluorescence of PS)

Conclusion

The morphological changes and the data of laser scanning microscopy demonstrate that the disorganization of tumour vascular bed is a key event in PDT with Photodithazine, which is probably due to the predominant accumulation of the drug in the tumor stroma. This indicates that targeting the vasculature of tumors is effective in enhancing the therapeutic efficacy of PDT [3].

The authors thank the Ministry of Education and Science of the Russian Federation (Project RFmEFI60414X0027, Contract № 14. B25.31.0015) and the Russian Foundation for Basic Research (grant №16-02-00974) for the financial support.

REFERENCES

1. *Yuzhakov V.V., Romanko Yu.S., Kaplan M.A., Galkin V.N., Majouga A.G., Grin M.A., Burmistrova N.V., Fomina N.K., Bandurko L.N., Sevankaeva L.E., Yakovleva N.D., Ingel I.E., Mozerov S.A., Starovoytova A.V.* Effect of photodynamic therapy with the bacteriochlorophyll a derivative on growth and functional morphology of rat sarcoma M-1. Almanac of Clinical Medicine. 2017 June; 45(4): 333–47. doi: 10.18786/2072-0505-2017-45-4-333-347 [in Russian].

2. *Kharroubi Lakouas D., Huglo D., Mordon S., Vermandel M.* Nuclear medicine for photodynamic therapy in cancer: Planning, monitoring and nuclear PDT. Photodiagnosis Photodyn Ther. 2017 Jun; 18: 236-243. doi: 10.1016/j.pdpdt.2017.03.002.

3. *Thomas E., Colombeau L., Gries M., Peterlini T., Mathieu C., Thomas N., Boura C., Frochot C., Vanderesse R., Lux F., Barberi-Heyob M., Tillement O.* Ultrasmall AGuIX theranostic nanoparticles for vascular-targeted interstitial photodynamic therapy of glioblastoma. Int J Nanomedicine. 2017 Sep 26; 12: 7075-7088. doi: 10.2147/IJN.S141559.

STUDY OF INHIBITORS OF GLYCOLYSIS IODOACETATE AND 2-DEOXYGLUCOSE AS ANTI-TUMOR AGENTS IN THE LEWIS LUNG CARCINOMA MODEL

D.A. Korshunov[1], I.A. Klimov[2], V.V. Ivanov[2], I.V. Kondakova[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Siberian State Medical University, Tomsk, Russia[2]

At present, the research of the metabolism of malignant tumors has led to the discovery of new targets of anti-tumor chemotherapy, which is based on blocking the energy production of the tumor cell. Inhibitors of glycolysis iodoacetate and 2-deoxyglucose have been studied as anti-tumor agents in the Lewis lung carcinoma model. Iodoacetate showed the effectiveness of anti-tumor and anti-metastatic action, however a high systemic toxicity was revealed for it. The 2-deoxyglucose inhibitor showed only an anti-tumor effect.

Keywords: warburg effect, glycolysis inhibitors, tumor.

One of the most important metabolic changes in malignant tumor cells is the formation of the Warburg effect that can be considered as one of the key moments of anti-tumor therapy [1, 2].

Advances in understanding the complex cellular and molecular mechanisms associated with the Warburg effect have become the basis for the creation of new selective and specific agents, glycolysis inhibitors, some of which have reached clinical trials [3]. Despite the successful preclinical trials, many new anti-tumor agents related to glycolysis inhibitors have failed in phase I or II clinical trials, demonstrating systemic toxicity [4]. This situation requires the search for selective anti-glycolytic agents with low toxicity, or the use of methods that reduce side effects of toxicity.

Two-deoxyglucose is one of the glycolysis inhibitors, which have moderate toxicity relative to other similar inhibitors. This analog of glucose is one of the most actively investigated inhibitors of glycolysis [5]. However, due to the fact that 2-deoxyglucose is a competitive inhibitor of glucose present in the blood at high concentrations, there are serious concerns about the existence of a sufficient therapeutic window for this compound [6].

Iodoacetate, which is practically unexplored glycolysis inhibitor, lacks the disadvantages of 2-deoxyglucose, but has a more pronounced systemic toxicity. Iodoacetate is a non-selective inhibitor and acts on key enzymes of glycolysis and pentose phosphate pathway [1, 5]. In this regard, new selective anti-glycolytic agents with low toxicity or methods that reduce their side effects of toxicity.

The purpose of this work was to evaluate the anti-tumor properties of inhibitors of carbohydrate metabolism of iodoacetate and 2-deoxyglucose as anti-tumor agents.

Material and Methods

The experiment was performed on mice of the line C57BL/6j (males) weighing 25-30 g, obtained from the nursery of the Research Institute of Pharmacology (TNRMC). The studies were conducted in compliance with the rules of laboratory practice in preclinical studies.

To transplant Lewis lung carcinoma (LLC), all animals were inoculated into the thigh muscle of LLC tumor cells. The hip volume was determined from the 3-day caliper and calculated from the formula $(x \cdot y \cdot z) \cdot \pi/6$, where x, y and z are the largest diameter of the tumor in three planes.

For visualization, photographs of the macro preparation were made in transmitted light using a Canon EOS 6D SLR camera.

In the study, the anti-tumor effect of glycolysis inhibitors used iodoacetate (IA, Sigma-Aldrich), 2-deoxyglucose (2-DG, Molekula). Methotrexate (MTX, Ebéve Pharma) was chosen as the reference drug. The drugs were administered intraperitoneally at MTD. The treatment of the animals started from the sixth day from the moment of inoculation of the tumor cells.

Tumor growth inhibition (TGI) and the level of inhibition of metastases (IM) in the lungs were calculated according to standards of the cancer research [7].

Statistical processing of data was carried out using a Mann-Whitney test.

Results

The efficacy of TGI in monoiodoacetate and 2-deoxyglucose averaged 15% and was comparable to that of methotrexate. The greatest therapeutic effect was recorded at the time of drug administration (Figure 1).

By the 21st day, the antitumor effect on the primary tumor was almost completely leveled in all the treated groups.

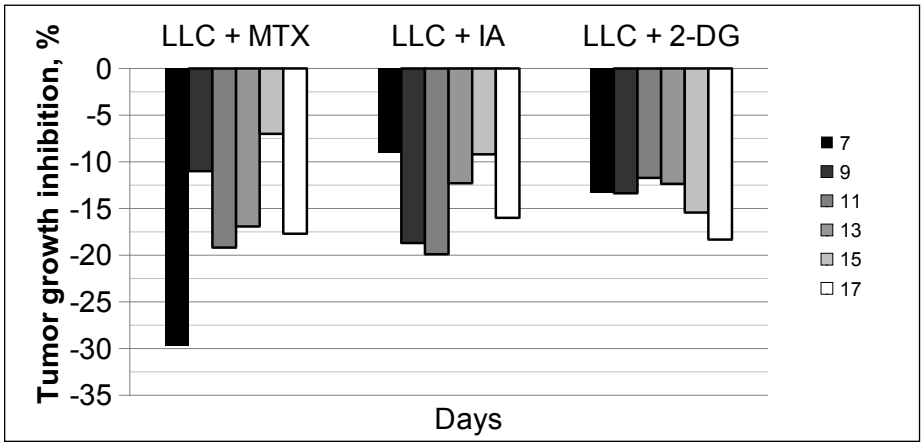


Figure 1. The effects of methotrexate (MTX), iodoacetate (IA) and 2-deoxyglucose (2-DG) on TGI of primary tumor of LLC. No difference in TGI between the drugs was found

The antimetastatic activity of the inhibitors varied. The IM level in the lungs with IA and MTX was stronger than that of the drugs on the primary tumor. The average for both treatment groups was 65%. 2-DG showed antimetastatic activity with a slight boost (Figures 2).

Figure 2. Right lung of mice with LLC: a - control, b - methotrexate, c - iodoacetate, d - 2-deoxyglucose

Perhaps this is due to the mechanism of cell death. The death of a cell under the action of IA occurs by necrosis, and in the case of 2-DG by apoptosis. Probably, under the action of 2-DG, signaling pathways are formed that stimulate resistant to apoptosis tumor cells to metastatic activity.

Conclusion

The glycolysis inhibitor IA showed an efficacy comparable to the reference preparation. 2-DG with MTD had an inhibitory effect on the primary tumor at LLC, but in respect of metastasis its effect was promoter. Therefore, it is worthwhile to carefully consider the therapeutic use of 2-DG for metastatic types of tumors and possible adverse effects in vivo, including malignant neoplasms in humans.

REFERENCES

1. *Korshunov D.A., Kondakova I.V.* Effect of anti-glycolytic agents on tumour cells in vitro. AIP Conference Proceedings. 2016; 1760(1). doi: 10.1063/1.4960254.

2. *Korshunov D.A., Korshunova Z.V., Kondakova I.V.* Inhibitors of carbohydrate metabolism in cancer therapy. Molekulyarnaya medicina. 2016; 14(1): 3-7.

3. *Yu L., Chen X., Wang L., Chen S.* The sweet trap in tumors: aerobic glycolysis and potential targets for therapy. Oncotarget. 2016 Jun; 7(25): 38908-26. doi: 10.18632/oncotarget.7676

4. *Cerella C., Dicato M., Diederich M.* Modulatory roles of glycolytic enzymes in cell death. Biochem. Biochem Pharmacol. 2014 Nov 1; 92(1): 22-30. doi: 10.1016/j.bcp.2014.07.005

5. *Granchi C., Minutolo F.* Anticancer agents that counteract tumor glycolysis. ChemMedChem. 2012 Aug; 7(8): 1318-50. doi: 10.1002/cmdc.201200176.

6. *Porporato P.E., Dhup S., Dadhich R.K., Copetti T., Sonveaux P.* Anticancer targets in the glycolytic metabolism of tumors: a comprehensive review. Front Pharmacol. 2011 Aug 25; 2: 49. doi: 10.3389/fphar.2011.00049.

7. *Mironov A.N.* Rukovodstvo po provedeniyu doklinicheskih issledovanij lekarstvennyh sredstv. Moskow: Grif i K; 2012. 944.

SOMATIC MUTATIONS IN CAROTID BODY TUMORS

A.V. Kudryavtseva[1], E.N. Lukyanova[1], D.V. Kalinin[2], G.S. Krasnov[1], A.V. Pokrovsky[2], A.L. Golovyuk[2], M.S. Fedorova[1], E.A. Pudova[1], V.S. Pavlov[1], A.A. Kobelyatskaya[1], M.V. Kiseleva[3], A.A. Dmitriev[1], A.V. Snezhkina[1]

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia[1]
Vishnevsky Institute of Surgery, Ministry of Health of the Russian Federation, Moscow, Russia[2]
National Medical Research Radiological Center, Ministry of Health of the Russian Federation, Moscow, Russia[3]

Carotid body tumors (CBTs) are the most frequent paragangliomas of the head and neck that arise in the bifurcation of the carotid artery. Paragangliomas are associated with germline and somatic mutations in at least 30 genes. However, the particular mechanisms of their initiation and progression remain unclear in many cases. Exome sequencing of six CBT samples with paired blood and lymph node tissues was performed in the study. Germline and somatic mutations were identified. Mutation load (ML) was estimated to be 0.07 to 0.47 per 1 Mb. CBT sample with the highest ML (0.47/Mb) did not contain germline mutations in known paraganglioma-causing genes. This sample carried twenty somatic mutations; eight of them were predicted as a potentially pathogenic and probably involved in the CBTs progression.

Keywords: carotid body tumor, mutations, mutational load, exome, high-throughput sequencing.

Carotid body tumors (CBTs) are rare neuroendocrine neoplasms that arise from carotid glomus at the common carotid artery bifurcation. These are the most frequent paragangliomas of the head and neck. According to the latest WHO classification published in 2017, paragangliomas belong to tumors with variable metastatic potential. Surgical resection is the main treatment for CBTs; however, it is associated with a high risk of postoperative neurovascular complications as these tumors are hypervascular masses with often involvement of cranial nerves.

The onset of paragangliomas is associated with germline or somatic mutations in at least 30 genes [1]. Up to 40 % of all paragangliomas are caused by germline mutations that affect reproductive fitness [24]. However, molecular mechanisms underlying development of these tumors have not been fully understood. Additionally, molecular gene expression study of CBTs is challenging as paired normal tissues are unavailable as well as due to rarity of the disease.

In this study, we performed exome sequencing of six CBTs with paired peripheral blood and lymph node tissues. Germline and somatic mutations were identified and analyzed, total mutational load was estimated.

Materials and methods

Formalin-fixed paraffin-embedded (FFPE) tumor and lymph node tissues as well as peripheral blood from six patients with CBTs were collected at Vishnevsky Institute of Surgery (Moscow) for exome sequencing. DNA was extracted from blood cells using a Nucleic Acid Isolation Kit I (Roche, Switzerland) on a MagNA Pure Compact Instrument (Roche); DNA from tumor and lymph node tissues was isolated with High Pure FFPET DNA Isolation Kit (Roche). Exome library preparation was performed with TruSeq Exome Library Prep Kit (Illumina, USA) according to manufacturer's instructions. The exome sequencing was performed on a NextSeq 500 System (Illumina) with paired-end reads (76×2 bp) for tumor and lymph node tissues and 151 bp for blood at average coverage of 300. The analysis of data was carried out as described earlier [5].

Results

We collected and sequenced a set of CBTs with paired lymph node tissues and peripheral blood. Mutations that were found either in the blood or lymph node (in any patients) were excluded. We also omitted X and Y chromosomes, as well as regions that were covered by less than 10 reads in any samples. Additionally, the data were filtered using 1000 Genomes Project, ESP 6500, and ExAC databases; and mutations with more than 1% frequency were excluded from the analysis. The ML was estimated as the number of somatic mutations per megabase of the target regions (predominantly, transcribed regions from TruSeq Exome Library Prep Kit). The ML values for six CBT samples were ranged from 0.07 to 0.47.

Among 30 somatic mutations identified, twenty mutations were found in CBT sample with the highest ML. Eight somatic mutations in *PRDM2, ASPM, OSBPL11, PCOLCE2, ZFAND5, NEK3,*

DYNC1H1, and *TP53* genes were predicted as potentially pathogenic according to several predicted algorithms (SIFT, PolyPhen2, MutationTaster, or LRT) (Table 1). It should be noted that in this sample, we did not find any germline mutations in known genes associated with paragangliomas (*VHL, SDHA, SDHB, SDHC, SDHD, NF1, RET, HRAS, KRAS, EPAS1 (HIF2A), ATRX, CSDE1, BRAF, FGFR1, FGFR2, FGFR3, FGFR4, FGFRL1, SETD2, ARNT, TP53, TP53BP1, TP53BP2, TP53I13, KMT2D, BAP1, IDH1, IDH2, SDHAF1, SDHAP2, FH, EGLN1, MDH2, TMEM127, MAX, KIF1B, MEN1, GDNF, GNAS, CDKN2A, BRCA1*, and *BRCA2*).

Table 1

Potentially pathogenic somatic mutations in the sample with the highest ML.

Gene	dbSNP ID	GenBank	Coordinate	Nucleotide change	Amino acid change	Genotype
PRDM2	rs756985448	NM_012231.4	chr1: 14,106,977	c.2687A>G	p.Tyr896Cys	Het
ASPM	-	NM_018136.4	chr1: 197,097,759	c.2797T>A	p.Phe933Ile	Het
OSBPL11	-	NM_022776.4	chr3: 125,2863,04	c.802T>G	p.Leu268Val	Het
PCOLCE2	-	NM_013363.3	chr3: 142,542,410	c.913G>A	p.Gly305Arg	Het
ZFAND5	-	NM_001102420.2	chr9: 74,970,993	c.518T>G	p.Leu173Trp	Het
NEK3	-	NM_002498.2	chr13: 52,722,548	c.603+2T>G	-	Het
DYNC1H1	-	NM_001376.4	chr14: 102,469,248	c.4829A>G	p.Lys1610Arg	Het
TP53	rs587781525	NM_000546.5	chr17: 7,577,096	c.842A>T	p.Asp281Val	Het

Six of eight potentially pathogenic somatic variants in *ASPM, OSBPL11, PCOLCE2, ZFAND5, NEK3*, and *DYNC1H1* genes have been previously described neither in databases nor in literature. The mutation NM_012231.4: c.2687A>G, p.Tyr896Cys (chr1: 14,106,977, rs756985448) in *PRDM2* was annotated in dbSNP with uncertain clinical effect. According to gnomAD database it is more likely non-pathogenic variant. The mutation NM_000546.5: c.842A>T, p.Asp281Val (chr17: 7,577,096, rs587781525) in *TP53* gene was described in dbSNP both as a germline and somatic mutation, and has a pathogenic clinical significance according to the ClinVar database.

Conclusion

Our results report several potentially pathogenic somatic mutations that can be involved in CBT pathogenesis.

This study was financially supported by the Russian Foundation for Basic Research, grant 16-04-01521a (tissue sequencing) and ICGEB project CRP/RUS15-01 (blood sequencing).

REFERENCES

1. *Zhikrivetskaya S.O., Snezhkina A.V., Zaretsky A.R., Alekseev B.Y., Pokrovsky A.V., Golovyuk A.L., Melnikova N.V., Stepanov O.A., Kalinin D.V., Moskalev A.A., Krasnov G.S., Dmitriev A.A., Kudryavtseva A.V.* Molecular markers of paragangliomas/pheochromocytomas. Oncotarget. 2017 Apr 11; 8(15): 2575625782. doi: 10.18632/oncotarget.15201.

2. *Kiseleva M.V., Abakushina E.V., Tsyb A.F.* Reproductive health of cancer patients: the state of the problem. Journal of N.N. Blokhin Russian Cancer Research Center RAMS. 2009; 20(2): 66. [in Russian]

3. *Kiseleva M.V., Karpeikina M.M., Komarova E.V., Malinova I.V., Denisov M.S., Chudakov K.V.* The ability to restore ovarian function in cancer patients. Journal of N. N. Blokhin Russian Cancer Research Center RAMS. 2013; 24(34): 4345. [in Russian]

4. *Kiseleva M.V., Komarova E.V., Malinova I.V., Karpeikina M.M., Denisov M.S.* Recovery of fertility in cancer patients by the method of retransplantation of vitrified ovarian tissue. Reproductive medicine. Scientific and practical journal. 2013b; 16(34): 3335. [in Russian]

5. *Snezhkina A.V., Lukyanova E.N., Kalinin D.V., Pokrovsky A.V., Dmitriev A.A., Koroban N.V., Pudova E.A., Fedorova M.S., Volchenko N.N., Stepanov O.A., Zhevelyuk E.A., Kharitonov S.L., Lipatova A.V., Abramov I.S., Golovyuk A.V., Yegorov Y.E., Vishnyakova K.S., Moskalev A.A., Krasnov G.S., Melnikova N.V., Shcherbo D.S., Kiseleva M.V., Kaprin A.D., Alekseev B.Y., Zaretsky A.R., Kudryavtseva A.V.* Exome analysis of carotid body tumor. BMC medical genomics. 2018 Feb 13; 11(Suppl 1): 17. doi: 10.1186/s12920-018-0327-0.

RECONSTRUCTIVE SURGERY FOR ORAL CAVITY CANCER

D.E. Kulbakin[1,2,4], E.L. Choinzonov[1,3,4], M.R. Mukhamedov[1,3], E.U. Garbukov[1], R.V. Vasilev[1,4], A.A. Shiyanova[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University, Tomsk, Russia[2]
Siberian State Medical University, Tomsk, Russia[3]
Tomsk State University of Control Systems and Radioelectronics, Tomsk, Russia[4]

Introduction. Treatment of patients with advanced oral cavity cancer remains challenging. **Material and Methods.** We analyzed 127 reconstructive operations performed in patients with oral cancer. We used free (90 cases - 71%) and pedicle (37 cases - 29%) flaps for the reconstruction**. Results.** Good functional results were achieved in most cases (85%). Adequate mobility of the tongue was restored by using skin-fascial flaps. In cases with maxillofacial reconstruction, the mandibular and maxillary continuity, natural facial contour and mouth opening were restored using bone flaps. **Conclusions.** To achieve good functional and cosmetic results, as well as to reduce postoperative complications of reconstructive surgery in patients with oral cavity tumors, an adequate reconstructive material should be selected.

Keywords: oral cavity cancer, reconstructive surgery, microsurgical reconstruction.

Introduction

In Russia, there has been a steady increase in the incidence of oral cancer. In the period from 2006 to 2016, the overall rise in the incidence of oral cancer was 29.93% for men and 54.61% for women [1]. Surgery is the main treatment for oral cancer, often requiring resection of the tongue, mandible, palate, oral/buccal mucosa, lip and the skin of the chin. Reconstructive surgery following extensive resections is an important aspect in the treatment of patients with oral cavity tumors [2]. Microsurgical reconstruction techniques allow the indications for radical surgical resections to be expended [3, 4]. However, the use of pedicle flaps in reconstructive surgery of the oral cavity has not lost its relevance. Management of patients with locally advanced oral cavity cancer, especially reconstructive surgery, remains a challenge [3, 5].

Material and Methods

Between 2008 and 2017, 115 patients with oral cancer were treated at the Department of Head and Neck Tumors of the Cancer Research Institute (Tomsk, Russia). All patients underwent surgical resection with reconstruction. A total of 127 reconstructive surgeries were performed (12 patients underwent two reconstructions). The distribution of patients according to the clinical stage was as follows: 16 patients with stage T2, 62 with T3, and 37 with T4. According to the presence of regional metastases in neck lymph nodes, there were 19 patients with N1 and 8 patients with N2. There were 44 women and 71 men. Primary cancer was detected in 63 cases (55%) and recurrent cancer in 52 cases (45%).

Distribution of tumor localization was as follows: tongue in 37 cases (32%), alveolar mucosa of the mandible in 24 (21%), mucosa of the mouth floor in 19 (17%), mucous cheeks in 14 (12 %), alveolar mucosa of the maxillary in 14 (12%), upper and lower lip in 7 cases.

In 90 cases (71%), free flaps were used as a reconstructive material: fibula flap in 40; ALT flap in 24; radial free flap in 12; medial sure artery perforator flap in 5; thoracodorsal flap in 4; osteocutaneous scapula free flap in 3; great omentum flap in 1; iliac crest free flap in 1. We used also pedicle flaps (37 cases): pectoral major myocutaneus flap in 17 cases and submental flap in 20 cases.

It should be noted that free flaps were mostly used to close large, composite (bone-soft-tissue) defects. Pedicle flaps (mostly submandibular flap) were used to reconstruct small defects of the tongue (hemigloselectomy), mucosa cheeks (up to 7×7 cm), or in a case of extensive defects in the tongue and oral cavity in patients with an unfavorable prognosis (pectoral flap).

Results

In most cases (85%), good functional results were achieved. In cases of tongue reconstruction using skin-fascial flaps, adequate mobility of the tongue was restored. Tongue reconstruction with skin-fascia flaps provided adequate tongue mobility and favorably influenced the speech and nutrition rehabilitation. Defects of the 1/2 tongue were reconstructed using free flaps (radial, medial sure artery perforator flap or ALT flap), or by pedicel flaps (submental flap). The ALT flap or pectoral major myocutaneus flap was used to reconstruct total defects of the tongue. It should be noted that the length of surgery was much less using a pedicle flap than a free flap (214.1 versus 580.88 minutes). In cases with reconstruction of *maxillary* and *mandibular* bone defects with *autotransplantation* and *bone* grafting, a successful restoration of the continuity of mandible and maxilla, natural contour of the face and mouth opening was achieved. The use of chimeric fibular flaps allowed restoration of extensive defects of the oral cavity, soft tissues of the buccal region and the mandible. The average length of a hospital stay following reconstructive surgery did not exceed 21 days (usually 14-18 days).

Flap necrosis was observed in 15 cases (12%). Necrosis of the fibula flap due to the formation of the saliva flowing to the area of microvascular anastomoses was the most common (10 cases,

8%). Partial necrosis of the flaps was noted in 5 cases (4%). Recurrences occurred in 25 cases (22%): local recurrence in 18 cases (16%) and neck lymph node metastases in 7 cases (6%). Distant metastases were observed in 2 cases (brain and skull bones).

Conclusion

Reconstructive surgery following radical surgical resection for oral cavity cancer contributes to the improvement of survival outcomes and social adaptation of the patients. To obtain good functional and cosmetic results, as well as to reduce postoperative complications, an apropriate reconstructive material should be selected. This choice should be based on the assessment of tumor involvement, reconstructive surgery extent and previous treatment.

The study was supported by a grant from the Russian Science Foundation (project № 16-15-00038)

REFERENCES

1. *Caprin A.D., Starinsky V.V., Petrova G.V.* The state of oncological care for the population of Russia in 2016. Moscow: MNIOI P.A.Gertsena is a branch of the National Medical Research Radiological Centre of the Ministry of Health of Russia, 2017. 236.
2. *Shah J.P., Patel S.G.* Head and neck surgery and oncology. Mosby; 2013. 713.
3. *Kulbakin D.E., Chonzonov E.L., Mukhamedov M.R. Garbukov E.U., Shtin V.I., Havkin N.M., Vasilev R.V.* Reconstructive plastic surgery in combined treatment of patients with locally advanced head and neck tumors. Questions of oncology. 2017; 63 (6): 862-6.
4. *Hanasono M.M.* Reconstructive Surgery for Head and Neck Cancer Patients. Adv Med. 2014; 2014: 795483. doi: 10.1155/2014/795483.
5. *Neligan P.C.* Head and Neck Reconstruction. Plastic and Reconstructive Surgery. 2013; 131: 260-9

LOW COUNT OF LYMPHOCYTES AND CD4T-CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH PRIMARY HODGKIN'S LYMPHOMA CORRELATES WITH UNFAVOURABLE DISEASE PROGNOSIS

E.G. Kuzmina, T.I. Bogatyreva, A.Yu. Terekhova, N.A. Falaleeva, T.Yu. Mushkarina, T.V. Konstantinova, I.A. Zamulaeva, S.V. Zatsarenko

A. Tsyb Medical Radiological Research Center – branch of the National Medical Research Radiological Center of the Ministry of Health of the Russian Federation, Obninsk, Russia

The prognostic significance of depletion of lymphocytes and of CD4T-cells was assessed in a retrospective cohort study of 162 Hodgkin lymphoma (HL) patients. The moderate CD4 lymphopenia (400-210 μL) was observed in all subgroups. The deep CD4 lymphopenia (≤200 μL) was found in 15% patients; it was associated with age ≥45 (p=0.031), advanced stage (p=0.03) and IPS score ≥4 (p=0.000). Overall survival (OS) and progression-free survival (PFS) for all 162 patients correlated with baseline CD4T-cells counts. At a median follow up of 60 months, all patients with CD4T-cells count ≤400 μL had lower progression-free survival (PFS) and lower overall survival (OS) compared with those without CD4 lymphopenia. In I-II favorable stages (n=13), progression occurred only in 1 patient with low CD4 count; OS was 100%. In I-II unfavourable stages (n=29), 6 patients with CD4 deficiency had PFS 50% vs. 95% in the rest, p=0.007; OS was 30% vs. 100%, p=0.001. Among 120 patients with stages III-IV, those with low CD4 count (n=53) had 5-year PFS 64% compared with 87% in patients without CD4 deficiency, p=0.006. Overall survival in advanced HL with low CD4 count was 70%, compared with 95%, p=0.004. Subset analysis in 94 patients with stages III-IV plus IPS 0-3 supported negative impact of CD4 lymphopenia. PFS in «low CD4» patients was 69% vs. 88%, p=0.054; OS was 76% vs. 97%, p=0.058.

Keywords: lymphocytes count, CD4T-cells, Hodgkin lymphoma, unfavourable prognosis.

Introduction

Initial pre-treatment lymphopenia (PL) is associated with negative prognosis in different malignancies, including lymphomas [1]. CD4 deficiency is known to accompany the impaired immunity in classical HL and has been recently shown to play a negative role in various solid tumors and Non-Hodgkin's lymphomas [2-4]. As part of reactive microenvironment, CD4T-cells play a key role in tumor-response regulation the activation of effector-cells. However, during progression the tumor acquires certain features that allow it to avoid immunological surveillance [5].

In a retrospective cohort study, long term relapse-free survival and overall survival were assessed in 162 HL patients with depletion of lymphocytes and CD4T-cells.

Methodology

In a database of MRRC (n=838 patients), we searched for 162 HL patients who had flow cytometry (95 females and 67 males) and were eligible for this study, 53 of them had low lymphocyte counts at presentation. Median age was 28 years (16-59). All diagnoses were made according to WHO classification. Pretreatment examination included thoracic and abdominal computed tomography; ultrasound examination of all peripheral lymph nodes, abdomen, retroperitoneal and small pelvis areas; bone-marrow biopsy. Stages of disease were defined by Ann Arbor classification. The patients were divided into 3 groups according to risk factors: group 1(n=13) included patients with HL I-II stages without risk factors; group 2 (n=29) - patients with HL I-II stages having risk factors (mediastinal bulk, isolated extranodal involvement, involvement of 3 or more lymphatic areas); group 3 (n=120) - patients with stages III-IV. Patients received risk-adapted combined modality therapy [6]. The median follow-up for all patients was 60 months. Immunophenotyping of peripheral blood CD4T-cells was performed by flow cytofluorimetry, using monoclonal antibodies to CD3, CD4.

Five-year overall survival and relapse-free survival were analyzed with regard to the initial level of CD4+T-cells. Kaplan-Meier method was applied to assess survival, the data were analyzed with statistical package SPSS 20 for Windows.

Results

Table 1 describes the baseline characteristics of the patients and the Ly=lymphocyte, LP=lymphocyte predominance, NS=nodular sclerosis, MC=mixed cellularity, LD=lymphocyte depletion, F=favourable, U=unfavourable, and the incidence of lymphopenia in different subgroups. The incidence of moderate CD4 lymphopenia (400-210 μL) was found in 36 (22%) of the 162 patients. The deep CD4 lymphopenia (≤200 μL) was found in 24(15%) of the 162 patients; it was associated with age ≥45 (p=0.031), advanced stage (p=0.03) and IPS score ≥4 (p=0.000). The overall survival (OS) and progression-free survival (PFS) for all 162 patients correlated with baseline CD4T-cells counts (Figure 1).

Table 1

Frequency of pretreatment lymphopenia in Hodgkin's lymphoma

Characteristics		No.(%)	Lymphocyte ≤1000 μL N (%)	*p* value	CD4 Ly ≤200 μL N (%)	*p* value	CD4 Ly ≤400 μL N (%)	*p* value
Gender	Male	67 (41)	16 (24)	**0.029**	7 (10)	0.276	16 (24)	0.815
	Female	95 (59)	37 (39)		17 (18)		20 (21)	
Age	≥ 45	17 (10)	8 (47)	0.093	6 (35)	**0.031**	3 (18)	0.864
	< 45	145 (90)	45 (31)		18 (12)		33 (23)	
Morphology	NS Gr I + MC	115	31 (27)	**0.014**	15 (13)	0.297	21 (18)	0.136
	NS Gr II + LD	42	19 (45)		9 (21)		13 (31)	
Stages	I-II F	13 (8)	0	**0.007**	0	**0.030**	1 (8)	0.281
	I-II U	29 (18)	5 (17)		1 (3)		5 (17)	
	III-IV	120 (74)	48 (40)		23 (19)		30 (25)	
IPS score	0-2	115 (71)	26 (23)	**0.000**	7 (6)	**0.000**	22 (19)	0.291
	3	21 (13)	10 (48)		3 (14)		7 (33)	
	≥4	26 (16)	17 (65)		14 (54)		7 (27)	

Bold characters designate a parameter with a significantly different distribution in the subgroups tested, defined by p<0.05.

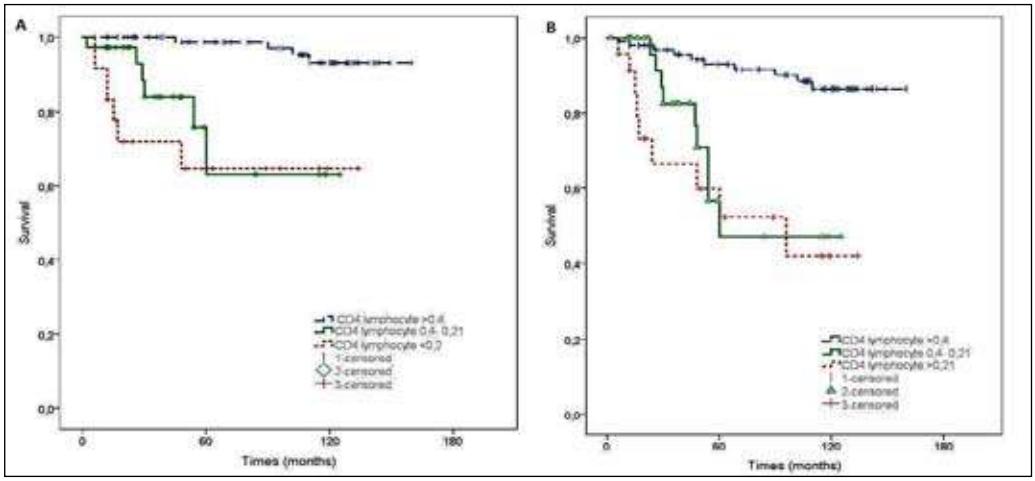


Figure 1. Overall (A) and progression-free (B) survival according to baseline CD4 lymphocyte counts in 162 patients with Hodgkin's lymphoma of I-IV stages.

At a median follow up of 60 months, all patients with CD4T-cells count ≤400 μL had lower progression-free survival (PFS) and lower overall survival (OS) compared with those without CD4 lymphopenia. In stages I-II, favorable (n=13) progression occurred only in a patient with low CD4 count; OS was 100%. In stages I-II unfavorable (n=29), six patients with CD4 deficiency had PFS 50% vs. 95% in the rest, p=0.007; OS was 30% vs. 100%, p=0.001. Among 120 patients with stages III-IV, those with low CD4 count (n=53) had 5-year PFS of 64% compared with 87% in patients without CD4 deficiency, p=0.006. Overall survival in advanced HL with low CD4 count was 70%, compared with 95%, p=0.004. Subset analysis in 94 patients with stages III-IV plus IPS 0-3 supported negative impact of CD4 lymphopenia. PFS in «low CD4» patients was 69% vs. 88%, p=0.054; OS was 76% vs. 97%, p=0.058.

Discussion

According to our earlier analysis, absolute lymphopenia (≤1000 μL) in previously untreated HL is relatively rare event with overall incidence of 14% (116 of 838 patients) [7]. Decreased lymphocyte counts were found in 1.4% of patients with favourable I-II stages, in 7.5% of patients with unfavorable I-II stages and in 18.6% of patients with III-IV stages. To our knowledge, in the course of different clinical trial no stratification is provided to account for possible adverse effect of pre-treatment lymphopenia. We studied prognostic significance of decreased CD4T-cell counts in the group of 162 patients with HL before treatment. An important conclusion from our study was the fact that a low CD4-cell count as a prognostic factor for overall and progression-free survival may be applied to patients with early-stage disease. It should be noted that in our study the incidence rate of CD4T-cell deficiency in patients without pre-treatment lymphopenia was 9%.

Conclusion

We suggest that the decreased CD4T-cell count should be considered when planning therapy for initial HL as an additional sign of unfavorable prognosis along with IPS factors and it can be used for early-stage HL as well. Presence of lymphopenia requires innovative therapeutic approaches that would improve the outcome in this patient group.

REFERENCES

1. *Hasenclever D., Diehl V.* A prognostic score for advanced Hodgkin's disease. International Prognostic Factors Project on Advanced Hodgkin's Disease. N. Engl. J. Med. 1998. 339: 1506–1514. doi: 10.1056/NEJM199811193392104.

2. *Judd J., Dulaimi E., Li T., Millenson M.M., Borghaei H., Smith M.R., Al-Saleem T.* Low Level of Blood CD4(+) T Cells Is an Independent Predictor of Inferior Progression-free Survival in Diffuse Large B-cell Lymphoma. Clin Lymphoma Myeloma Leuk. 2017 Feb; 17(2): 83-88. doi: 10.1016/j.clml.2016.11.005.

3. *Péron J., Cropet C., Tredan O., Bachelot T., Ray-Coquard I., Clapisson G., Chabaud S., Philip I., Borg C., Cassier P., Labidi Galy I., Sebban C., Perol D., Biron P., Caux C., Menetrier-Caux C., Blay J.Y.* CD4 lymphopenia to identify end-of-life metastatic cancer patients. Eur J Cancer. 2013 Mar; 49(5): 1080-9. doi: 10.1016/j.ejca.2012.11.003.

4. *He L., Liang J.H., Wu J.Z., Li Y., Qin S.C., Miao Y., Wu Y.J., Wang Y., Wang L., Fan L., Li J.Y., Xu W.* Low absolute CD4(+)T cell counts in peripheral blood are associated with inferior survival in follicular lymphoma. Tumour Biol. 2016; 37(9): 12589-12595. doi: 10.1007/s13277-016-5124-9.

5. *Vardhana S., Younes A.* The immune microenvironment in Hodgkin lymphoma: T cells, B cells, and immune checkpoints. Haematologica. 2016 Jul; 101(7): 794-802. doi: 10.3324/haematol.2015.132761.

6. *Bogatyreva T.I., Pavlov V.V.* Long-term results of risk-adapted therapy for advanced. Hodgkin's lymphoma: one-center experience (1998-2012). Hematologica. 2013; 98(Suppl.2): 8.

7. *Bogatyreva T., Pavlov V. Konova O., Chait S.* Absolute lymphopenia: factor of unfavorable prognosis in all stages of previously untreated Hodgkin's lymphoma. Hematology and Transfusiology. 2012; 57(S3): 32. [in Russian]

INSOLUBLE NI^{+2} COMPOUNDS INDUCE GENOTOXICITY AND GENE SILENCING, DISRUPTING 14 SIGNAL TRANSDUCTION PATHWAYS, AND LEADING TO DIFFERENTIAL EXPRESSION OF 144 GENES AND MORPHOLOGICAL, A. I., AND NEOPLASTIC TRANSFORMATION OF C3H/10T1/2 MOUSE EMBRYO CELLS

J.R.Jr. Landolph[1,2,3,4], J. Grondin[1,3,4], C.T. Salazar[1,2,4], S. Khaksar[1,2,4], M.A. Horton[1,2,4]

Lab. of Chemical Carcinogenesis/Molecular Oncology[1]
Dept. of Molecular Microbiology/Immunol[2]
Dept. of Pathology[3]
USC/Norris Comprehensive Cancer Center, Norris Topping Tower Keck School of Medicine, Univ. of Southern California, Los Angeles, USA[4]

Workers who inhaled dusts of nickel (Ni) sulfidic ores and smoked cigarettes in Ni refineries had increased incidences of nasal/lung cancers. Inhalation of nickel subsulfide (Ni_2S_3) and green (high temperature) nickel oxide (NiO) induced respiratory cancer in rats. Our laboratory showed Ni_2S_3, green NiO, and black NiO were phagocytosed into immortal, non-transformed C3H/10T- 1/2 (10T1/2) mouse embryo cells and induced chromosomal aberrations, cytotoxicity, and morphological, anchorage-independent (A. I.), and neoplastic transformation (Tx) in 10T- 1/2 mouse embryo cells. We used mRNA differential display to show that: Nickel (Ni^{+2})-transformed (Tx) and 3-methylcholanthrene (MCA)-Tx 10T1/2 cell lines had 1) Ect-2 gene amplification, higher steady-state levels of ect-2 mRNA/protein, and consequently higher steady-state levels of micro- tubules (MTs), aggregation of MTs in regions of the Tx cells, and consequently altered cell shapes. 2) β-centaurin-2 mRNA was detected in 10T1/2 cells, but not in Ni^{+2} -Tx nor in MCA-Tx 10T1/2 cell lines. Consequently, microfilaments (MFs) were expressed in 10T1/2 cells, but there were higher levels of MFs and aggregation of MFs in Ni-Tx and MCA-Tx 10T1/2 mouse embryo cell lines, altering shapes of Ni-Tx and MCA-Tx cell lines. 3) Ni-Tx and MCA-Tx cells also had higher steady-state levels of calnexin mRNA and protein. 4) Non-Tx cells had mRNA from the Vitamin D interacting protein (DRIP80) encoding gene, but Ni-Tx and MCA-Tx 10T1/2 cell lines had no detectable DRIP80 mRNA. Events #3 and #4 altered distributions of Ca^{+2} ions in Ni^{+2}-Tx and MCA-Tx cell lines. mRNA differential display showed that in Ni-Tx and in MCA-Tx cell lines, 6 driver genes were over-expressed, causing over-expression of an additional 52 genes. Nine tumor suppressor-like genes were under-expressed/not expressed in Ni^{+2}-Tx and MCA-Tx 10T1/2 cell lines, causing under-expression/no detectable expression of 77 additional genes. In total, these events disrupted 14 signal transduction pathways and caused differential expression of 144 genes between non-Tx and Ni-Tx or MCA-Tx 10T1/2 cell lines, resulting in induction/main- tenance of Tx phenotypes in Ni^{+2}-Tx and MCA-Tx 10T1/2 cell lines. This work was supported by:Grant R01 ES03341/NIEHS/NIH/USA (P. I., JRL); contracts from Nickel Producers Environ-mental Res. Association (NiPERA), U. S. A. (P. I., JRL); discretionary funds from the M. S. Trai- ning Program, Dept. of Mol. Microbiol./Immun., USC, to JRL; discretionary funds from the USC/Norris Comprehensive Cancer Center (NCCC) (P. I., JRL); and by the Molecular/Cell Biol- ogy Support Core (Faculty Supervisor, JRL) and the Cell Imaging Core (Faculty Supervisor, Dr. David Hinton), of Cancer Center Core Support Grant 5 P30 09320 NCI/NIH/USA, to USC/-NCCC.

Keywords: signal transduction, metal, neoplastic transformation.

REFERENCES

1. *Shuin T., Billings P.C., Lillehaug J.R., Patierno S.R., Roy-Burman P., Landolph J.R.* Enhanced expression of c-myc and decreased expression of c-fos proto-oncogenes in chemically and radiation transformed C3H/10T1/2 Cl 8 mouse embryo cell lines. Cancer Res. 1986; 46: 5302-5311.

2. *Billings P.C., Shuin T., Lillehaug J.R., Miura T., Roy-Burman P., Landolph J.R.* Enhanced expression and state of the c-myc oncogene in chemically and x-ray transformed C3H/10T1/2 Cl 8 mouse embryo fibroblasts. Cancer Res. 1987; 47: 3643-3649.

3. *Miura T., Patierno S.R., Sakuramoto T., Landolph J.R.* Morphological and neoplastic transformation of C3H/10T1/2 Cl 8 mouse embryo cells by insoluble carcinogenic nickel compounds. Environ. Mol. Mutagenesis. l989; 14: 65-75.

4. *Landolph J.R.* Molecular mechanisms of transformation of C3H/10T1/2 mouse embryo cells and diploid human fibroblasts by carcinogenic metal salts. Environ. Health Perspect. l994; 102: 115-119.

5. *Landolph J.R., Verma A., Ramnath J., Clemens F.* Molecular biology of de-regulation of gene expression in transformed C3H/10T1/2 mouse embryo cell lines induced by specific insoluble, carcinogenic nickel compounds. Environ. Health Perspect. 2002; (Suppl. 5): 845-850.
6. *Clemens F., Landolph J.R.* Genotoxicity of samples of nickel refinery dust. Toxicol. Sci. 2003; 73: 114-123.
7. *Verma A., Ramnath J., Clemens F., Kaspin L.C., Landolph J.R.* Molecular biology of nickel carcinogenesis: Identification of differentially expressed genes in morphologically transformed C3H/10T1/2 Cl 8 mouse embryo fibroblast cell lines induced by specific insoluble nickel compounds. Mol. Cell. Biochem. 2004; 255: 203-1216.
8. *Clemens F., Verma R., Ramnath J., Landolph J.R.* Amplification of the ect2 proto-oncogene and over-expression of ect-2 mRNA and protein in nickel compound- and methylcholanthrene-transformed 10T1/2 mouse fibroblast cell lines. Toxicol. Applied Pharmacol. 2005; 206: 138-149.
9. *DeSilva A.T., Verma R., Landolph J.R.* Silencing of the beta centaurin 2 and the FAD synthetase genes in nickel-transformed C3H/10T1/2 cell lines. Metal Ions in Biology and Medicine, 2008, 10: 63-67.
10. *Landolph J.R., Verma R., Ramnath. J., Jobling F.* The Cell and Molecular Biology of Nickel Compound-Induced Neoplastic Transformation and Carcinogenesis. Toxicology and Risk Assessment. 2015. 599-628.

THE INFLUENCE OF CISPLATIN ON TRANSCRIPTIONAL PROFILE OF TUMOR-ASSOCIATED MACROPHAGES OF BREAST CANCER AND COLON CANCER

I.V. Larionova[1,2], L. Tengfei[3], A.M. Kiselev[4], E.V. Denisov[1,2], N.V. Cherdyntseva[1,2], J.G. Kzhyshkowska[1,3,5]

Laboratory of translational cellular and molecular biomedicine, Tomsk State University, Tomsk, Russia[1]
Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[2]
University of Heidelberg, Medical Faculty Mannheim, Institute of Transfusion Medicine and Immunology, Mannheim, Germany[3]
Institute of Molecular Biology and Genetics, Almazov National Medical Research Centre, St. Petersburg, Russia[4]
German Red Cross Blood Service Baden-Württemberg–Hessen, Mannheim, Germany[5]

In the present study we demonstrated the whole transcriptome analysis of model tumor-associated macrophages (TAM) of breast and colon cancer with using next-generation sequencing data. We identified the most significantly increased and down-regulated genes as well as differentially expressed genes for breast and cancer TAM. Our results showed that TAM can be reprogrammed by chemotherapeutic agents and obtain mostly pro-inflammatory program.

Keywords: Tumor-associated macrophages, chemotherapy, cisplatin, cancer, next-generation sequencing.

Introduction

The application of chemotherapeutic agents is the most effective approach to the treatment of major oncological diseases [1]. We showed that an increased number of tumor-associated macrophages (TAM) correlated with a poor response to neoadjuvant therapy in breast cancer patients [2]. Identification of the phenotypic and functional characteristics of TAM during therapy is necessary to improve the effectiveness of chemotherapy, identify new mechanisms for poor response to NAC and to develope personalized approaches to the treatment of breast cancer and colon cancers [3, 4]. In the present study we demonstrated the analysis of whole transcriptome sequencing of samples of model breast and colon cancer TAM which indicated the most activated pathways under cisplatin treatment.

Material and Methods

Human primary monocytes-derived macrophages were isolated with using CD14+positive selection and stimulated ex vivo by IL4 and TGFb and supernatants of breast adenocarcinoma cell line MCF-7 and colorectal carcinoma cell line Colo206F to model cancer-specific TAM. Cisplatin treatment was performed on day 6 of macrophage differentiation. The whole transcriptome sequencing was performed. The libraries were prepared with the NEXT flex Rapid Directional qRNA-Seq Kit. Ribosomal RNA was removed from the NEBNext® rRNA Depletion Kit (Human/Mouse/Rat). Prepared libraries were then pooled and sequenced with using Illumina NextSeq500 instrument. The Hallmark, Reactome, KEGG databases were used for the experiment.

Results

The genes were selected according to their functional component and the highest value of differential expression (log2FoldChange values was at least 2, p-value <0.000001). At the transcriptional level, it was significantly increased the expression of genes involved in the inflammatory response,

interferon-dependent pathways, p53-dependent apoptosis, genes responsible for DNA damage, and genes that participate in the response to cisplatin and tamoxifen and in transplant rejection. The expression of genes involved in lipid and fatty acid metabolism, cholesterol homeostasis, glycolysis, oxidative phosphorylation, MTORC1 signaling, NOTCH signaling decreased, that indicates that the metabolism of macrophages changes in the direction of inhibiting certain metabolisms under the influence of the chemotherapeutic agent.

This approach helped to identify differences in the programming effect of chemotherapeutic agents on model TAM of breast adenocarcinoma and colon cancer. The most pronounced differences were cisplatin stimulation of the proto-oncogen KRAS signaling pathway, activation of the IRF7 interferon regulatory factor, DNA repair, and cisplatin suppression of angiogenesis, glycolysis, glucose metabolism, hypoxia, insulin metabolism, platelet activation, epithelial-mesenchymal transition in tumor-associated macrophages of intestinal adenocarcinoma. In the TAM of breast carcinoma it was mostly activated the pathways associated with the G protein-coupled receptor (signal transmission in the cell), the epithelial-mesenchymal transition, the activation of the proto-oncogenic protein MYC, the metabolism of RNA, including mRNA, regulation of translation, regulation of the mitochondrial transport.

Conclusions

The global influence of chemotherapeutic agents on the transcriptional profile of model tumor-associated macrophages was first established. We demonstrated that tumor-associated macrophages can be reprogrammed by chemotherapeutic agents. Macrophage response to cisplatin had a similarity to the antiviral response and can trigger an inflammatory antitumor targeting program.

This study was supported by grand RNF № 14-15-00350.

REFERENCES

1. *Stakheyeva M., Riabov V., Mitrofanova I., Litviakov N., Choynzonov E., Cherdyntseva N., Kzhyshkowska J.* Role of the immune component of tumor microenvironment in the efficiency of cancer treatment: perspectives for the personalized therapy. Curr Pharm Des. 2017; 23(32): 4807-4826. doi: 10.2174/1381612823666170714161703.

2. *Liu T., Larionova I., Litviakov N., Riabov V., Zavyalova M., Tsyganov M., Buldakov M., Song B., Moganti K., Kazantseva P., Slonimskaya E., Kremmer E., Flatley A., Klüter H., Cherdyntseva N., Kzhyshkowska J.* Tumor-associated macrophages in human breast cancer produce new monocyte attracting and pro-angiogenic factor YKL-39 indicative for increased metastasis after neoadjuvant chemotherapy. Oncoimmunology. 2018 Mar 13; 7(6): e1436922. doi: 10.1080/2162402X.2018.1436922.

3. *Mantovani A., Allavena P.* The interaction of anticancer therapies with tumor-associated macrophages. J Exp Med. 2015 Apr 6; 212(4): 435-45. doi: 10.1084/jem.20150295.

4. *Russell, J.S., Brown J.M.* The irradiated tumor microenvironment: role of tumor-associated macrophages in vascular recovery. Front Physiol. 2013 Jul 17; 4: 157. doi: 10.3389/fphys.2013.00157.

MELANOMA CELL GROWTH AND MIGRATION ALTERATIONS UNDER MIR-204-5P INHIBITOR APPLICATION

S.N. Lavrentev, M.B. Aksenenko, N.V. Palkina, A.V. Komina, T.G. Ruksha

Federal State Budget Educational Institution of Higher Education «Krasnoyarsk State Medical University named after Professor V.F. Voyno-Yasenetsky» of the Ministry of Health of the Russian Federation, Krasnoyarsk, Russia

MicroRNAs are regulatory molecules which play a role in melanoma biology as well as in other malignancies. MicroRNAs can affect multiple protein expression as posttranscriptional modulators. Therefore these molecules are considered as plausible therapeutic targets in cancer. The aim of the present study was to determine the effects of microRNA miR-204-5p specific inhibitor on melanoma cell growth and migration capacities.

C57Bl6 mice with transplanted melanoma B16 cells were kept in conditions of natural light without any restrictions on access to water and food. Inhibition of this microRNA we realized by inserting into the neck crease inhibitor LNA 25 mg/kg twice on the 7th and 14th day after the transplantation. After that we produced the observation of animals with measurement of the sizes of tumor node every day. We finished the experiment on the 19th day after the transplantation.

miR-204-5p specific inhibitor application resulted in the increase of melanoma cell proliferation that confirms miR-204-5p action in melanoma as oncosupressor. Besides, melanoma cells showed decreased migration rates under miR-204-5p inhibition.

miR-204-5p may be implicated in melanoma pathogenesis and act as oncosupressor.

Keywords: melanoma, LNA inhibitor, microRNA, miR-204-5p.

Introduction

Skin cancer is one of the leading malignancies in Russian Federation. Over the last decade, melanoma incidence rates have increased among pale-skinned populations. Although localized melanoma is highly curable, disseminated forms of the disease remain fatal. Recent approvals of immune check-point and BRAF inhibitors allowed treatment efficacy to be increased, however metastatic melanoma is currently under investigation.

MicroRNAs are a class of small non-coding RNAs of 20-22 nucleotides involved in the regulation of protein-coding genes at posttranscriptional level. MicroRNAs can exert oncogenic or/ and oncosuppressive properties. One of microRNAs with oncosupressive features is miR-204-5p, the levels of which are diminished in several cancer types [1, 2]. In the present study we evaluated miRNA 204-5p role in melanoma cell viability, proliferation, and migration.

Material and Methods

Melanoma B16-bearing mice were randomly divided into three groups: control group animals received phosphate buffered solution (n=3), negative control group animals (n=6) received antisence nucleotides, and miR-204-5p LNA inhibitor group (n=5). On days 7 and 14, after B16 melanoma cell transplantation, an LNA inhibitor mmu-miR-204-5p miRCURY LNA™ (Exiqon, Vedbaek, Denmark), a negative control solution Negative control A miRCURY LNA™ miRNA Inhibitor Control (Exiqon, Vedbaek, Denmark), and a PBS solution Phosphate Buffered Saline (Amresco, Solon, OH, USA) were injected subcutaneously, respectively, to the aforementioned animals. Physical activity of animals was evaluated daily as well as tumor volume was determined once in three days. The experiment melanoma cells were isolated and placed in a culture medium RPMI-1640 containing L-glutamine (Gibco, Life Technologies, Paisley, UK), 10% fetal bovine serum (Gibco, Life Technologies, Paisley, UK), and antibiotic-antimycotic solution (Gibco, Life Technologies, Paisley, UK). The cells were cultured for 7 days in a CO_2 incubator at 37 °C and a 5% CO_2.

MTT test was done to determine melanoma cells viability/proliferation under LNA inhibitor mmu-miR-204-5p treatment. For this purpose the culture medium was replaced, and 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) was added to a final concentration of 0.5 mg/ml. After four hours, the culture medium was removed and the amount of formazan formed in the cells was evaluated. To accomplish this, a solution of DMSO was added to the fixed cells to lyse and dissolve formazan. Light absorption at a wavelength of 560 nm was evaluated on an EFOS-9305 spectrophotometer (Shvabe photosystems, Moscow, Russia). The viability/proliferation of cells was considered directly proportional to the absorption of the light wave. The experiment was made three times.

Scratch test was performed to estimate the migratory ability of melanoma cells. Cells were plated into 6-well plates to a final concentration of 1×10^7. 24 hours later when the cells demonstrated 100% confluence, a sterile tip was used to make a line which covering by cells were estimated every 12 hours. The images randomly selected were captured with the microscope Olympus BX-41 and proceeded by the Infinity v.6 software (Lumenera Corporation Ottawa, ON, Kanada).

Statistical analysis of the data was carried out using the Kruskal-Wallis nonparametric H-test for three or more independent groups by the Statistica 6.1 software (StatSoft, Moscow, Russia). Differences were considered significant at $P<0.05$.

MTT showed the increase of melanoma cell metabolic activity under LNA inhibitor miR-204-5p treatment as compared to negative control after 96 hours ($p=0.016$, Figure 1). This observation may be in the line with miR-204-5p oncosuppressive function as it was reported earlier. Scratch test demonstrated reduction in melanoma cell migration capacities as compared to PBS group ($p = 0.027$).

To conclude, the application of the LNA inhibitor miR-204-5p leads to melanoma B16 cell functional activity alterations, which are expressed as the short-term change in the migratory ability and proliferation rate. To conclude, miR-204-5p might be implicated in both processes regulation: migration and proliferation. More significant alterations we observed in cell proliferation rate. Migrated capacities of melanoma cells may be under control of various regulators as the miR-204-5p impact on it was not stable.

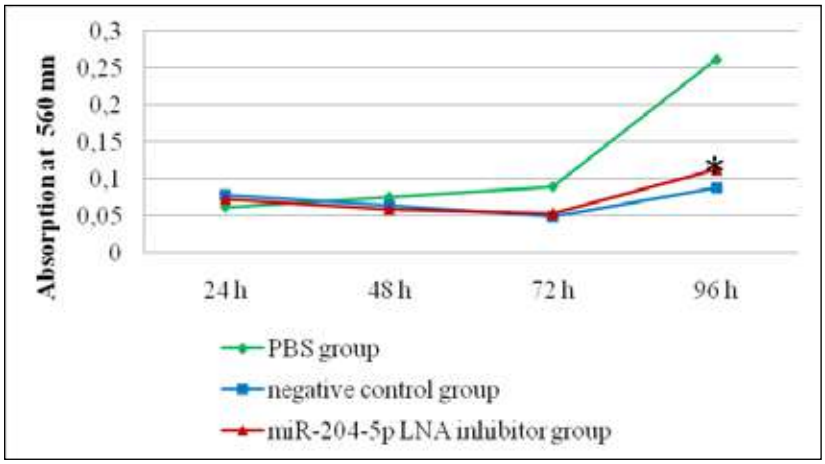


Figure 1. MTT test: *significant differences in viability/metabolic activity between LNA inhibitor group versus negative control group (p=0,016).

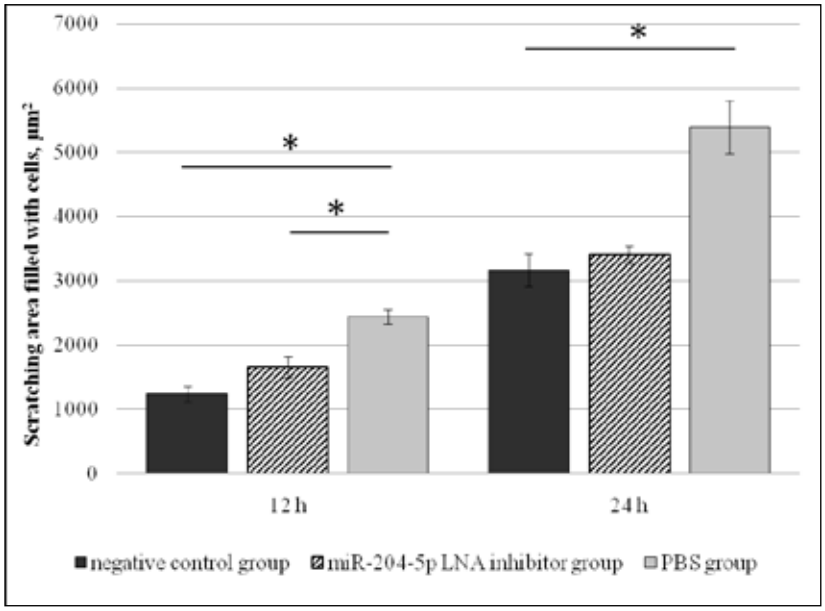


Figure 2. Results of scratch test * significant differences in migration ability (p<0.05)

The study was supported by Grant of the Russian Science Foundation (project № 14-15-00074-P).

REFERENCES

1. *Longwen S., Zongxin Z., Yunxiang C.* MicroRNA-204 inhibits cell migration and invasion in human cervical cancer by regulating transcription factor 12. 2017 Nov; 15: 161-166. doi: 10.3892/ol.2017.7343.

2. *Wei G., Yongyan W., Xiaoling H., Chunming Z., Meixia Z., Bo C., Qingqing L., Xukuan Q., Weiyan L., Shuxin Wen., Binquan W.* MicroRNA-204-5p inhibits invasion and metastasis of laryngeal squamous cell carcinoma by suppressing forkhead box C1. 2017 May; 8(12): 2356-2368. doi: 10.7150/jca.19470.

TARGETING EXOSOME-MEDIATED SECRETION OF HEAT SHOCK PROTEIN-90ALPHA (HSP90α) BY TUMOR CELLS

W. Li

Department of Dermatology and the Norris Comprehensive Cancer Center, University of Southern California Keck Medical Center, Los Angeles, USA

Mammals have two isoforms of heat shock protein-90 (Hsp90α and Hsp90β) to deal with environmental stress, especially ischemia. These functions of Hsp90 are taken advantage of by tumor cells to cope with the paucity of oxygen and nutrient supply within the harsh microenvironment

during tumorigenesis. Hsp90β acts as a chaperone to maintain the integrity of the entire intracellular signaling networks. In contrast, Hsp90α is secreted into and acts from the extracellular environment. Tumor-secreted Hsp90α binds to the cell surface LRP-1 receptor to prevent tumor cell death under hypoxia and, thereafter, to promote tumor cell invasion and metastasis. Since 1999, targeting intracellular Hsp90β has proven too toxic in clinical trials and few inhibitors have advanced to FDA approval for clinical use. However, in contrast to the equal importance of the intracellular Hsp90β in both tumor and normal cells, secreted Hsp90α is non-essential for maintaining homeostasis. Selective inhibition of tumor-secreted Hsp90α function inhibits both *de novo* tumor formation and expansion of already formed tumors of various origins in CDX tumor mouse models. We hypothesize that tumor-secreted Hsp90α is more effective and safer target for anti-tumor therapeutics than targeting the intracellular Hsp90β chaperone. will discuss 1) the mechanism of exosome-mediated secretion of Hsp90α and 2) the therapeutic potential of inhibiting tumor-secreted Hsp90α with monoclonal antibody USC-1G6-D7. Because of its extracellular location of tumor-secreted Hsp90α, we are presented with an opportunity to develop tumor-secreted Hsp90α into a new anti-tumor drug candidate with less toxicity and high clinical efficacy.

Keywords: exosome, heat shock protein, cancer.

REFERENCE

1. *Li W., Li Y., Guan S., Fan J., Cheng C.F., Bright A., Chinn C., Chen M., Woodley D.T.* Extracellular heat shock protein-90alpha: linking hypoxia to skin cell motility and wound healing. EMBO J. 2007 Mar 7; 26(5): 1221-33. doi: 10.1038/sj.emboj.7601579.

2. *Cheng C.F., Sahu D., Tsen F., Zhao Z., Fan J., Kim R., Wang X., O'Brien K., Li Y., Kuang Y., Chen M., Woodley D.T., Li W.* A fragment of secreted Hsp90alpha carries properties that enable it to accelerate effectively both acute and diabetic wound healing in mice. J Clin Invest. 2011 Nov; 121(11): 4348-61. doi: 10.1172/JCI46475.

3. *Sahu D., Zhao Z., Tsen F., Cheng C.F., Park R., Situ A.J., Dai J., Eginli A., Shams S., Chen M., Ulmer T.S., Conti P., Woodley D.T., Li W.* A potentially common peptide target in secreted heat shock protein-90α for hypoxia-inducible factor-1α-positive tumours. Mol Biol Cell. 2012 Feb; 23(4): 602-13. doi: 10.1091/mbc.E11-06-0575.

4. *Jayaprakash P., Dong H., Zou M., Bhatia A., O'Brien K., Chen M., Woodley D.T., Li W.* Hsp90α and Hsp90β together operate a hypoxia and nutrient paucity stress-response mechanism during wound healing. J Cell Sci. 2015 Apr 15; 128(8): 1475-80. doi: 10.1242/jcs.166363.

5. *Dong H, Zou M, Bhatia A., Jayaprakash P., Hofman F., Yin Q., · Chen M., Woodley D.T., Li W.* Breast Cancer MDA-MB-231 Cells Use Secreted Heat Shock Protein-90alpha (Hsp90α) to Survive a Hostile Hypoxic Environment. Sci Rep. 2016 Feb 5; 6: 20605. doi: 10.1038/srep20605.

6. *Zou M., Bhatia A., Dong H., Jayaprakash P., Guo J., Sahu D., Hou Y., Tsen F., Tong C., O'Brien K., Situ A.J., Schmidt T., Chen M., Ying Q., Ulmer T.S., Woodley D.T., Li W.* Evolutionarily conserved dual lysine motif determines the non-chaperone function of secreted Hsp90alpha in tumor progression. Oncogene. 2017 Apr; 36(15): 2160-2171. doi: 10.1038/onc.2016.375.

7. *Guo J., Jayaprakash P., Dan J., Wise P., Jang G.B., Liang C., Chen M., Woodley D.T., Fabbri M., Li W.* PRAS40 Connects Microenvironmental Stress Signaling to Exosome-Mediated Secretion. Mol Cell Biol. 2017 Sep 12; 37(19). pii: e00171-17. doi: 10.1128/MCB.00171-17.

8. *Li W., Sahu D., Tsen F.* Secreted heat shock protein-90 (Hsp90) in wound healing and cancer. Biochim Biophys Acta. 2012 Mar; 1823(3): 730-41. doi: 10.1016/j.bbamcr.2011.09.009.

COLON- AND RECTAL CANCER ARE DIFFERENT TUMOR ENTITIES ACCORDING TO EPIDEMIOLOGY, CARCINOGENESIS, MOLECULAR- AND TUMORBIOLOGY, SURGERY/MULTIMODAL THERAPY, RESULTS QUALITY, AND PRIMARY AND SECONDARY PREVENTION

K.H. Link[1,3], S. Jafarov[3], L. Staib[3], M. Kornmann[2,3]

Surgical Center and Asklepios Tumor Center, Asklepios Paulinen Klinik, Wiesbaden, Germany[1]
Dept.General/Visceral Surgery (Chir.I), Univ.Ulm, Ulm, Germany[2]
FOGT, Univ.Ulm, Ulm, Germany[3]

Colon and Rectal Cancer (CC, RC) are different entities from a clinical and tumorbiological point of view. Up to now, both, CC and RC, are synonymously called "Colorectal Cancer" (CRC). We analyzed the actual available data from the literature and our own results from the Ulm based study group FOGT to proof or reject our hypothesis.

The following evident differences were recognized:

1. Anatomically, the risk to develop RC 4× higher than for CC.
2. Molecular changes in carcinogenesis in CC are different from RC.
3. Physical activity helps to prevent CC, not RC.
4. Pathologically there are differences between RC and CC.
5. Surgical topography and procedures are different.

6. Multimodal treatment (MMT) approaches are different.
7. RC in MMT is less sensitive to chemotherapy than CC.

Prognostic factors for the spontaneous course and for success of MMT are different (e.g. TS or DPD).

CC´sand RC´s definitely are different in parameters of causal and formal carcinogenesis, effectivity of primary prevention by physical activity, conventional and molecular pathology, clinical/biological behaviour, surgical and multimodal treatment concepts, sensitivity to chemotherapeutic protocols within multimodal therapy and the prognostic value of various parameters. Actually there are even observations, that in palliative chemotherapy the response rates of metastases from CC (e.g. right hemicolon) are differing from those deriving from RC. According to our findings we can demand that CC and RC are two different tumor entities. "CRC" should no longer be used in basic and clinical research and other fields of cancer classification. CC is not the same as RC.

Keywords: colon cancer, rectal cancer, epidemiology, carcinogenesis, prevention.

ANGIOGENESIS IN EXPERIMENTAL HEPATOCARCINOMA-29

V.V. Makarova, N.P. Bgatova, Yu.S. Taskaeva, Yu.I. Borodin

Laboratory of ultrastructural research, Research Institute of Clinical and Experimental Lymphology - Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

Hepatocellular carcinoma is one of the most aggressive human tumors, with a high prevalence and mortality. Tumor microenvironment, in particular blood vessels, plays a large role in the progression of tumor growth. In connection with the important role of angiogenesis in tumor progression and metastasis, it is important to develop new approaches of hepatocarcinoma treatment by suppressing tumor angiogenesis. The aim of the study was to identify blood vessels during the development of experimental hepatocarcinoma-29. Light microscopy revealed that capillaries with narrow lumens permeate the entire volume of tumor tissue, dividing it into small segments. Immunohistochemical analysis showed a poor degree of CD31 expression. Ultrastructural analysis revealed that the walls of the vessels are formed by endotheliocytes and tumor cells. Apparently, neoangiogenesis in hepatocarcinoma-29 follows the path of vasculogenic mimicry.

Keywords: hepatocarcinoma-29, angiogenesis, CD 31, vasculogenic mimicry.

Hepatocellular carcinoma is one of the most aggressive human tumors, and despite the advances in diagnosis and treatment, it remains the fifth most common and third-largest mortality rate in the world due to the resistance to polychemotherapy [1, 2]. Tumor microenvironment, in particular the blood vessels, plays a large role in the progression of tumor growth [3, 4]. In connection with the important role of angiogenesis in tumor progression and metastasis, it is important to develop new approaches of hepatocarcinoma treatment by suppressing tumor angiogenesis. The aim of the study was to identify blood vessels during the development of experimental hepatocarcinoma-29 (HC-29).

Material and Methods

Experiments were performed on CBA line male mice from the Institute of Cytology and Genetics SB RAS. Mice weighted 18–20 g and were three months of age. Work with animals was performed according to the principles of humanity stated in directions of EC (86/609/EEC) and Declaration of Helsinki. To model the tumour process, we used HP-29. HC-29 was obtained and verified by the staff of the Institute of Cytology and Genetics, SB RAS.

HC-29 cells were injected into the intact muscle of the right thigh. The sampling of the material for the studies was carried out on the 30^{th} day of the experiment. For light microscopy, tumor fragments were fixed in a 4% solution of paraformaldehyde and encapsulated in paraffin according to a standard histological procedure. Paraffin sections were stained with Mayer's hematoxylin and with using anti-CD31 antibody (Abcam). Evaluation of the results was carried out with a LEICA DME light microscope (Germany), with a 400-fold magnification, photographs were obtained using the computer program «Avigion». For electron microscopic examination, samples of tumor tissue were fixed in a 1% solution of OsO_4, dehydrated with increasing concentrations of ethanol and imbedded into Epon (Serva, Germany).Semithin sections (1 μM) were cut with an EM UC7

Leica ultratome (Germany/Switzerland), stained with toluidine blue, and visualized under a LEICA DME light microscope (Germany). Ultrathin sections 70-100 nm thickness were contrasted with a saturated water solution of sodium uranyl acetate and lead citrate, and analyzed with a JEM 1010 electron microscope.

Results and Discussion

In the study of tumor tissue samples, capillaries with narrow lumens were observed, which permeated the tumor tissue, dividing it into small segments, and necrosis was not observed, which indicates a very good blood supply to the tumor. However, in the immunohistochemical analysis of sections stained with the CD31 endothelial cell marker in blood vessels, it was found that not all vessels visualized by staining with Meyer's hematoxylin were detected. On ultrathin preparations it was found that the walls of the vessels were not completely, but only partially formed by endothelial cells, and partly by tumor cells. There is evidence that neoangiogenesis in tumor tissue can follow the path of vasculogenic mimicry (VM), some newly formed vessels are lined with tumor cells rather than endothelial cells [5]. The results obtained require further study, including staining of PAS [6], to detect VM in tumor tissue with the aim of further developing methods for suppressing tumor growth by inhibiting angiogenesis, including the formation and growth of VM-vessels (7).

REFERENCES

1. *Shen Y., Cao D.* Hepatocellular carcinoma stem cells: origins and roles in hepatocarcinogenesis and disease progression. Biosci. 2012 Jan; 1(4): 1157–1169.

2. *Qu B., Sheng G., Guo L., Yu F., Chen G., Lu Q., Wang R., Han B., Lu Y.* MIG7 is involved in vasculogenic mimicry formation rendering invasion and metastasis in hepatocellular carcinoma. Oncol Rep. 2018 Feb; 39(2): 679-686. doi: 10.3892/or.2017.6138.

3. *Ansell S.M., Vonderheide R.H.* Cellular composition of the tumor microenvironment. Am Soc Clin Oncol Educ Book. 2013. doi: 10.1200/EdBook_AM.2013.33.e91.

4. *Bgatova N.P., Borodin Y.I., Makarova V.V., Pozhidaeva A.A., Rachkovskaya L.N., Konenkov V.I.* Effects of nanosized lithium carbonate particles on intact muscle tissue and tumor growth. Bull Exp Biol Med. 2014 May; 157(1): 89-94. doi: 10.1007/s10517-014-2499-8.

5. *Cheng R., Cai X.R., Ke K., Chen Y.L.* Notch4 inhibition suppresses invasion and vasculogenic mimicry formation of hepatocellular carcinoma cells. J Huazhong Univ Sci Technolog Med Sci. 2017 Oct; 37(5): 719-725. doi: 10.1007/s11596-017-1794-9.

6. *Grigorieva I.N., Kharatishvili T.K., Baryshnikov A.Yu.* An alternative mechanism in tumor vascularization: vasculogenic mimicry. Russian Biotherapeutic Journal. 2011; 10(3): 25–30. [in Russian]

7. *Zhang F., Zhang C.M., Li S., Wang K.K., Guo B.B., Fu Y., Liu L.Y., Zhang Y., Jiang H.Y., Wu C.J.* Low dosage of arsenic trioxide inhibits vasculogenic mimicry in hepatoblastoma without cell apoptosis. Mol Med Rep. 2018 Jan; 17(1): 1573-1582. doi: 10.3892/mmr.2017.8046

THE INFLUENCE OF ORGANIC SOLVENTS ON THE EPIGENETIC REGULATION OF GENE EXPRESSION

V.P. Maximova[1], O.P. Lizogub[2], D.A. Fedorov[2], E.M. Zhidkova[1], E.A. Lesovaya[1,3], A.V. Kholodova[1], G.A. Belitsky[1], M.G. Yakubovskaya[1], K.I. Kirsanov[1,4]

Blokhin NMRCO, Department of Chemical Carcinogenesis, Moscow, Russia[1]
First Moscow State Medical University, Moscow, Russia[2]
Ryazan State Medical University, Ryazan, Russia[3]
RUDN University, Moscow, Russia[4]

In this study we screened 22 organic solvents for epigenetic activity using the HeLa TI model system. We demonstrated that acetone, acetonitrile, methanol, mercaptoethanol, DMSO and toluene affect epigenetic mechanisms of acetylation and methylation of H3 and H4 histones, thereby participating in the regulation of gene expression. The mechanism of chloroform epigenetic action is most likely not associated with histone modifications.

Keywords: organic solvents, epigenetic activity, HeLa TI.

Introduction

Organic solvents are widely used in industry (degreasing and dilution) and are often found in everyday life (in products such as paints, pharmaceuticals, adhesives, printing inks, cosmetics and pesticides) [1]. There are three routes of entry of organic solvents into the body: skin contact, inhalation, and ingestion. Skin absorption is a major route of entry for organic solvents that are readily soluble in lipids and water. Organic solvents have the potential to cause adverse health effects that range from skin and lung irritation to the depression of the central nervous system

[2]. Epidemiological studies have shown a link between the action of solvents and deleterious effects on respiratory, hematological and thyroid functioning [3]. Alcohols, for example, depress the central nervous system, and cause irritation to the eyes and upper respiratory tract. Aromatic hydrocarbons, such as xylene and toluene cause skin and lung irritation and act as a strong depressant to the central nervous system. Aliphatic hydrocarbons can have a narcotic effect after large exposures. Ketones depress the central nervous system; irritate the eyes and upper respiratory tract [2]. Despite the extensive study of the effect of solvents on the human body, the epigenetic effect of organic solvents has not been studied to date. In order to reduce occupational risks when working with these chemical agents, it is necessary to take into consideration all possible routes of exposure of solvents to humans, toxic and non-toxic effects, which include epigenetic mechanisms of transcription regulation.

The aim of this work was to study the influence of some organic solvents on epigenetic mechanisms of transcription regulation using the HeLa TI model system.

Material and Methods

The determination of the epigenetic activity of organic solvents was carried out by flow cytometry on the HeLa TI cell line with an epigenetically repressed vector containing GFP gene. Cell line HeLa TI was obtained from The Epigenetic Department of the Fox Chase Cancer Center (Philadelphia, USA). To analyze epigenetic activity of the solvents the cells were treated with the agents for 24 hours and analyzed with flow cytometer FASCCanto II and BD FascDiva Software (Becton Dickinson, USA) in the next 48 hours. The fluorescence of GFP-positive cells was recorded in the green fluorescent dye fluorescein isothiocyanate (FITC) mode. Untreated cells were used as a negative control. Histone deacetylase inhibitor –Trichostatin A (TSA) – was used as positive control. Results of flow cytometry were compared with the images of fluorescence microscopy before starting work to accurately adjust the parameters. The degree of histone modifications at specific residues were monitored by western blotting using site specific antibodies (H3acK9, H4acK12, H3me3K9, H4me3K20) (Abcam, Great Britain).

Results

On the first step we screened the organic solvents, which are most often used in molecular biological and analytical experiments, and are widely used in industry, for their epigenetic activity. The study included 21 solvents from various chemical classes: acetone, acetonitrile, benzene, hexane, ammonium hydroxide, dimethylsulfoxide (DMSO), dimethylformamide (DMF), dichloromethane, diethyl ether, isoamyl alcohol, isopropanol, xylene, mercaptoethanol, methanol, n-octane, toluene, chloroform, ethanol, ethyl acetate, 1,4-dioxane (Table 1).

Table 1

Results of the analysis of the solvents' epigenetic activity by flow cytofluorimetry

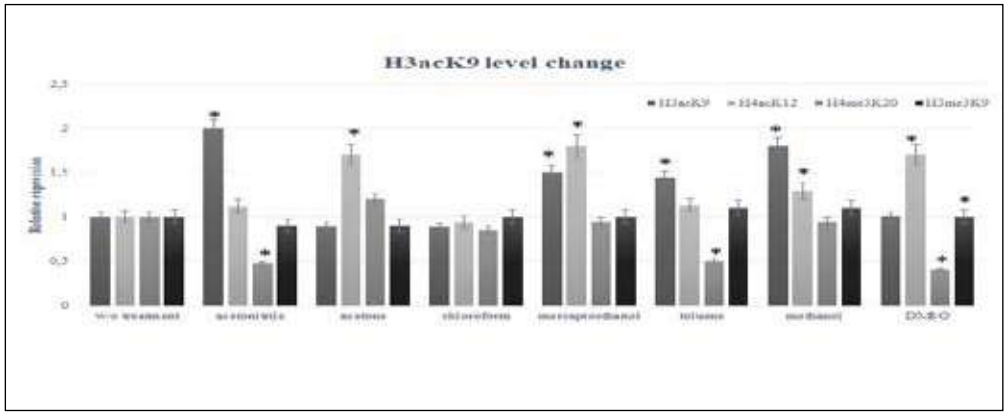
Agent name	% GFP-positive cells ± SD (n=5)	Agent name	%GFP-positive cells ± SD (n=5)
w/o treatment	3.5±0.2	Isoamyl alcohol	4.8±0.3
TSA	42.8*±2.5	Isopropyl alcohol	3.6±0.3
Acetone	13.5*±0.8	Xylene	3.9±0.2
Acetonitrile	22.3*±1.1	Mercaptoethanol	10.8*±0.7
Benzene	3.8±0.1	Methanol	13.8*±1.2
Hexane	3.4±0.2	n-Octane	3.6±0.1
Ammonium Hydroxide	4.1±0.1	1,4-dioxane	16.6*±1.2
DMSO	17.3*±0.8	Toluene	10*±0.5
DMF	3.6±0.3	Chloroform	11.5*±1.0
Dichloromethane	3.9±0.2	Ethanol	3.6±0.2
Diethyl Ether	3.5±0.1	Ethyl Acetate	3.4±0.3

n – the number of biological replicates, *p<0.05 compared to cells without treatment

Thus, we showed that acetone, acetonitrile, DMSO, methanol, chloroform, mercaptoethanol and toluene possess epigenetic activity. Solvents exhibited this effect in low-toxic (or non-toxic) concentrations.

On the next step we studied the ability of the solvents to affect level of global H3 and H4 acetylation (Picture 1). It was shown that acetonitrile and toluene increase the degree of histone H3 acetylation, while acetone and DMSO influenced histone H4 acetylation; mercaptoethanol and methanol cause a significant increase in acetylation of both histones H3 and H4. In addition, we revealed decrease in the level of lysine 20 trimethylation of histone H4 (H4K20) (typical for

non-transcribed chromatin) after the treatment with acetonitrile. Changes in level of H3me3K9 were not detected after treatment with any of studied organic solvents.



Picture 1. Determination of the level of post-translational modifications by Western blotting (densitometric analysis of western blotting results).

Conclusion

In summary, acetone, acetonitrile, methanol, mercaptoethanol, DMSO and toluene affect such epigenetic mechanisms as acetylation and methylation of histones H3 and H4, thereby participating in the regulation of gene expression. The mechanism of chloroform epigenetic action is most likely not associated with H3 and H4 histone modifications.

This work was supported by Russian Scientific Foundation (№ 18-75-00115).

REFERENCES

1. *Occupational* exposure to organic solvents: effects on human reproduction. Health Council of the Netherlands. 2008.
2. *Schenker M.B., Jacobs J.A.* Respiratory effects of organic solvent exposure. Tuber Lung Dis. 1996 Feb; 77(1): 4-18.
3. *Uzma N., Salar M., Kumar S., Aziz N., David M., Reddy D.* Impact of Organic Solvents and Environmental Pollutants on the Physiological Function in Petrol Filling Workers. Int J Environ Res Public Health. 2008 Sep; 5(3): 139–146.

MALIGNANT PERIPHERAL NERVE SHEATH TUMORS: PATTERNS OF RECURRENCE AND SURVIVAL AND THE ROLE OF POLY (ADP) RIBOSE POLYMERASE INHIBITION

I.E. McCutcheon

M. D. Anderson Cancer Center, Houston, Texas, USA

Objective

Malignant peripheral nerve sheath tumors (MPNSTs) are an aggressive group of soft tissue sarcomas. We retrospectively acquired outcome data on sporadic, NF1-associated, and radiation (RT)-associated MPNSTs in a large series of such tumors. Poly (ADP) ribose polymerase (PARP) inhibitors treat malignancies with defects in DNA repair genes, *e.g.*, *BRCA1/2*. While no specific mutations in *BRCA1/2* are known in MPNSTs, they might be effectively targeted with PARP inhibitor due to their complex karyotype and high level of genomic instability. Here we show differences in outcome between MPNST etiologies; assess expression of PARP1 and PARP2 in MPNST and efficacy of tumor control with PARP inhibition; and correlate these findings with overall survival.

Methods

In MPNST (n=289) subtypes, adverse predictors of recurrence and survival were identified. In a clinically annotated tissue microarray containing primary (n=38) and recurrent (n=32) MPNSTs we assessed markers for cell proliferation, cell cycle arrest, inhibitor specificity, and apoptosis. Cell lines derived from NF1-associated MPNST (S462, ST88) and sporadic MPNST (STS26T, MPNST724) were cultured, as were Schwann cells, and PARP1/PARP 2 activity determined by Western blot analysis and colorimetric assay. Cell growth was determined by MTS and clonogenic assay, and cell cycle progression by FACS analysis, before and after treatment by the PARP inhibitor AZD2281 (Olaparib). *In vivo* animal models of local tumor growth and lung metastasis were

created in SCID mice by injection of tumor cell suspension into the flank and tail vein respectively, and tumor was measured in animals treated with vehicle only vs. AZD2281.

Results

Five-year local recurrence-free survival (LRFS), distant recurrence-free survival (DRFS), and disease-specific survival (DSS) estimates were 56.6%, 49.6%, and 53.6% respectively. Five-year DSS was lower in NF1-associated and RT-associated MPNST than in sporadic MPNST (52%, 47%, and 67% respectively, $p = 0.140$). Patients with RT-associated MPNST had worse 5-year LRFS than those with the sporadic and NF1-associated subtypes (RT-associated vs sporadic, $p = 0.010$; RT-associated vs NF1-associated, $p = 0.232$). Truncally located tumors, positive surgical margins, local recurrence, and metastasis predicted adverse DSS in multivariate analysis. PARP1 and PARP2 were highly expressed in MPNST, and PARP activity was enhanced in MPNST cell lines. Decreased cell proliferation and enhanced apoptosis occurred *in vitro* with AZD2281 treatment. AZD2281 significantly reduced local growth of MPNST xenografts, decreased development of lung metastases, and increased survival of mice with metastases.

Conclusions

RT-associated MPNSTs are associated with poorer local recurrence-free and disease-specific survival than sporadic and NF1-associated tumors. NF1-associated MPNSTs may have worse survival outcomes owing to large tumor size, truncal location, and less frequent negative resection margins compared with sporadic tumors. AZD2281 may be effective against MPNST and its clinical use should be further investigated.

Keywords: malignant peripheral nerve sheath tumor, distant recurrence-free survival, disease-specific survival.

REFERENCES

1. *Watson K.L., Al Sannaa G.A., Kivlin C.M., Ingram D.R., Landers S.M., Roland C.L., Cormier J.N., Hunt K.K., Feig B.W., Ashleigh Guadagnolo B., Bishop A.J., Wang W.L., Slopis J.M., McCutcheon I.E., Lazar A.J., Torres K.E.* Patterns of recurrence and survival in sporadic, neurofibromatosis type 1-associated, and radiation-associated malignant peripheral nerve sheath tumors. J Neurosurg. 2017 Jan; 126(1): 319-329. doi: 10.3171/2015.12.JNS152443.

2. *Kivlin C.M., Watson K.L., Al Sannaa G.A., Belousov R., Ingram D.R., Huang K.L., May C.D., Bolshakov S., Landers S.M., Kalam A.A., Slopis J.M., McCutcheon I.E., Pollock R.E., Lev D., Lazar A.J., Torres K.E.* Poly (ADP) ribose polymerase inhibition: a potential treatment of malignant peripheral nerve sheath tumor. Cancer Biol Ther. 2016; 17(2): 129-38. doi: 10.1080/15384047.2015.1108486.

3. *Peacock J.D., Cherba D., Kampfschulte K., Smith M.K., Monks N.R., Webb C.P., Steensma M.* Molecular-guided therapy predictions reveal drug resistance phenotypes and treatment alternatives in malignant peripheral nerve sheath tumors. J Transl Med. 2013 Sep 17; 11: 213. doi: 10.1186/1479-5876-11-213.

4. *Fang Y., Elahi A., Denley R.C., Rao P.H., Brennan M.F., Jhanwar S.C.* Molecular characterization of permanent cell lines from primary, metastatic and recurrent malignant peripheral nerve sheath tumors (MPNST) with underlying neurofibromatosis type 1. Anticancer Res. 2009 Apr; 29(4): 1255-62.

5. *Bryant H.E., Schultz N., Thomas H.D., Parker K.M., Flower D., Lopez E., Kyle S., Meuth M., Curtin N.J., Helleday T.* Specific killing of *BRCA2*-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005 Apr 14; 434(7035): 913-7.

MORPHOLOGICAL CHANGES OF THE TRANSPLANTED KIDNEY TUMORS AFTER ADMINISTRATION OF FLAVONOID-CONTAINING EXTRACT GRATIOLA OFFICINALIS L.

N.A. Navolokin, A.B. Bucharskaya, D.A. Mudrak, N.V. Polukonova, G.N. Maslyakova

Saratov State Medical University named after V. I. Razumovsky, Saratov, Russia

The antitumor activity of flavonoid-containing extract of Gratiola officinalis was examined in vivo by oral and intramuscular administration in a dosage of 110 mg/kg. The white outbred rats with transplanted kidney tumors were investigated by morphological and biochemical methods. Tumor growth inhibition index was up to 71%. The morphological changes in tumor tissue are more pronounced after intramuscular administration of extract with marked necrobiotic and atrophic changes in the tumor cells, proliferative activity of tumor cells have disappeared (proliferation index Ki67 was equal to 0 , compared with group without any treatment , where it was equal to 48%). We also observed the absence of mitosis and decrease of nuclear RNA expression. These facts confirm the lock mitotic processes at the nuclear level. Thus, Gratiola officinalis's extract induces the pathomorphosis of kidney tumor more pronounced at intramuscular administration.

Keywords: bioflavonoids, extract of Gratiola officinalis, pathomorphosis, kidney tumor, apoptosis.

Relevance

Oncological diseases are the leading cause of death in the world. However, there are no currently safe anticancer drugs that have no side effects. In this regard, the search for anticancer drugs based on plant extracts with minimal side effects is of particular relevance [1, 2]. We developed the author's method of obtaining flavonoid-containing extract from Gratiola officinalis flowers and leaves, which demonstrated anti-inflammatory, antioxidant and immunomodulating properties [3-10].

The aim of the study was to investigate the antitumor activity of the flavonoid-containing extract of Gratiola officinalis and its effect on the transplanted kidney tumors in rats.

Material and Methods

30 male white outbred rats weighing 150 ± 50 g were subcutaneously injected into the scapula region with 0.5 ml of 25% tumor cells solution. The animals were divided randomly into 3 groups - 2 experimental and a comparison group (10 rats in each group). The comparison group consisted of animals with transplanted tumors, but without any exposure. 72 hours after transplantation, the experimental groups of rats received perorally or intramuscularly respectively daily for 2 weeks extract of Gratiola officinalis in a dosage of 110 mg/kg. After administration, all animals were withdrawn from the experiment and tumor samples were taken. Standard morphological methods, histochemical staining and immunohistochemical staining using markers Ki67 and p53 were used.

Results

In the comparison group, the tumor weigh at the end of the experiment was 11.52±2.34. The tumor growth inhibition index by weight of the tumor was up 71% for oral administration (3.29±1.23 g) and 73% for the intramuscular administration (3.29 ± 1.23 g) of the extract.

Histological examination revealed extensive zones of necrosis and dystrophic changes of tumor cells under the influence of Gratiola officinalis extract, apoptotic corpuscles and positive expression of the p53 marker were also determined after extract administration (Figure 1).

The expression of nuclear RNA in tumor tissue was reduced after both oral and intramuscular administration that might indicate a decrease in its transcriptional activity.

At immunohistochemical staining using Ki67, the proliferative activity of tumor cells disappeared after extract administration. The proliferation index was equal to 0, compared with the group without any treatment, where it was equal to 48%).

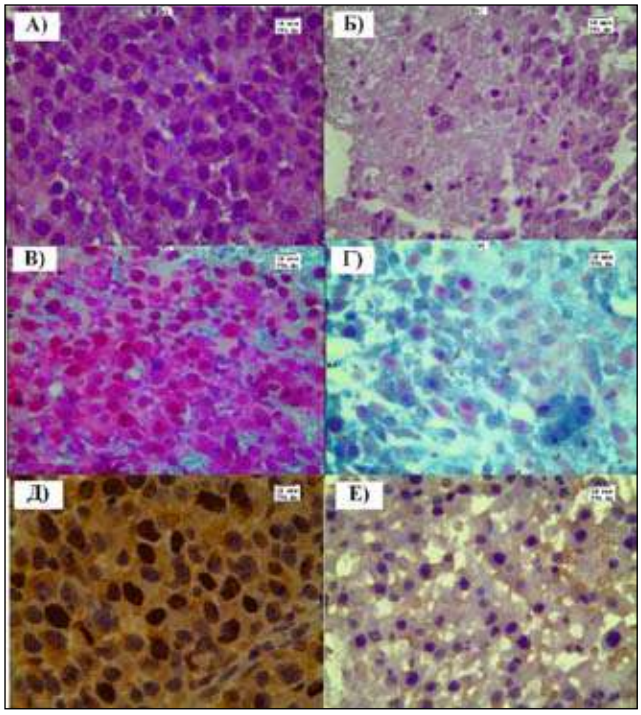


Figure 1. (А) comparison group, hematoxylin and eosin, ×774; (Б) after intramuscular administration of extract, hematoxylin and eosin, ×774; (В) comparison group, histochemical staining of DNA and RNA; (Г) - expression of color on DNA by intramuscular introduction of the extract (Д) comparison group, nuclear expression of Ki67 of tumor cells; (Е) after intramuscular administration of Gratiola officinalis extract, absence of Ki67 expression.

Conclusion

The intramuscular administration of Gratiola officinalis extract causes inhibition of tumor growth in rats with transplanted kidney cancer and leads to the development of necrobiotic and dystrophic processes in tumors, the appearance of apoptosis, a decrease in proliferative activity in tumor cells.

The work of NVP was funded by RFBR according to the research project № 18-015-00298 A. The work of NAN was supported by state assignment of Russian Ministry of Health.

REFERENCES

1. *Goldberg E.D., Razin TG, Zueva E.P.* Plants in complex therapy of tumors Moscow: Publishing house RAMS, 2008. 232. [in Russian]
2. *Mironov A.N., Bunatyan N.D., Vasiliev A.N.* Guidelines for preclinical drug research. Part one. Moscow: Grif and K; 2012. 855. [in Russian]
3. *Navolokin N.A., Mudrak D.A., Polukonova N.V., Tychina S.A., Korchakov N.B., Bucharskaya A.B., Maslyakova G.N.* Evaluation of the antitumor and anticachexia activity of gratiola officinalis l. extract in rats with transplanted sarcoma 45. Siberian Journal of Oncology. 2016; 15(1): 37-43. doi: 10.21294/1814-4861-2016-15-1-37-43. [in Russian]
4. Navolokin N.A., Polukonova N.V., Maslyakova G.N., Bucharskaya A.B., Durnova N.A. The morphology of internal organs and tumors in laboratory rats with transplanted liver cancer Pc-1 when administered orally flavonoid containing extracts of Gratiola Officinalis L. and Zea Mays L. Saratov Journal of Medical Scientific. 2013; 9(2): 213-220. [in Russian]
5. *Navolokin N.A., Skvortsova V.V., Polukonova N.V., Manaenkova E.V., Pankratova L.E.* Antituberculous in vitro activity of Gratiola officinalis extract Russian Journal of Experimental and Clinical Pharmacology. 2015; 78: 10–13. [in Russian]
6. *Polukonova N.V., Navolokin N.A., Rajkova S.V., Maslyakova G.N.* Anti-inflammatory, antipyretic and antimicrobial activity of flavonoid-containing extract of Gratiola officinalis l. Experimental and Clinical Pharmacology. 2015; 78 (1): 34-38.
7. *Navolokin N.A., Polukonova N.V., Maslyakova G.N., Bucharskaja A.B.* Effect of extracts of Gratiola officinalis and Zea mays on the tumor and the morphology of the internal organs of rats with trasplanted liver cancer. Russian Open Medical Journal. 2012; 1: 0203. doi: 10.15275/rusomj.2012.0203
8. *Polukonova N.V., Kurchatova M.N., Navolokin N.A., Bucharskaja A.B., D.A. Dronova.* A new extraction method of bioflavanoids from poisonous plant (Gratiola officinalis L.). Russian Open Medical Journal. 2014; (3): 0304. doi: 10.15275/rusomj.2014.0304.
9. *Tkachenko N., Pravdin A., Terentyuk G., Navolokin N., Kurchatova M., Polukonova N.* Inhibiton of photodynamic haemolysis by gratiola officinalis l. extract. Proc. SPIE. 2015; 9448: 94480P. doi: 10.1117/12.2179862.
10. *Tkachenko N.V., Bykova E.V., Pravdin A.B., Navolokin N.A., Polukonova N.V., Bucharskaya A.B., Prilepskii A.Y.* Comparison of membrane-protective activity of antioxidants quercetine and Gratiola Officinalis L. extract under conditions of photodynamic haemolysis. Proc. SPIE. 2016; 9917: 99170L. doi:10.1117/12.2228291.

ASSOCIATION OF SURVIVAL OF PATIENTS WITH UVEAL MELANOMA WITH MOLECULAR GENETIC ABERRATIONS: LONG-TERM FOLLOW-UP

V.V. Neroev[1,2], S.V. Saakyan[1,2], A.G. Amiryan[1], A.Iu. Tsygankov[1,2], A.M. Burdenny[3], V.I. Loginov[3], M.R. Khlgatyan[1]

Moscow Helmholtz Research Institute of Eye Diseases, Moscow, Russia[1]
Medical State University of Medicine and Dentistry, Moscow, Russia[2]
Institute of General Pathology and Pathophysiology, Moscow, Russia[3]

Aim: to determine the survival of patients with UM after enucleation, depending on molecular - genetic aberrations. **Material and Methods**. 30 patients with UM at the age from 23 to 83 years were examined and treated. In all cases, enucleation was performed. The removed eyes underwent morphological and molecular genetic and cytogenetic analysis (loss of heterozygosity on chromosomes 1, 3 and 8, methylation of the *RASSF1A* gene, mutations in *GNAQ/11* genes, polymorphism of the *ABCB1* gene). The median follow-up was 61 months. **Results**. The overall 3-year survival rate of patients with UM was 77.8% ± 8.0%, and the 5-year survival rate was 63.0% ± 9.0%. The average survival time was 52.8 months ± 3.9 months. Patients with chromosome 3 monosomy showed significantly lower 5-year survival rates than patients with partial monosomy and without loss of heterozygosity in chromosome 3 (Log-rank test, $\chi 2 = 14{,}111$, $p = 0.001$). The loss of heterozygosity on chromosomes 1 and 8, the methylation of the *RASSF1A* gene, the mutations in *GNAQ/11* genes and the polymorphism of the *ABCB1* gene were not associated with the worst vital prognosis. **Conclusion**. Molecular - genetic aberrations play an important role in predicting the course of the tumor process and determining the risk of hematogenous metastasis in patients with UM. The significant role of monosomy of chromosome 3 has been proved. Due to the relatively small cohort (30 patients) and the time factor (analysis of 5-year survival), the role of other molecular genetic changes has not confirmed, therefore, the assessment of not only genetic, but also clinical, echographical and morphological prognostic factors, is required.

Keywords: uveal melanoma, genetics, survival, molecular genetic changes, monosomy of chromosome 3, deletion of chromosome 1, deletion of chromosome 8, *RASSF1A* methylation, *GNAQ / GNA11* gene mutations, *ABCB1* gene polymorphism.

Introduction

In recent years, molecular genetic prognostic factors for uveal melanoma (UM) have become very important. It should be taken into account that UM is not a monogenic but a multifactorial disease with a complex molecular structure. In clinical practice, molecular genetic methods are used to identify patients with a high risk of developing metastases [1]. Despite the possibility of achieving good short-and medium-term local tumor control and in some cases, long-term follow-up, the incidence of metastasis remains about 50% after 5 years regardless of treatment [2]. To predict the course of the disease, several clinical, histological and genetic factors have been identified, the most important of which is monosomy of chromosome 3 and a specific set of tumor genes [3].

Aim: to determine the survival of patients with UM after enucleation, depending on molecular - genetic aberrations.

Material and Methods

The study included 30 patients with UM at the age of 23 to 83 years (mean 51.3 ± 12), 18 (60%) women and 12 (40%) men treated in 2012. Prominence of UM varied from 2.6 to 13.8 mm (average - 8.6 ± 2.9 mm), the basal diameter - from 8.2 to 21.9 mm (average - 15 ± 3.4 mm). In all cases, enucleation was performed. The removed eyes underwent morphological and molecular genetic and cytogenetic analysis (loss of heterozygosity on chromosomes 1, 3 and 8, methylation of the *RASSF1A* gene, mutations in *GNAQ*/*11*genes, polymorphism of the *ABCB1* gene). According to pathohistological data, spindle cell type of melanoma was established in 16 (53.3%) patients, mixed cell type in 9 (30%) patients and epithelioid cell type in 5 (16.7%). The median follow-up was 61 months. For this period of observation, 10 people (1/3) died from metastatic disease. The statistical analysis was carried out using the packages of Microsoft Windows® 7, IBM SPSS Statistics 23.0.

Results

The 3-year survival rate of UM patients was 77.8% ± 8.0%, and the 5-year survival was 63.0%±9.0%. The mean survival time was 52.8 months ± 3.9 months [95% CI, 50.5-65.9]. Patients with full monosomy of chromosome 3 showed significantly lower 5-year survival rates than patients with partial monosomy (log-rank test, $\chi 2=14,111$, $p=0.001$). Monosomy of chromosome 3 was determined in 12 patients. Loss of heterozygosity on chromosome 1p was detected in 9 patients with UM, 4 of which died from distant metastases. The overall survival rate in patients without loss of heterozygosity on chromosome 1p after 5 years of dynamic observation was 66.7% ± 11.0%, and in patients with loss of heterozygosity on chromosome 1p - 50.0% ± 18.0%. The mean survival time in patients without and with loss of heterozygosity on chromosome 1p was 60.1 months ± 3.13 months [95% CI, 54.0-66.2] and 44.8 months ± 8.8 months [95 % CI, 27.4-62.1], respectively. Cumulative proportion of survivors under 5-year observation in patients without loss of heterozygosity on chromosome 8p was 63.6% ± 0.10, and in patients with loss of heterozygosity - 50.5 % ± 0.25. When analyzing the mean survival time, in patients without loss of heterozygosity on chromosome 8p, it was 56.1 months ± 4.2 months [95% CI, 47.9-64.3], and in patients with heterozygosity loss 53.3 months ± 8.8 months [95% CI, 36.0-70.5]. Of 10 deaths from metastases, RASSF1A gene methylation was determined only in 3 patients. The accumulated proportion of survivors under 5-year follow-up in patients with the presence of RASSF1A gene methylation was 62.5% ± 17.0%, and in patients without RASSF1A gene methylation 63.2% ± 11.0% ($p\leq0.05$). The cumulative proportion of survivors in the absence of a mutation in the GNAQ gene (Gln209) was 63.6% ± 10.0%, while in patients with a mutation in the exon it was 50.0% ± 25.0%. Mean values for survival time were 54.6 months ± 4.4 months [95% CI, 46.0-63.2] and 62.2 months ± 3.3 months [95% CI, 55.5-68.5] in patients having no mutation in the GNAQ gene (Gln209) and in patients with this mutation, respectively. Survival analysis revealed no significant differences in survival curves in patients with GNAQ gene mutations. The accumulated proportions of survivors were 46.2% ± 13.8% and 76.9% ± 11.7% in the group of patints without mutation of the GNA11 gene (Gln209) and in the group of patients with the mutation of the GNA11 gene, respectively. Survival time was 53.2 months ± 4.8 months [95% CI, 43.7-62.6] in patients without mutations and 58.1 months ± 5.7 months [46.8-69.3] in patients having mutations. The accumulated proportions of survivors under 5-year follow-up in patients with the CC and CT genotypes of polymorphic marker C3435T of the ABCB1 gene were nearly identical and amounted to 62.5% ± 12.0% and 60.0% ±

15.0%, respectively. In this case, the median survival of patients with UM with the CC genotype was 56.6 months ± 4.2 months [95% CI, 48.4-64.8], and the median survival of patients with the CT genotype was 53.3 months ± 7.1 [95% CI, 39.7-67.5]. Thus, it was not possible to reveal statistical differences in the 5-year survival of patients depending on the CC and CT genotype.

Conclusion

The molecular-genetic aberrations play an important role in predicting the risk of hematogenous metastasis in patients with UM. A significant role of monosomy of chromosome 3 has been proved. Due to the relatively small cohort (30 patients) and the time factor (analysis of 5-year survival), the role of other molecular genetic changes has not confirmed, thus requiring the assessment of not only genetic, but also clinical, echographical and morphological prognostic factors.

REFERENCES

1. *Saakyan S.V., Amiryan A.G., Tsygankov A.Iu., Sklyarova N.V., Zaletaev D.V.* Clinical, pathomorphological and molecular genetic aspects of uveal melanoma with high metastatic risk. Russian ophthalmological journal. 2015; 9(2): 47-52 [in Russian].

2. *Damato B.E., Heimann H., Kalirai H., Coupland S.E.* Age, survival predictors, and metastatic death in patients with choroidal melanoma. JAMA Ophthalmol. 2014; 132(5): 605-613. doi: 10.1001/jamaophthalmol.2014.77.

3. *Saakyan S.V., Tsygankov A.Iu., Amiryan A.G., Loginov V.I., Burdennyy A.M.* Loss of heterozygosity on chromosomes 1, 3 and 8 in uveal melanoma patients. Molecular medicine. 2018; 16 (2): 37–40. doi: 10.29296/24999490-2018-02-05 [in Russian].

INVESTIGATION OF THE ASSOCIATION OF POLYMORPHIC VARIANTS 481C> T, 590G> A AND 857G> A OF N-ACETYLTRANSFERASE 2 GENE (*NAT2*) WITH THE DEVELOPMENT OF LUNG CANCER IN THE YAKUTS

V.M. Nikolaev[1], F.V. Vinokurova[1], S.I. Sofronova[1], S.D. Efremova[1], S.A. Fedorova[1]

Yakut Scientific Center of Complex Medical Problems, Yakutsk, Russia

The associations of polymorphic variants 481C> T, 590G> A and 857G> A of *NAT2* gene with development of lung cancer in Yakutia have been studied for the first time. Genetic markers of increased and decreased risk of lung cancer in the Yakuts have been identified. It has been revealed that the markers of increased risk of developing lung cancer for the Yakuts are the NAT2 * 857A allele and the NAT2 * 857G/A genotype, the NAT2 * 857G allele, the NAT2 * 857G/G genotype are markers of reduced risk.

Keywords: lung cancer, polymorphic variant, N-acetyltransferase-2.

Relevance

Lung cancer, like many other oncological diseases, is a multifactorial disease, in the development of which an important role is played by both external (smoking, asbestos, radon, arsenic, etc.) and genetic factors. Some authors have shown that the polymorphic variants of the *NAT2* gene contribute to the development of oncological diseases, including lung cancer [1, 2].

The *NAT2* gene is localized on the short arm of chromosome 8 (8p23.1), has a length of about 9900 bp, contains 2 exons and is predominantly expressed in the liver and intestine. The N-acetyltransferase-2 enzyme, encoded by this gene, is a 33 kD protein consisting of 290 amino acid residues. This enzyme, localized in the cytoplasm, participates in the biotransformation process of aromatic amines that are present in the environment. The source of aromatic amines is industrial waste, pollution of water, air, and a number of medicines [3, 4].

Material and Methods

We examined 60 patients with lung cancer (43 male and 17 female), Yakut ethnicity, who received treatment in the Republican oncological dispensary of Yakutsk, Republic Sakha (Yakutia), Russia. The mean age of the patients was 58.86 ± 8.72 yrs. As a control, a group of healthy individuals without cancer, corresponding to a group of patients on ethnic origin and sex, consisting of 60 people (mean age 49.5 ± 5.75) was studied.

Results

In order to identify possible associations of polymorphic variants of the *NAT2* gene with the development of lung cancer, we performed an analysis of the frequency distribution of alleles and genotypes of the polymorphic variants 481C> T and 590G> A in patients with lung cancer and in people without oncological diseases. We did not find statistically significant differences in the distribution of frequencies of alleles and genotypes between the control group and the group of patients with lung cancer.

The most statistically significant differences in the frequencies of alleles and genotypes were observed by the polymorphic variant 857G> A. In patients compared with healthy ones, there is a decrease in the incidence of mutant NAT2 * 857G allele - 64.2% and 78.3%, respectively, χ^2 = 42.52; p = 0.000 ...; OR = 2.02; 95% CI = 1.10-3.78 and an increase in the frequency of wild NAT2 * 857A allele-35.8%, 21.7%, respectively χ^2 = 42.52; p = 0.000 ...; OR = 6.47; 95% CI = 3.52-11.98.

In the group of patients, the frequency of the NAT2 * 857A allele (35.8%, χ^2 = 42.52, p = 0.000 ..., OR = 6.47, 95% CI = 3.52-11.98), and the heterozygous genotype NAT2 * 857G/A (71.6%, χ^2 = 13.43, p = 0.0002, OR = 0.23, 95% CI = 0.10-0.53), but the frequency of homozygous genotype decreased NAT2 * 857G/G (28.4%, χ^2 = 10.95, p = 0.0009, OR = 3.79, 95% CI = 1.66-8.78) compared with the control - 21.7%, 36.6% and 60.1% respectively.

Conclusion

In the analysis of associations of polymorphic variants 481C> T, 590G> A and 857G> A of the *NAT2* gene with the development of lung cancer in Yakutia, allelic variants and genotypes of the *NAT2* gene were established, contributing to the development of lung cancer in individuals of Yakut ethnicity. The markers of increased risk of developing lung cancer in the Yakuts are the NAT2 * 857A allele and the NAT2 * 857G/A genotype; the NAT2 * 857G allele, the NAT2 * 857G/G genotype are low-risk markers.

REFERENCES

1. *Fraumeni J.F.* Respiratory carcinogenesis: an epidemiologic appraisal. J Natl Cancer Inst. 1975 Nov; 55(5): 1039−46.

2. *Janerich D.T., Thompson W.D., Varela L.R., Greenwald P., Chorost S., Tucci C., Zaman M.B., Melamed M.R., Kiely M., McKneally M.F.* Lung cancer and exposure to tobacco smoke in the household. The New England Journal of Medicine. 1990 Sep 6; 323(10): 632-6. doi: 10.1056/NEJM199009063231003.

3. *Glotov A.S., Gra O.A., Goldenkova-Pavlova I.V., Bruskin S.A., Agafonova E.E., Markarova E.V., Abdeev R.M., Korsunskaia I.M., Piruzian A.L., Barskiĭ V.E., Zasedatelev A.S., Nasedkin T.V.* Analysis of NAT2 Point Mutations with Biological Microchips, Molecular Biology. 2007 Jul-Aug; 41(4): 725-33.

4. *Pompeo F., Brooke E., Kawamura A., Mushtaq A., Sim E.* The pharmacogenetics of NAT: structural Paspects. Pharmacogenomics. 2002 Jan; 3(1): 19-30. doi: 10.1517/14622416.3.1.19.

GLUTATHIONE AND HEAT SHOCK PROTEINS 27 AND 70 AS MOLECULAR TARGETS FOR REGULATION OF APOPTOSIS IN JURKAT CELLS

O.L. Nosareva, E.A. Stepovaya, E.V. Shakhristova

Siberian State Medical University, Tomsk, Russia

The article presents the data on the role of reduced and oxidized glutathione and heat shock proteins 27 and 70 in regulating apoptosis in Jurkat cells.

Keywords: Jukart tumor cells, apoptosis, oxidative stress, glutathione, Hsp 27, Hsp 70, refolding.

Rationale

Currently, identification of molecular mechanisms and molecular targets of apoptosis regulation is a crucial area of fundamental research. In the pathogenesis of tumor growth, impairment of programmed cell death is associated with development of oxidative stress and accumulation of reactive oxygen species (ROS). The latter causes the oxidative modification of protein and lipid molecules, damage to DNA, and damage to the cell membrane structure. Proteins act as key traps for ROS due to the presence of sulfur-containing and aromatic amino acids in the structure of their polypeptide chain. The interaction between the cell proteome and ROS results in oxidative modifications that change the functional activity of proteins. To ensure vital activity of tumor cells

under oxidative stress, refolding of proteins that are involved in metabolism is warranted along with proper functioning of the antioxidant protection system, the glutathione-dependent system, in particular [1]. Consequently, the functional state and survival of the tumor cell directly depend on the redox balance and activity of heat shock proteins. According to modern understanding, proteins of the Hsp 70 family have enzymatic properties that can alter conformational changes in proteins by means of ATP energy. Besides, they contribute to transmembrane translocation of proteins and are among the first to react to oxidative stress development [2]. Refolding of proteins by means of Hsp 70 is more efficient after protein aggregates associate with low-molecular-weight heat shock proteins, Hsp 27, in particular [3].

The aim of this work is to establish a relationship between the changes in the reduced and oxidized glutathione concentrations and Hsp 70 and Hsp 27 levels and implementation of apoptosis in Jurkat cells.

Material and Methods

The study was carried out on Jurkat cells ("human T-lymphoblastic leukemia") incubated in the semi-open system at +37 °C with 5% CO2 in sterile conditions in the complete growth medium RPMI-1640 and intact blood lymphocytes isolated from the mononuclear leukocyte fraction of healthy donors (8 men and 8 women aged 20-45). In the experiment we used the cell cultures containing not more than 5% of dead cells. Cell vital activity was evaluated by microscopy with the 0.4% tryptan blue solution ("Serva", USA).

Qualitative assessment of the cells that entered apoptosis was conducted by flow cytometry with Annexin V-FITC, and propidium iodide ("TREVIGEN", USA). The intracellular ROS level was evaluated with 2,7-dichlorofluorescein diacetate ("Sigma-Aldrich", USA), in accordance with the manufacturer's instructions.

The levels of reduced and oxidized glutathione were measured by spectrophotometry with recirculation of the enzyme and blocking of the -SH group of reduced glutathione with 2-vinylpyridine ("Wako", Japan) [4]. The heat shock protein concentration was determined by Western blot analysis with phospho-Hsp 27 ("Abcam", USA) and Hsp 70 ("R&D Systems", USA) primary monoclonal antibodies, according to the manufacturer's instructions. The conclusion about the level of the studied protein in the cell was made according to the changes in the sought protein labeling signal β-actin cytoskeleton signal ratio ("Sigma-Aldrich", USA) [5].

The normality of quantitative data distribution was tested using the Shapiro-Wilk test. The statistical significance of the independent samples was evaluated by the nonparametric Mann-Whitney test. The Spearman's rank correlation technique was applied to establish a relationship between the variables. The differences were considered statistically significant at $p<0.05$.

Results and Discussion

Following the conducted study, we pointed out a change in the pro/antioxidant ratio under tumor progression. Thus, in the Jurkat cells the level of ROS was 6.2 times higher ($p<0.05$), the concentration of reduced glutathione was 2.7 times higher ($p<0.05$) and the level of oxidized thiol was 1.5 times higher ($p<0.05$) than similar parameters in the intact blood lymphocytes. The glutathione system makes an essential contribution to maintaining cellular redox-status. Its key component is reduced glutathione, which acts as a non-enzymatic trap for free radicals.

Under generated oxidative stress, the Hsp 27 level in the Jurkat cells was 1.9 times higher ($p<0.05$) and the level of Hsp 70 was 2.9 times higher ($p<0.05$) than the same parameters in the intact blood lymphocytes. It proved the need for refolding of oxidatively modified proteins. In the meantime, the Jurkat cells were characterized by inhibition of apoptosis. We obtained 4.4 times ($p<0.05$) less Annexin V positive cells among the Jurkat cells, as opposed to the intact blood lymphocytes. Following the correlational analysis of the obtained findings, we identified a negative relationship between the number of Annexin V positive cells and the Hsp 27 level in the Jurkat cells ($r = -0.67$; $p<0.05$).

Conclusions

Therefore, the components of the glutathione system and heat shock proteins 27 and 70 are potential targets for regulation of apoptosis in Jurkat cells.

REFERENCES

1. *Nosareva O.L., Stepovaya E.A., Ryazantseva N.V., Shakhristova E.V., Egorova M.Y., Novitsky V.V.* The role of the glutathione system in oxidative modification of proteins and dysregulation of apoptosis in Jurkat cells. Bull. Exp. Biol. Med. 2017 Dec; 164(2): 199-202. doi: 10.1007/s10517-017-3957-x.

2. *Shuhong G., Wharton W., Moseley P., Shi H.* Heat shock protein 70 regulates cellular redox status by modulating glutathione-related enzyme activities. Cell Stress Chaperones. 2007. Autumn; 12(3): 245-54. doi:10.1379/CSC-265.1.

3. *Haslbeck M., Franzmann T., Weinfurtner D., Buchner J.* Some like it hot: the structure and function of small heat-shock proteins. Nat. Struct. Mol. Biol. 2005. Oct; 12(10): 842-6. doi:10.1038/nsmb993.

4. *Kojima S., Nakayama K., Ishida H.* Low dose gamma-rays activate immune functions via induction of glutathione and delay tumor growth. J. Radiat. Res. 2004. Mar; 45(1): 33-9.
5. *Nosareva O.L., Stepovaya E.A., Ryazantseva N.V., Zakirova E.V., Mazunin I.O., Litvinova L.S., Sokhonevich N.A., Vesnina O.N., Shakhristova E.V.* Disruption of expression of mRNA Hsp27 and ubiquitin as a mechanism of escaping from apotosis of Jurkat line tumor cells. Bulletin of Siberian Medicine. 2015. 14(1): 66-72.

INTRAOPERATIVE RADIOTHERAPY IN COMBINED TREATMENT OF SINONASAL CARCINOMA

V.A. Novikov[1], R.V. Vasiljev[1,2], O.V. Gribova[1], E.L. Choynzonov[1], V.I. Shtin[1], P.V. Surkova[1], Zh.A. Startseva[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University of Control Systems and Radioelectronics, Tomsk, Russia[2]

Intraoperative radiation therapy allows a higher radiation dose to be delivered to the tumor bed, while sparing normal surrounding tissues and reducing the risk of fibrosis. Sinonasal tumors represent a convenient object for intraoperative radiotherapy application (surface location, relatively small size tumors, good surgical access). Treatment was conducted using a mobile compact betatron (MIB-6E) using a 10-12 Gy single radiation dose. There were no pathological clinical and laboratory reactions to IORT in the early postoperative period. Electron-beam IORT allowed prevention of radiation side effects and achievement of the 5-year- disease-free survival in 66% patients.

Keywords: IORT, sinonasal carcinoma, head and neck tumors.

Introduction

Sinonasal tumors are a highly heterogeneous group of tumors that account for less than 1% of all cancers. Despite advances in diagnosis and cancer treatment, early detection of nasal cavity and paranasal sinuse cancers remains challenging. More than 80% of patients are diagnosed with locally advanced forms of the disease (T3 – T4), requiring a combined approach to treatment [1-3].

Large, multi-center clinical trials have shown the benefit of combination radiotherapy and radical surgery [4-7]. Despite aggressive tactics, long-term treatment outcomes remain unsatisfactory: five-year survival rates range from 37 to 60%, local relapses are observed within the first two years in 50-80% of cases. Currently, intraoperative radiotherapy (IORT) is widely used in the treatment of different types of malignant tumors. It allows a large single dose of irradiation to be delivered directly to the tumor or to the «bed» of the removed tumor. Electron beams of different energies produced by acelerators or betatrons are used for IORT. To date, a special equipment for performing IORT for cancer patients have created in a number of countries in the United States, Italy, Germany, Russia [7-9].

Fast electrons have been used in clinical radiology for more than half a century and have a number of important advantages due to the physical characteristics of electron radiation therapy: the depth of penetration of electrons is proportional to the average energy and can be adjusted depending on the task; the maximum of absorbed dose is at a given depth; a sharp drop of the dose of fast electrons after reaching a maximum, which significantly reduces the irradiation of adjacent healthy tissues

Material and Methods

Forty-three patients with newly diagnosed malignant tumors of the nasal cavity and paranasal sinuses received combined modality treatment. Preoperative hypofractionated external beam radiation therapy (EBRT) was given at a daily dose of 3 Gy, 5 fractions per week to a total focal dose of 36 Gy (isoeffective dose = 44 Gy). Surgery with 10Gy IORT was performed 5-10 days after the completion of preoperative radiotherapy. The total EBRT and IORT dose in this group of patients was 60-65 Gy, i.e. it corresponded to the dose of the radical course. Intraoperative radiotherapy was performed using a small-size betatron (MIB-6E) with the energy of 6 MeV.

Results

The treatment was well tolerated in all patients. Preoperative EBRT in combination with IORT had no adverse effect on the healing process of postoperative wounds, the frequency and severity of local complications did not differ significantly from those in the control group. Radiation-induced brain injury was not observed in the immediate postoperative period and in the long term follow-up. The analysis of relapses after combined treatment with IORT and EBRT showed that the additional irradiation of the «risk» zones in the operating cavity resulted in reduction in the

frequency of relapses in these zones and significantly improved survival rates of patients. The 5-year recurrence-free survival rates in the study and control groups were 66.2 ± 8.8% and 40 ± 9.2%, respectively.

Conclusion

The developed method of combined treatment of tumors of the nasal cavity and paranasal sinuses resulted in reduction in the frequency of relapses and increase in the life expectancy of patients. The IORT session prolonged surgery for 30 minutes. No pathological clinical and laboratory reactions to the IORT in the early postoperative period were observed. The procedure is possible to perform in a standard operating unit, does not require the use of special security measures for the patient and staff. Electrn-beam IORT made it possible to avoide radiation-induced reactions and to achieve the 5-year recurrence-free survival in 66% of the patients.

The study was supported by a grant from the Russian Science Foundation (project № 16-15-00038).

REFERENCES

1. *Shah J.P., Patel S.G., Singh B.* Head and neck surgery and oncology. Elsevier Health Sciences. 2013. 104-140.
2. *Gribova O.V., Musabaeva L.I., Choinzonov E.L., Novikov V.A., Lisin V.A.* The use of fast neutrons in treatment of malignant tumors of the head and neck. Voprosy onkologii. 2015; 1: 149-153. [in Russian].
3. *Velikaya V.V., Gribova O.V., Musabaeva L.I., Startseva Zh.A., Simonov K.A., Aleinik A.N., Lisin V.A.* Ozone therapy for radiation reactions and skin lesions after neutron therapy in patients with malignant tumors. Voprosy onkologii. 2015; 61(4): 571-574. [in Russian].
4. *Mallick S., Benson R., Julka P.K., Rath G.K.* Altered fractionation radiotherapy in head and neck squamous cell carcinoma. J Egypt Natl Canc Inst. 2016 Jun; 28(2): 73-80. doi: 10.1016/j.jnci.2016.02.004.
5. *Ashraf M., Biswas J., Dam A., Bhowmick A., Jha., Sing V., Nayak S.* Results of Treatment of Squamous Cell Carcinoma of Maxillary Sinus: A 26-Year Experience. World J Oncol. 2010 Feb; 1(1): 28-34. doi: 10.4021/wjon2010.02.191w.
6. *Won H.S., Chun S.H., Kim B., Chung S.R., Yoo I.R., Jung C.-K., Kim Y.-S., Sun D., Kim M.S., Kang J.-H.* Treatment outcome of maxillary sinus cancer. Rare Tumors. 2009; 1(2): e36. doi: 10.4081/rt.2009.e36.
7. *Zavyalov A.A., Musabaeva L.I., Lisin V.A., Choinzonov E.L., Novikov V.A., Kolomiets L.A., Tuzikov S.A., Afanasyev S.G., Dubski C.V., Anisenya I.I., Tyukalov Yu.I., Miller S.V., Dobrodeev A.Yu., Chivchish L.N., Nechitailo M.N., Geravin A.A.* Pyatnadtsatiletnii opyt primeneniya intraoperatsionnoi luchevoi terapii. Siberian Journal of Oncology. 2004; 2-3: 75-84. [in Russian]
8. *Calvo F.A., Hoekstra H.J., Lehnert T.* Intraoperative radiotherapy: 20 years of clinical experience, technological development and consolidation of results. European journal of surgical oncology: the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2000; 26: S1-4.
9. *Azinovic I., Martinez Monge R., Javier Aristu J., Salgado E., Villafranca E., Fernandez Hidalgo O., Amillo S., San Julian M., Villas C., Manuel Aramendía J., Calvo F.A.* Intraoperative radiotherapy electron boost followed by moderate doses of external beam radiotherapy in resected soft-tissue sarcoma of the extremities. Radiother Oncol. 2003 Jun; 67(3): 331-7.

INTRAOPERATIVE IMAGING OF SENTINEL LYMPH NODES IN ENDOMETRIAL CANCER USING ALOTECH, A NEW RADIOPHARMACEUTICAL

M.O. Ochirov[1], L.A. Kolomiets[1,2], V.I. Chernov[1,3]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Siberian State Medical University, Tomsk, Russia[2]
National Research Tomsk Polytechnic University, Tomsk, Russia[3]

Purpose. To evaluate the effectiveness of intraoperative imaging of sentinel lymph nodes (SLN) in patients with stage I endometrial cancer using ^{99m}Tc-Al_2O_3 (Alotech), a new radiopharmaceutical. **Material and Methods**. The study included endometrial cancer patients treated at the Gynecological Department of the Cancer Research Institute, Tomsk National Research Medical Center in the time period from 2017 to 2018. Alotech, a new radiopharmaceutical, was used for identification of sentinel lymph nodes in all patients. **Results.** The measurements of local concentrations of Alotech were carried out in 46 patients. The average accumulation of ^{99}mTc-Al_2O_3 in SLN was 6% by SPECT and 8% by gamma probe. In intraoperative detection of SLN, bilateral accumulation of the radiopharmaceutical was revealed in 37% of patients (n=17), unilateral in 56.5% (n=26). **Conclusion.** The use of Alotech allows successful intraoperative assessment of sentinel lymph nodes due to the high level of accumulation in SLNs of patients with endometrial cancer. SPECT and intraoperative gamma probe-guided identification performed after injection of ^{99m}Tc-Al_2O_3 enable the detection of SLNs with 93.4 % sensitivity.

Keywords: endometrial cancer, sentinel lymph nodes, radiopharmaceutical.

Endometrial cancer is the most common gynecologic cancer in Russia. More than 24,000 new cases are diagnosed annually. From 2005 to 2015, the standardized incidence rate per 100,000 was 17.5 [1]. Stage I endometrial cancer was detected in 65.6 % of cases with 5-year survival rate of 90%. However, in patients with lymph node metastases, the 5-year survival rate did not exceed 60% [2].

Detection of sentinel lymph nodes (SLN) is the most important prognostic factor of long-term disease outcome survival [3]. The method of detection of sentinel lymph nodes is included in the recommendations of some cancer societies such as NCCN and EANM as an alternative to lymphadenectomy. The detection of SLNs allows optimization and personalization of surgical treatment as well as evaluation of lymph node status.

The most common method of detecting SLN is the isotope-associated method using radiopaque radioactive lymphotropic colloid substance that can penetrate the walls of the lymph capillaries. Currently, nanocolloid labeled with technetium-99m is widely used for identification of SLN. However, this radiopharmaceutical has several disadvantages: low level of accumulation (1-2%), it can be accumulated in level II-III lymph nodes, and it has not been registered in the territory of the Russian Federation [5].

The purpose of our research was to evaluate the effectiveness of intraoperative imaging of SLN in patients with stage I endometrial cancer using $^{99m}Tc\text{-}Al_2O_3$ (Alotech) radiopharmaceutical.

Material and Methods

The study included patients with stage I endometrial cancer, treated at the Gynecological Department of the Cancer Research Institute, Tomsk National Research Medical Center, in the time period from 2017 to 2018. All patients underwent total hysterectomy with bilateral salpingo-ovariectomy and pelvic lymph node dissection with the detection of SLN. In 75% of cases (n=34), SLNs were detected using a portable gamma scanner GammaFinder II®. The accumulation level of the radiopharmaceutical was measured after removing lymph nodes from the abdominal cavity. The detected SLN were marked separately. In 25% of cases (n=12), SLNs were detected using Read Pointer-gamma, a new system for the detection of SLNs, which allowed the measurement of the accumulation level of the radiopharmaceutical during laparoscopic surgery. The detected SLN were removed from the abdominal cavity and sent for urgent cytological examination.

Results

The mean age of patients was 57±7.2 years. The average accumulation of Alotech in SLN was 6% by SPECT and 8% by gamma probe. Internal iliac vessels were the most common site for SLN detection (37%, n=17), followed by external iliac vessels (18.7%, n=9), and common iliac vessels (17.3%, n=8). A single SLN was located in the same region of the obturator fossa and the aortic bifurcation and iliac arteries. Findings of intraoperative SLN detection were consistent with SPECT results in all patients. In intraoperative detection of SLN, bilateral accumulation of the radiopharmaceutical was revealed in 37% of patients (n=17), unilateral in 56.5% of patients (n=26).

Conclusions

The SLN detection is an alternative to lymphadenectomy. An important aspect is the search for optimal radiopharmaceutical for the detection of SLN in surgical treatment of stage I endometrial cancer. The use of Alotech, a new radiopharmaceutical, allows successful identification of intraoperative assessment of lymph node status, due to the high level of accumulation in the SLN in patients with endometrial cancer. SPECT and intraoperative gamma probe-guided identification performed after injection of $^{99m}Tc\text{-}Al_2O_3$ enabled the detection of SLNs with 93.4 % sensitivity.

REFERENCES

1. *Kaprin A.D., Starinskii V.V., Petrova G.V.* Malignant neoplasms in Russia in 2015 (morbidity and mortality). M.: MNIOI im. P.A. Gertsena – filial FGBU «NMIRTs» Minzdrava Rossii, 2017. 250. [in Russian]

2. *Lewin S.N.* Revised FIGO staging system for endometrial cancer. Clin Obstet Gynecol. 2011 Jun; 54(2): 215-8. doi: 10.1097/GRF.0b013e3182185baa.

3. *Colombo N., Creutzberg C., Amant F., Bosse T., González-Martín A., Ledermann J., Marth C., Nout R., Querleu D., Mirza M.R., Sessa C.; ESMO-ESGO-ESTRO Endometrial Consensus Conference Working Group.* ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment and follow-up. Int J Gynecol Cancer. 2016 Jan; 26(1): 2-30. doi: 10.1097/IGC.0000000000000609.

4. *Kolomiyets L.A., Chernyshova A.L., Churuksaeva O.N., Villert A.B., Molchanov S.V.* Optimization of modern approaches in the treatment of gynaecological cancer of major localizations. Malignant tumors. 2017; 3-S1: 66-72.

5. *Chernov V.I., Afanasyev S.G., Sinilkin I.G., Titskaya A.A., Augustinovich A.V.* Radionuclide diagnosis for detection of sentinel lymph nodes. Siberian Journal of Oncology. 2008; 4(28): 5-10. [in Russian]

MIR-204-5P INHIBITOR APPLICATION AFFECTS MELANOMA CELL PROLIFERATION RATES AND IMMUNOCOMPETENT CELLS PRESENCE IN MELANOMA METASTASIS TARGET ORGAN

N.V. Palkina[1], A.V. Komina[1], M.B. Aksenenko[1], A.S. Moshev[2], A.A. Savchenko[2], T.G. Ruksha[1]

Department of pathophysiology, Krasnoyarsk State Medical University, Krasnoyarsk, Russia[1]
Laboratory of cell molecular physiology and pathology, Federal Research Center «Krasnoyarsk Science Center» of the Siberian Branch of the Russian Academy of Sciences, Krasnoyarsk, Russia[2]

Activating mutations in BRAF, NRAS or KIT genes in melanoma were shown to be important targets for forceful treatment by specific inhibitors though the acquired resistance complicates their efficacy [1]. Therefore the search for novel therapeutic approaches to disseminated forms of melanoma is under study. Previously we demonstrated that microRNA-204-5p (miR-204-5p) expression levels decreased in melanoma as compared to melanocytic nevi. This observation inspired us to perform a functional study on miR.

Melanoma cells BRO and SK-MEL-1 were cultured in RPMI-1640 medium with L-glutamine (Gibco, Life Technologies, Paisley, UK) and 10% fetal bovine serum (Gibco, Life Technologies, Paisley, UK) at 37 °C in 5% CO_2 in CO_2-incubator Sanyo MSO-5AC (Sanyo, Electric Co., Ltd., Osaka, Japan). Flow cytometric analysis of cell apoptosis was provided by the use of Annexin V – FITC/7AAD kit (Immunotech, Beckman Coulter, Marseille, France). Cell viability/cell proliferation rates were evaluated by MTT-test. Migration and invasion experiments were performed with CytoSelect™ 24-Well Cell Migration and Invasion Assay (Cell Biolabs, Inc., San Diego, CA, USA). DIANA-mirPath v.3.0 was used for the KEEG pathway analysis of miRNA signature. MiRNA targets were predicted based on DIANA-microT-CDS. The data on cell proliferation, viability, apoptosis, migration and invasion assay were analyzed by using nonparametric Mann-Withney U-test. The results were considered as significant at $p < 0.05$.

Melanoma cell line B16 was obtained from Research Institute of Fundamental and Clinical Immunology (Novosibirsk, Russia). C57Bl6 mice were obtained from Research Institute of Fundamental and Clinical Immunology (Novosibirsk, Russia). Animals were kept in conditions of natural light without any restrictions on access to water and food. Mice were randomly divided into three groups: control group treated with sterile 0.01M PBS (n=3), negative control group treated with negative control of LNA inhibitor Negative control A miRCURY LNA™ miRNA Inhibitor Control (Exiqon, Vedbaek, Denmark) (n=6), and experimental group which was treated with the inhibitor mmu-miR-204-5p miRCURY LNA™ (Exiqon, Vedbaek, Denmark) (n=5). The solutions were injected intracutaneously on the 7th and 14th day after transfection.

The study was approved by Krasnoyarsk State Medical University Local Ethic committee (protocol № 70/2016 issued by June 6th, 2016).

Bioinformatic analysis showed BCL2, TGFBR1, and SIRT1 as possible gene targets for miR-204-5p, further expression analysis revealed their expression alterations confirming functioning as gene targets for the miR. MiR-204-5p inhibitor application in vitro resulted in diminishing of melanoma cell proliferation and colony formation, stimulated their migration. In vivo study of miR-204-5p inhibitor application revealed transient increase in tumor volume of B16-melanoma bearing mice. Melanoma metastasis target organs were characterized by decreased number of CD45-positive cells.

To assess LNA-inhibitor toxicity for the animal, some parameters were estimated in dynamics during the experiment: weight, physical activity, amounts of food and water consumed. It was shown similarity of these data for the mice treated with LNA-miR-204-5p-inhibitor to ones in PBS group. At the same time physical activity of LNA-inhibitor treated mice was significantly decreased from day 12th to 16th of the experiment without alterations in animals activity. Organs weigth after mouse euthanasia did not reveal any differences between groups studied that allowed to conclude about the absence in significant negative effect of LNA-inhibitor on animals.

SIRT1 as target gene of miR-204-5p may affect melanoma cell proliferation rates by targeting several signaling pathways including transforming growth factor family [2]. Bioinformatic analysis showed that miR-204-5p involved in T-cell receptor and B-cell receptor signaling pathways regulation that may explain immunocompetent cell down-regulation in melanoma metastasis target organs under miR-204-5p specific inhibitor treatment.

Keywords: cell migration, cell proliferation, melanoma, microRNA-204-5p.

The study was supported by a grant from the Russian Science Foundation (project № 14-15-00074).

REFERENCES

1. *Dietel M., Jöhrens K., Laffert M.V., Hummel M., Bläker H., Pfitzner B.M., Lehmann A., Denkert C., Darb-Esfahani S., Lenze D., Heppner F.L., Koch A., Sers C., Klauschen F., Anagnostopoulos I.* A 2015 update on predictive molecular pathology and its role in targeted cancer therapy: a review focussing on clinical relevance. Cancer Gene Ther. 2015 Sep; 22(9): 417-30. doi: 10.1038/cgt.2015.39.

2. *Wang L., Zhang T., Xi Y., Yang C., Sun C., Li D.* Sirtuin 1 promotes the proliferation of C2C12 myoblast cells via the myostatin signaling pathway. Mol Med Rep. 2016 Aug; 14(2): 1309-15. doi: 10.3892/mmr.2016.5346.

MACROPHAGE POLARIZATION MARKERS OF BLOOD MONOCYTES IN PATIENTS WITH BREAST CANCER

M.R. Patysheva[1], M.N. Stakheyeva[1], N.A. Tarabanovskaya[1], E.S. Grigoryeva[1], E.M. Slonimskaya[1], J. Kzhyshkowska[2], N.V. Cherdyntseva[1,2]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University, Tomsk, Russia[2]

Polarized monocytes can have diagnostic value in breast cancer, including a triple negative subtype. We examined the markers of M2- polarization of monocytes: CD163 and stabilin1 and the recently described chitinase-like proteins: SI-CLP, YLK39, YLK40, in breast cancer patients and healthy volunteers. The features of polarized monocytes in breast cancer were revealed depending on the molecular subtype of the tumor.

Keyworlds: monocytes polarization, chitinase-like proteins, inflammation, breast cancer.

Introduction

The appearance of polarized monocytes is noted in malignant neoplasms which are accompanied by inflammation in most cases. Such monocytes express marker molecules of macrophages exhibiting pro- (M1) or anti-inflammatory (M2) activity. A diagnostic value for CD163 + monocytes associated with M2-polarization was shown for breast cancer [1]. Recently, a number of studies have found new markers on tumor-associated macrophages that may be of clinical significance for the pathogenesis of breast cancer: the transmembrane protein stabilin1 and the associated stabilin interacting chitinase-like protein (SI-CLP), as well as the recently described chitinase-like proteins (CLP) YLK39 and YLK40 [2]. In the primary cultures of human macrophages Stabilin1 and CLP appear on M2-macrophages activated by the alternative pathway. CLP in blood monocytes are not known, but based on the results of studies of monocytes in vitro, they are markers of M2-polarization. Although not all producers of YLK39, YLK40 in the human body are established, a significant role in this process is assigned to tissue macrophages and blood monocytes.

For a triple negative breast cancer (TNBC), a special significance of the evaluation of the tumor microenvironment is shown [3]. There are few studies on circulating monocytes in TNBC. However, this subtype represents the most popular object for immunotherapy, which shows the relevance of the study of the effector mechanisms of the immune system in TNBC.

Thus, the goal was to evaluate the expression of markers of M2-polarization of monocytes in breast cancer taking into account the TNBC.

Subjects and Methods

Twenty-five patients diagnosed with primary local breast cancer and 6 healthy volunteers were included into the study. We examined monocyte populations and gene expression within them in peripheral blood of cancer patients before any treatment.

Phenotypic features of monocytes were assessed by flow cytometry using mAb (BD Pharmingen, USA) against CD45 and CD163 and kindly provided mAb Stabilin1 produced in Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, University of Heidelberg (Mannheim, Germany). Monocytes fraction from whole blood were subjected to positive CD14 Magnetic Cell Sorting using CD14 Magnetic beads (Miltenyi Biotech, Germany). Total RNA from the monocytes was isolated using RNAesy Mini plus kit (Qagen, USA). Real-time RT-PCR was performed with Taq-man technology on a Rotor-gene 6000 (Corbet, Australia). The Pfaffl method

was used to assess the target transcript in a patients group relative to that of an health donors control group using expression of an internal control GAPH-DH to normalize data.

Statistical analysis was performed using Statistica 10.0 software package. Differences between groups were evaluated using the non-parametric Mann-Witny U-test. The Spearman rank correlation coefficients were calculated by comparing the prevalence of monocytes subpopulations to the gene expression. The results are present as medians with interquartile ranges, Me (25-75%). Differences were considered significant where $p<0.05$.

Results

There were no differences in the content of M-2 polarized CD163 + monocytes in patients with breast cancer in comparison with healthy subjects: 1.55 (0.82-4.95) % and 2.05 (2.00-2.60)%, respectively. Stabilin1+ monocyte evaluation was performed only in women with breast cancer: it was -28.35 (13.20-58.30) %. However, the presence of the oncologic process affected the expression of the genes of CLP YLK40, YLK39 and SI-CLP, which was significantly decreased in comparison with the group of healthy individuals, amounting to 0.64, 0.7, and 0.54 with respect to healthy controls, respectively. Correlation analysis showed that the expression of SI-CLP ($r = -0.8$, $p> 0.05$), YLK39 ($r = 0.8$, $p> 0.05$) and YLK40 ($r = 0.4$, $p>0.05$) was not associated with the number of M2-polarized CD163 + monocytes in BC. This fact reveals a difference in the mechanisms that induce the synthesis of CLP and the CD163 molecule in monocytes.

It is known that TNBC is characterized by a more pronounced reactivity of the immune system compared to other molecular subtypes [3, 4]. The results obtained in our study also confirmed this thesis. Thus, the content of M2-polarized CD163 + monocytes was lower for TNBC (5.70 (3.30-9.90) %) compared with other molecular genetic subtypes (1.45 (0.65-4.10) %) (Figure 1). The content of Stabilin1 + monocytes in TNBC (9.60 (0.10-19.10) %) was higher for a triply negative subtype - 43.35 (25.85-66.85) % (Figure 1), however, differences in gene expression CLP (the putative markers of M2-polarization of monocytes), as a function of the molecular subtype, were not detected (Figure 1).

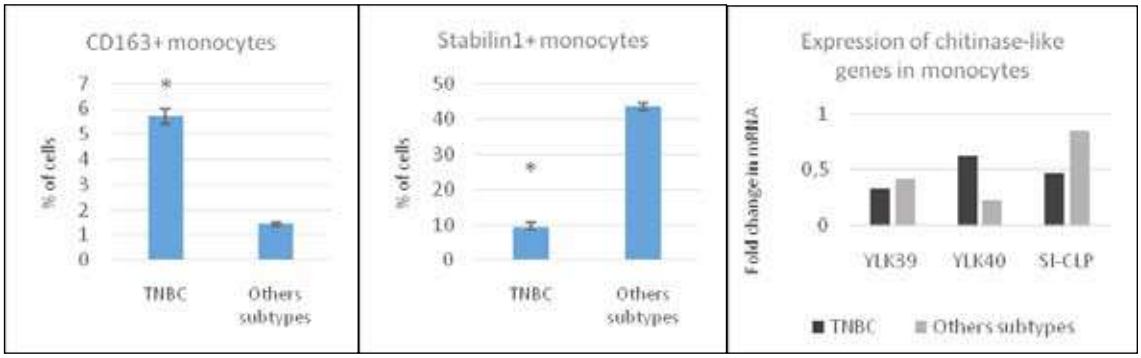


Figure 1. Features of the PB monocytes in patients with TNBC (p-level: * - p<0,05).

Conclusions

In general, the diagnostic significance of CD163+ and Stabilin1+ mnocytes has not been established for breast cancer. However, their significance for the characterization of TNBC is shown. Data were obtained on the presence of CLP in monocytes of both healthy people and patients regardless of the molecular subtype of the tumor, which may indicate the polarization of monocytes at the level of transcription of CLP genes. At the same time, the tumor process can have a negative effect on the expression of these genes in monocytes, reducing it. The obtained data reveal the significance of the study of CLP as markers of monocyte polarization. Identified features of TNBC indicate new diagnostic parameters for this subtype.

This research was supported by Russian Foundation for Basic Research (Grant № 16-54-76015)

REFERENCES

1. *Zhang B., Cao M., He Y., Liu Y., Zhang G., Yang C., Du Y., Xu J., Hu J., Gao F.* Increased circulating M2-like monocytes in patients with breast cancer. Tumour Biol. 2017 Jun; 39(6): 1010428317711571. doi: 10.1177/1010428317711571.

2. *Mitrofanova I., Zavyalova M., Telegina N., Buldakov M., Riabov V., Cherdyntseva N., Kzhyshkowska J.* Tumor-associated macrophages in human breast cancer parenchyma negatively correlate with lymphatic metastasis after neoadjuvant chemotherapy. Immunobiology. 2017; 222(1): 101-109. doi: 10.1016/j.imbio.2016.08.001, IF 2.72.

3. *Loi S., Michiels S., Salgado R., Sirtaine N., Jose V., Fumagalli D., Kellokumpu-Lehtinen P.L., Bono P., Kataja V., Desmedt C., Piccart M.J., Loibl S., Denkert C., Smyth M.J., Joensuu H., Sotiriou C.* Tumor infiltrating lymphocytes are prognostic in triple nega-

tive breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol. 2014 Aug; 25(8): 1544-50. doi: 10.1093/annonc/mdu112.

4. *Lehmann B.D., Pietenpol J.A.* Identification and use of biomarkers in treatment strategies for triple negative breast cancer subtypes. J Pathol. 2014 Jan; 232(2): 142-50. doi: 10.1002/path.4280. doi: 10.1002/path.4280.

SIGNAL TRANSDUCTION AND THERAPEUTIC EFFECTS OF HUMAN MENINGIOMA EMBOLIZATION

M.L. Perepechaeva, E.V. Vorontsova, S.M. Pyatov, A.Y. Grishanova

Federal publicly funded scientific institution Federal Research Center for Fundamental and Translational Medicine, Novosibirsk, Russia

Hypoxia plays an important role in the pathogenesis of meningiomas, and hypoxia can stimulate apoptosis of tumor cells; on the other hand, hypoxia may contribute to meningioma progression. To clarify the molecular basis for the differences in the outcomes of these two therapeutic strategies, we investigated the mRNA expression of *HIF-1α*, *AhR*, *ARNT*, *NcoA2*, and of HIF-1α-controlled genes *VEGF-A*, *GLUT1*, and *c-Myc* in tumor samples of *benign meningiomas* from two groups of patients: where the tumor was removed either without or after endovascular embolization. The study showed that in *meningiomas* exposed to hypoxia as a result of embolization, the mRNA levels of *AhR*, *ARNT*, and *NcoA2* were significantly lower as compared to unembolized meningiomas. It is likely that AhR can be an independent therapeutic target in meningiomas without the intermediation of endovascular-embolization–induced hypoxia.

Keywords: meningiomas, AhR, HIF-1α, hypoxia, embolization, therapeutic target.

Relevance

Hypoxia plays an important role in the mechanisms of development of meningiomas, and hypoxia can stimulate apoptosis of tumor cells; on the other hand, hypoxia can contribute to meningioma progression. The central role in the development of hypoxia is played by the transcription factor HIF-1α and its downstream signaling pathways mediating angiogenesis, glucose metabolism, and cell proliferation. In the nucleus, HIF-1α forms a complex with the ARNT protein [*arylhydrocarbon receptor (*AhR) *nuclear* transporter], and *AhR can compete* with HIF-1α for ARNT. *AhR is known* to control the expression of genes of cytochrome P450 family 1 and to participate in the molecular cascades that promote cell differentiation and apoptosis and inhibit proliferation. It is known that the transcription coactivator NcoA2 regulates activation and inhibition of proteins of the basic helix-loop-helix (*bHLH*)/*PAS family,* which includes HIF-1α, ARNT, and AhR. The aim of the study was to investigate HIF-1α- and AhR-dependent signaling pathways in unembolized and embolized human meningiomas to reveal the molecular basis of a tumor response to hypoxia. This knowledge can be relevant to the search for molecular markers of different responses to hypoxia and ultimately the choice of therapeutic tactics.

Material and Methods

The object of the study was tissue samples of *meningiomas*, a type of *benign* brain tumor, from two groups of patients with the diagnosis of Meningioma WHOGr1, where the tumor was removed either without (n = 10) or after (n = 7) endovascular embolization. The mRNA expression of *HIF-1α*, *AhR*, *ARNT*, and *NcoA2* and of HIF-1α-controlled genes *VEGF-A*, *GLUT1*, and *c-Myc* was determined in the samples. The gene expression was evaluated on the iCycler CFX96 real-time PCR detection system (Bio-Rad Laboratories, USA) according to the TaqMan principle. *EIF2B1* served as an internal control (housekeeping gene). The $2^{-\Delta Ct}$ method was used to analyze the data. All the calculations were performed in the Statistica software package (StatSoft, Inc., USA). Data were subjected to analysis of variance (ANOVA) with the *Bonferroni post hoc* test.

Results

The study showed that there were no statistically significant differences in the mRNA levels of *HIF-1a*, *VEGF-A*, *GLUT1*, and *c-Myc* between the unembolized and embolized meningiomas. Nevertheless, in the *meningiomas* exposed to hypoxia as a result of embolization, the mRNA levels of *AhR*, *ARNT*, and *NcoA2* were significantly lower as compared to the unembolized meningiomas.

Conclusions

Thus, in the mechanism of action of hypoxia on meningiomas (as a result of endovascular embolization), the AhR-dependent pathway plays a more important role than does the HIF-1α-dependent

pathway. Given the major involvement of the AhR signaling pathway in the cancer progression of human meningiomas (shown in a study on meningiomas biopsy samples [1]), the therapeutic effect of hypoxia may be related to its inhibitory influence on the *AhR* gene expression. It is likely that AhR can be an independent therapeutic target in meningiomas without the intermediation of endovascular-embolization–induced hypoxia.

REFERENCES

1. *Talari N.K., Panigrahi M.K., Madigubba S., Phanithi P.B.* Overexpression of aryl hydrocarbon receptor (AHR) signalling pathway in human meningioma. J Neurooncol. 2018 Apr; 137(2): 241-248. doi: 10.1007/s11060-017-2730-3.

2. This work was supported by the Russian Foundation for Basic Research (project № 16-04-00754) and involved equipment from *the Proteomic Analysis Center of the Institute of Molecular Biology and Biophysics of the* Federal Research Center for Fundamental and Translational Medicine

EXPRESSION OF *BRCA1* GENE IN BREAST TUMOR: ASSOCIATION WITH DISEASE PROGNOSIS

A.M. Pevzner, M.M. Tsyganov, I.V. Deryusheva, M.K. Ibragimova, E.E. Perminova, P.V. Kazantseva, E.M. Slonimskaya, N.V. Litviakov

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia

Most modern researches are limited to only germinal mutations of the *BRCA1* gene (more often 5382insC) and there are a few studies that characterize various somatic alterations of the *BRCA1* gene in a tumor, namely expression of this gene and its correlation with chemotherapy response. Taking into account the data on the association between the genetic mutation of *BRCA1* with high efficiency of platinum medication, we may suggest that expression of the *BRCA1* gene is also associated with high sensitivity of the tumor to platinum-based drugs. **Aim:** to evaluate the correlation between the expression of the *BRCA1* gene in breast cancer and response to neoadjuvant chemotherapy (NACT). The study included 86 patients with breast cancer (BC). We evaluated the expression of *BRCA1* in tumor sample before and after NACT. We established that objective response to NACT was associated with high level of *BRCA1* in the general group of patients (p=0.04) and in case of taxotere monotherapy (p=0.03).

Keywords: breast cancer, genes expression, *BRCA1*, neoadjuvant chemotherapy, chemotherapy efficacy, prognosis.

Introduction

The *BRCA1* gene is situated on the long arm of the 17 chromosome (17q21.31) and it codes nuclear phosphoprotein, which partakes in reparation of DNA within a cell and in regulation of the cell cycle (PubMed; OMIM 113705).

Modern studies of this gene are limited to the study of only germinal mutations. There are few studies that characterize the different somatic changes in the *BRCA1* gene in a breast tumor and, in particular, the expression of this gene. A low level of gene expression (due to epigenetic modification, down-regulation, effects of microRNAs or other causes) as well as a mutation can lead to a deficiency in the repair system, which can play an important role in the sensitivity of the tumor to treatment. Thus, the aim of this study was to study the correlations between *BRCA1* expression in a breast tumor and disease prognosis.

Material and Methods

The study included 105 BC patients with $T_{1-4}N_{0-3}M_0$ IIA–IIIB stages with morphologically verified diagnosis, in the age range 22 to 68 (median age 47.7±0.9 years). According to the Consensus Conference on Neoadjuvant Chemotherapy in Carcinoma of the Breast (April 26–28, 2003, Philadelphia, Pennsylvania), all patients received 2–6 courses of neoadjuvant chemotherapy (NACT) with FAC regimen (fluorouracil, doxorubicin, cyclophosphan), CAX (cyclophosphan, doxorubicin, xeloda), CP (cyclophosphan, cisplatin), and taxotere monotherapy. Surgery was followed by 2 courses of adjuvant chemotherapy according to the FAC regimen. Biopsy tumor samples were used as a test material before treatment and following NACT. Total RNA was isolated from 105 pair samples before treatment and after NACT. The level of expression of the *BRCA1* gene was assessed by RT-qPCR.

Results and Discussion

At the first stage of our study we evaluated the level of *BRCA1* expression before and after NACT. The average value of expression for this gene before treatment equaled to 0.85±0.1, and 0.65±0.08 for the post-surgery material.

Evaluation of the relationship between the expression of the studied gene and response to NACT showed that patients who underwent CAX therapy (p=0.04) and patients who underwent taxotere monotherapy (p=0.03) had statistically significant differences between the group with objective response (CR+PR) and the group with no response to NACT (SD+P) (Table 1). In both cases, the expression level in the studied gene was higher in the group of patients with objective response to chemotherapy.

Table 1

Correlation of *BRCA1* expression with the main clinical and morphological parameters of patients

Clinical and morphological parameters			Expression before treatment (Mean±SE)	p-level
Age	≤45		0,80±0,17	0,92
	>45		0,90±0,15	
Menstrual status	Perimenopause		0,79±0,13	0,35
	Postmenopause		0,97±0,20	
Tumor size	T_{1}-T_{2}		0,87±0,12	0,73
	T_{3}-T_{4}		0,87±0,28	
Lymph node status	N_{0}		0,97±0,24	0,82
	N_{1-3}		0,80±0,11	
Molecular subtype	Luminal B		1,04±0,16	0,03
	Triple negative		0,56±0,09	
	HER2-positive		0,41±0,17	
Histological form	Unicentric		0,80±0,15	0,40
	Multicentric		0,98±0,17	
Efficiency of neoadjuvant therapy	All patients	CR+PR	0,53±0,10	0,07
		SD+P	0,97±0,17	
	FAC	CR+PR	0,87±0,16	0,75
		SD+P	0,84±0,17	
	Taxotere	CR+PR	1,29±0,22	0,03
		SD+P	0,51±0,22	
	CAX	CR+PR	1,12±0,24	0,04
		SD+P	0,85±0,52	
	CP	CR+PR	0,41±0,18	1,00
		SD+P	1,62±1,62	

It is important to note that mRNA expression is variable. Thus, to more accurately determine the correlation between the expression of the gene being studied and the prognosis by statistical analysis, the expression level of *BRCA1* was divided into groups.

The association of the postoperative level of expression of *BRCA1* with metastasis-free survival was revealed when the overexpression group (more than 1) of the investigated gene was isolated (Figure 1).

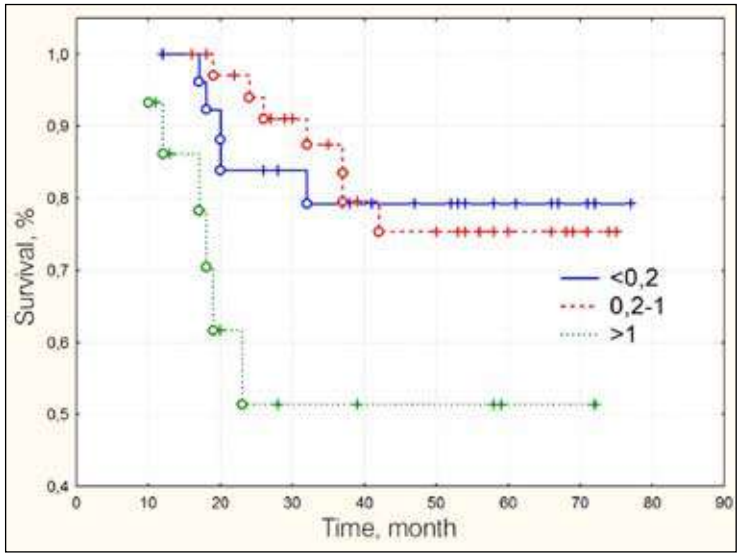


Figure 1. The parameters of the metastasis-free survival of breast cancer patients, depending on the expression level of *BRCA1* (log-rank test p <0.05)

There was a statistically significant increase in metastasis-free survival values with a *BRCA1* expression level of less than 0.2 (p=0.05) and 0.2-1 (p=0.01) as compared to a *BRCA1* value of more than 1.

Thus, the association of *BRCA1* gene expression in breast tumor with the prognosis of the disease was demonstrated. Based on the data obtained, it can be assumed that the expression of *BRCA1* may also be a marker of the effectiveness of treatment of breast cancer patients, but this requires further study.

The work was carried out within the framework of the competition in 2018-2020 for the scholarship of the President of the Russian Federation to young scientists and graduate students (Order № 231 of April 3, 2018).

UP-REGULATION OF FLOTILLINS, NEW MARKER OF METASTATIC DEVELOPMENT, INDUCES CELL INVASION AND METASTATIC DEVELOPMENT

D. Planchon[1], E.R. Morris[1], M. Genest[1], F. Comunale[1], S. Vacher[2], I. Biche[2], E.V. Denisov[5,6], L.A. Tashireva[5], V.M. Perelmuter[5], S. Linder[4], Ph. Chavrier[3], S. Bodin[1], C. Gauthier-Rouviиre[1]

CRBM, Univ Montpellier, CNRS, Montpellier, France[1]
Department of Genetics[2]
Cell Dynamics and Compartmentalization Unit, Institut Curie, Paris, France[3]
Institut für Medizinische Mikrobiologie, Virologie und Hygiene, University Medical Center Eppendorf, Martinistr. Hamburg, Germany[4]
Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[5]
Tomsk State University, Tomsk, Russia[6]

Flotillin 1 and 2 are two ubiquitous, highly conserved and related proteins present in many cellular membrane compartments. They exist as hetero-tetramers associated with specific caveolin-independent membrane microdomains rich in cholesterol and glycosphingolipids. Flotillin hetero-tetramers assemble in large oligomers to form molecular membrane scaffolds known to participate in membrane proteins clustering [1]. When overexpressed, as it occurs in many invasive carcinoma and sarcoma, they induce the formation of plasma membrane invaginations and of intracellular vesicles and modify the trafficking of several cargos; promoting the Upregulated Flotillin-Induced Trafficking (UFIT) pathway [2-3].

Flotillin overexpression is detected in many invasive carcinoma and sarcoma and is a marker of poor prognosis associated with a higher metastatic risk [4-8]. How flotillins participate in the acquisition of invasive and metastatic properties remains to be determined.

Our study aims at identifying how the UFIT pathway influences the membrane remodeling and modifies the trafficking of cargo leading to the acquisition of invasive properties.

We show that flotillins downregulation in invasive cancer cells dramatically inhibit their invasive properties as monitored *in vitro* using a 3D-collagen invasion assay and *in vivo* using zebrafish xenografts. Reciprocally, ectopic up-regulation of flotillins in non-tumoral cells is sufficient to induce their invasive behavior *in vitro* and *in vivo*. We show that flotillins are critical regulators of the trafficking of several cargo amongst them MT1-MMP, a key metalloproteinase responsible for the proteolytic activity of invadopodia [9].

Keywords: flotillins, cancer marker, metastasis, cell invasion.

REFERENCES

1. *Bodin S., Planchon D., Rios Morris E., Comunale F., Gauthier-Rouviere C.* Flotillins in intercellular adhesion - from cellular physiology to human diseases. J Cell Sci. 2014 Dec 15; 127(Pt 24): 5139-47. doi: 10.1242/jcs.159764.

2. *Frick M., Bright N.A., Riento K., Bray A., Merrified C., Nichols B.J.* Coassembly of flotillins induces formation of membrane microdomains, membrane curvature, and vesicle budding. Curr Biol 2007, 17:1151–1156. doi: 10.1016/j.cub.2007.05.078.

3. *Glebov O.O., Bright N.A., Nichols B.J.* Flotillin-1 defines a clathrin-independent endocytic pathway in mammalian cells. Nat Cell Biol. 2006; 8: 46–54. doi: 10.1038/ncb1342.

4. *Cao S., Cui Y., Xiao H., Mai M., Wang C., Xie S., Yang J., Wu S., Li J., Song L., Guo X., Lin C.* Upregulation of flotillin-1 promotes invasion and metastasis by activating TGF-β signaling in nasopharyngeal carcinoma. Oncotarget. 2016 Jan 26; 7(4): 4252-64. doi: 10.18632/oncotarget.6483.

5. *Koh M., Yong H.Y., Kim E.S., Son H., Jeon Y.R., Hwang J.S., Kim M.O., Cha Y., Choi W.S., Noh D.Y., Lee K.M., Kim K.B., Lee J.S., Kim H.J., Kim H., Kim H.H., Kim E.J., Park S.Y., Kim H.S., Moon W.K., Choi Kim H.R., Moon A.* A novel role for flotillin-1 in H-Ras-regulated breast cancer aggressiveness: Flotillin-1 is Important for Breast Cancer Aggressiveness. Int J Cancer. 2016 Mar 1; 138(5): 1232-45. doi: 10.1002/ijc.29869.

6. *Liu Y., Lin L., Huang Z., Ji B., Mei S., Lin Y., Shen Z.* High expression of flotillin-2 is associated with poor clinical survival in cervical carcinoma. Int J Clin Exp Pathol. 2015; 8: 622–628.

7. *Li H., Wang R.-M., Liu S.-G., Zhang J.-P., Luo J.-Y., Zhang B.-J., Zhang X.-G.* Abnormal expression of FLOT1 correlates with tumor progression and poor survival in patients with non-small cell lung cancer. Tumour Biol. 2014 Apr; 35(4): 3311-5. doi: 10.1007/s13277-013-1434-3.

8. *Wang X., Yang Q., Guo L., Li X.H., Zhao X.H., Song L.B., Lin H.X.* Flotillin-2 is associated with breast cancer progression and poor survival outcomes. J Transl Med. 2013 Aug 15; 11: 190. doi: 10.1186/1479-5876-11-190.

9. *Castro-Castro A., Marchesin V., Monteiro P., Lodillinsky C., Rosse C., Chavrier P.* Cellular and Molecular Mechanisms of MT1-MMP-Dependent Cancer Cell Invasion. Annu Rev Cell Dev Biol. 2016 Oct 6; 32: 555-576. doi: 10.1146/annurev-cellbio-111315-125227.

CIRCULATING DNA-MARKERS IN LUNG CANCER: CHANGES IN RETROTRASPOSONS METHYLATION STATUS IN RESPONSE THERAPY AND DURING THE POST-TREATMENT FOLLOW-UP

A.A. Ponomaryova[1,3], A.Y. Dobrodeev[1], A.A. Bondar[2], N.V. Cherdyntseva[1,4], A.A. Zavyalov[1], S.A. Tuzikov[1], V.V. Vlassov[2], P.P. Laktionov[2,5], E.Y. Rykova[2,6]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia[2]
Tomsk Polytechnic University, Tomsk, Russia[3]
Tomsk State University, Tomsk, Russia[4]
Meshalkin Novosibirsk Research Institute of Circulation Pathology, Novosibirsk, Russia[5]
Novosibirsk State Technical University, Novosibirsk, Russia[6]

Background

Malignant cell transformation is accompanied by two processes of DNA methylation changes: hypermethylation in CpG islands of tumor suppressor genes and global hypomethylation in repetitive DNA sequences (retrotransposons) [1, 2]. The composition of circulating DNA (cirDNA) from plasma and cell-surface-bound cirDNA (csb-cirDNA) was shown earlier to be altered in the blood of cancer patients due to accumulation of tumor-specific aberrantly methylated DNA fragments, which are currently considered valuable cancer markers [3, 4].

Material and Methods

The present study compared LINE-1 retrotransposon methylation patterns in free-cirDNA and csb-cirDNA from healthy subjects (n=33) and lung cancer (LC) (n=32) patients, and also from

LC patients during the post-treatment follow-up period. Concentrations of methylated LINE-1 region 1 (LINE-1met) were assayed by real-time methylation-specific PCR. In order to normalize the LINE-1 methylation level, the LINE-1 region 2 concentration was evaluated, which was independent of the methylation status (LINE-1Ind).

Results

The LINE-1 methylation level, determinated as the ratio LINE met/LINE Ind, in csb-cirDNA from LC patients was significantly lower than in csb-cirDNA from healthy subjects (P=0.005). In the total group of LC patients, LINE-1 methylation level was shown to be significantly increased during the follow-up after chemotherapy (P<0.05, paired test) and after surgery comparaed to the methylation level before treatment (P<0.05, paired t-test). The revealed association between LINE-1 methylation level and effect of antitumor therapy was more pronounced in squamous cell lung cancer compared with adenocarcinoma (P<0.05 and P>0.05, respectively). All relapse-free patients within the follow-up period (n=19) were characterized by an increase in LINE-1 methylation level, and patients who experienced disease recurrence (n=13) had decreased levels that corresponded to those observed before treatment.

Conclusion

Our data demonstrate that LINE-1 methylation level determination represents a valuable tool for evaluation of cancer treatment efficiency and post-treatment monitoring.

Keywords: lung cancer, diagnosis, prognosis, oncomarkers, methylation, circulating DNA.

The study was supported by the fundamental research program of the Presidium of the Russian Academy of Sciences «Fundamental research for the development of biomedical technologies» (2014-208), the program of the Presidium of the Russian Academy of Sciences «Molecular and Cellular Biology», the Russian Foundation for Basic Research (№ 17-29-06002), scholarship of the RF President (№ SP-1549.2018.4; 2018-2020).

REFERENCES

1. *Plongthongkum N., Diep D.H., Zhang K.* Advances in the profiling of DNA modifications: cytosine methylation and beyond. Nat Rev Genet. 2014 Oct; 15(10): 647-61. doi: 10.1038/nrg3772.

2. *Warton K., Mahon K.L., Samimi G.* Methylated circulating tumor DNA in blood: power in cancer prognosis and response. Endocr Relat Cancer. 2016 Mar; 23(3): R157-71. doi: 10.1530/ERC-15-0369.

3. *Rykova E.Y., Morozkin E.S., Ponomaryova A.A., Loseva E.M., Zaporozhchenko I.A., Cherdyntseva N.V., Vlassov V.V., Laktionov P.P.* Cell-free and cell-bound circulating nucleic acid complexes: mechanisms of generation, concentration and content. Expert Opin Biol Ther. 2012 Jun; 12 Suppl 1: S141-53. doi: 10.1517/14712598.2012.673577.

4. *Ponomaryova A., Rykova E., Cherdyntseva N., Skvortsova T., Dobrodeev A, Zav'yalov A., Bryzgalov L., Tuzikov S., Vlassov V., Laktionov P.* Potentialities of aberrantly methylated circulating DNA for diagnostics and post-treatment follow-up of lung cancer patients. Lung Cancer. 2013 Sep; 81(3): 397-403. doi: 10.1016/j.lungcan.2013.05.016.

AUTOMATED IMAGE ANALYSIS IN ASSESSMENT OF E-CADHERIN DOWN-REGULATION DURING EPITHELIAL-MESENCHYMAL TRANSITION IN PROSTATE CANCER

M. Puchinskaya

Minsk City Clinical Oncologic Dispensary, Minsk, Belarus

Experience of using automated image analysis with Aperio software for evaluation of E-cadherin expression is presented. Advantages and limitations of the method are described. Automated analysis makes it possible to distinguish changes in marker expression that may be difficult to assess visually.

Keywords: automated image analysis, Aperio, E-cadherin, epithelial-mesenchymal transition.

Automated image analysis (AIA) is becoming increasingly popular in pathology as it provides some opportunities that are difficult or even impossible to realize by visual staining assessment [1-3]. It is used for a wide variety of applications, but, anyway, can't yet substitute classic pathology.

During epithelial-mesenchymal transition (EMT) epithelial cells transiently acquire some features of mesenchymal ones, this leads to higher invasion, migration and therapeutic resistance.

E-cadherin (E-cad) is an epithelial marker implied in adhesion and signaling pathways (MAPK, EGFR, PI3K). Its down-regulation is an important feature of EMT and as so, E-cad expression can be used as one of morphological signs of EMT.

The aim of the study is to describe personal experience in assessing E-cad expression in prostate cancer (PCa) using AIA.

Material and Methods

Evaluation of E-cad expression in PCa samples was performed in microphotographs (MPG) (×200 magnification) by AperioImageScope v.11.0.2.780 software (Leica Biosystems).

Results

E-cad exhibited mostly homogenous membranous staining, strong in non-cancerous glands, but weaker to various extent in PCa. Besides, cytoplasmic staining was present in some PCa cells. When choosing the best way to quantitatively evaluate E-cad staining for future analysis the following methods were taken into consideration.

1. Visual semiquantitative assessment. It is widely used in different works, usually with 3-point scale (weak, moderate or strong staining). But this method seems rather subjective, as in borderline cases it is difficult to assess the staining correctly. Besides, if different intensities of staining are present in one case, there are discrepancies on how to evaluate them. Some authors consider the strongest staining, while others prefer to calculate indexes evaluating both intensity and the percentage of stained cells. In the latter case the formula vary in different works, and there are no definite guidelines on how to choose the best one.

2. As staining was predominantly membranous, an algorithm assessing it, but originally developed for another marker, for example, Her2/neu, could be used, but most of them can't be downloaded and used for free.

3. AIA with specialized software. AperioImageScope software was chosen as an easy and effective tool. Freely available analysis algorithms were used. Brightness of each pixel is assessed as a continuous variable, and after applying certain thresholds, that can be chosen by a user, pixels are marked as weak, moderate or strong staining. Default thresholds for diaminobenzidine as a chromogen were used. A number of parameters are calculated automatically and can be used for further analysis:

a) positivity is used by most authors. It evaluates the number of stained pixels divided by the total number of pixels in the analyzed area. Its serious limitation is dependence on the amount of stained histologic structures per field, cell density and cell size or the presence of gland lumina, necrotic areas and so on. "Negative selection" tool in the program makes it possible to outline areas that should be excluded from analysis, but may be difficult to apply in some cases.

b) average intensity of stained pixels (I_{avg}). It is calculated as a weighed mean of staining intensities of all positive pixels assessed in a 3-point scale. Opposite to positivity it doesn't count negative pixels and doesn't depend on area, occupied by analyzed structures. It may be thought of as an automated and more objective counterpart of visual assessment with quantification of mean staining index. This method was used for evaluation of E-cad expression.

AIA has the following advantages in evaluation of various markers staining:

1. AIA facilitates the assessment of small differences in staining intensities in different areas of the slide or of the same MPG. Using this, it was possible to show statistically significant differences in E-cad expression in histological structures, present in PCa (higher in intraductal cancer and cribrosis, lower in glomeruloids). The possibility of obtaining such results by visual assessment is doubtful, taking into consideration that staining intensity was changing rather gradually.

2. AIA is a good method of staining evaluation in cases where assessment of staining intensity (and not the percentage of stained cells) is critical (as in the present case, as even in PCa absence of E-cad staining was a rare event) and where staining changes gradually. In such cases semiquantitative assessment with 3-point scale can be used, but seems less accurate.

At the same time AIA has a number of limitations, that should be taken into consideration while planning a research or interpreting its results.

1. No distinction can be automatically made between staining of different subcellular compartments (e.g., membranous, cytoplasmic or nuclear), if it is present in the same slide. It leads to some limitations in analysis of markers, that are normally (or as a sign of a disease) expressed in different compartments, as well as can't aid in evaluation of subcellular redistribution of different proteins during the disease progression. Automatic assessment of cytoplasmic E-cad was not possible in the present work, so both membranous and cytoplasmic staining were evaluated together.

2. If background or false-positive staining is present, it can't be excluded from analysis. Using "negative selection" tool can partially resolve this problem, but if false-positive staining is present

within small structures or in structures, adjacent to those that should be evaluated this may be technically challenging. This was the case with another EMT marker, N-cadherin, staining. Granular cytoplasmic staining from weak to strong was present in most cells of PCa and noncancerous glands, it challenged the correct evaluation of percentage of cells with true cytoplasmic staining and prevented using AIA for marker evaluation, that's why N-cadherin expression was assessed visually.

3. If it is necessary to study a marker in a subset of cells present in one MPG, negative selection tool should be carefully applied. But if these structures are too close or too small this may be challenging. For example, one more EMT marker, vimentin, is normally expressed in stroma, but during EMT appears in epithelium; trying to outline the border between these two compartments is technically difficult as can exclude parts of cell membranes from analysis.

4. The correct work of the algorithm should be checked. In the present work in many cases pixels in the small area in the left upper part of many (but not all) MPGs were considered weak positive after analysis, though they were definitely negative on original MPGs. To avoid bias this area was excluded from analysis if this artifact was present.

5. As AIA is a sensitive method, and as so careful observation of staining procedures at all stages is crucial to avoid variants in staining due to the procedure, that may influence analysis results. Fixation should be uniform throughout the sample, areas of artifacts (for example, false-positive stronger staining at sample edges) should not be analyzed.

Conclusions

AIA with AperioImageScope software gives quantitative evaluation of E-cad expression in PCa, that is suitable for further statistical analysis of the differences in E-cad levels as a sign of EMT, as well as its correlations with other tumor parameters. Due to some limitations of the method, however, it can't be uniformly used for evaluation of all studied EMT markers. Awareness of both positive sides and limitations of AIA makes it possible to correctly apply the method to obtain reliable and significant results.

REFERENCES

1. *Prilepskaya E.A., Kovylina M.V., Govorov A.V., Glotov A.V., Vasilyev A.O., Kolontarev K.B., Nikitaev V.G., Pronichev A.N., Pushkar D.Y.* Possibilities of automated image analysis in pathology. Arkh Patol. 2016 Jan-Feb; 78(1): 51-55. doi: 10.17116/patol201678151-55.

2. *Mulrane L., Rexhepaj E., Penney S., Callanan J.J., Gallagher W.M.* Automated image analysis in histopathology: a valuable tool in medical diagnostics. Expert Rev Mol Diagn. 2008 Nov; 8(6): 707-25. doi: 10.1586/14737159.8.6.707.

3. *Higgins C.* Applications and challenges of digital pathology and whole slide imaging. Biotech Histochem. 2015 Jul; 90(5): 341-7. doi: 10.3109/10520295.2015.1044566.

4. *Kalluri R., Weinberg R.A.* The basics of epithelial-mesenchymal transition. J Clin Invest. 2009 Jun 1; 119(6): 1420-28. doi: 10.1172/JCI39104.

THE STUDY OF DELITION POLYMORPHISM OF GSTM1 AND GSTT1 GENES IN YAKUT PATIENTS WITH LUNG CANCER

E.K. Rumyantsev, E.V. Tsypandina, V.M. Nikolaev, S.D. Efremova, F.V. Vinokurova, S.I. Sofronova

Yakutian Scientific Center of Complex Medical Problems, Yakutsk, Russia

The results of the study of the polymorphism of the GSTT and GSTM1 genes in 36 patients with lung cancer and 65 healthy inhabitants of the Sakha (Yakutia) with Yakut nationality are presented. It was first established that the risk of developing lung cancer in the Yakut population is associated with the zero genotype of the GSTM1 gene, which may indicate a high risk of cancer for carriers of this genotype.

Keywords: glutathione-S-transferase, deletion, oncopathology, xenobiotics, polymorphism.

Polymorphism in the genes GSTM1, GSTT1, which encodes the isoforms μ, t of the glutathione-S-transferase enzyme characterized by deletion in both alleles leads to complete absence of synthesis of the protein product and resulting in a deep suppression of the enzyme function. According to the reports, there is conflicting data on the association of deletion-genotype GSTM1 with the risk of developing lung cancer [1].

Subject and methods

Molecular genetic studies were conducted in 36 Yakut lung cancer patients. The control group consisted of 65 age-and ethnicity matched people. The main criterion for inclusion into the control group was the absence of cancer.

A standard phenolic-chloroform extraction method was used to isolate DNA. The analysis of polymorphic variants of specific sections of the genes GSTM1, GSTT1 was carried out using polymerase chain reaction methods and while using the primer structure described in the works (Gronau et al., 2003).

When comparing genotype frequencies, the standard χ^2 criterion was used. The relative risk (OR) of the development of the disease with a specific genotype was calculated using the standard formula: OR = a / bxd / c, where a, b - the number of patients with and without mutant genotype, respectively, and d, c - the number of people in the control group with and without mutant genotype. The 95% *confidence interval* (CI) is used to estimate the precision of the OR.

Results

The frequency of the *GSTM1 0/0 genotype* in the group of patients was significantly higher (66.6%) than in the group of healthy individuals (43.0%) (χ^2 = 5.16, p = 0.023, OR = 0.67 (0.26-1.69).

The analysis of deletion polymorphism of the GSTT1 gene did not reveal any significant differences in the frequency distribution of genotypes depending on oncopathology. The frequency of deletion polymorphism was 41.7% and 32.3% in the patient group and control group, respectively (χ^2 = 0.88, p = 0.347, OR = 0.61 (0.26-1.69)).

Obviously, the GST genes, especially GSTM1, are involved in the pathogenesis of various types of cancer and act as modifiers and risk factors for a variety of diseases associated with adverse environmental factors.

According to published data of other authors, the influence of polymorphism of GST-genes is associated with the development of numerous malignant tumors: lung cancer [2], bladder [3], rectum [4], stomach [5], esophagus [6], breast [7], ovaries and skin [8]. There are studies showing the relationship between the polymorphism of genes encoding the enzymes of the second phase of detoxification and chronic and hereditary diseases: chronic bronchitis, bronchial asthma [9]. Moreover, it was shown that the deletion of the GSTM1 and GSTT1 genes led to increased sensitivity to xenobiotics (in particular, to thermal decomposition products of tobacco).

Our study showed that the increased risk of developing lung cancer in the Yakut population can be related to the GSTM1 null *genotype*.

Conclusion

The risk of developing lung cancer in the Yakut population was found to be associated with the GSTM1 null *genotype, thus i*ndicating an increased cancer risk for carriers of this genotype.

REFERENCES

1. *Klusek J., Nasierowska-Guttmejer A., Kowalik A., Wawrzycka I., Lewitowicz P., Chrapek M., Gluszek S.* GSTM1, GSTT1, and GSTP1 polymorphisms and colorectal cancer risk in Polish nonsmokers. Oncotarget. 2018 Apr 20; 9(30): 21224-21230. doi: 10.18632/oncotarget.25031.

2. *Minina V.I.., Soboleva O.A., Glushkov A.N., Voronina E.N., Sokolova E.A., Bakanova M.L., Savchenko Y.A., Ryzhkova A.V., Titov R.A., Druzhinin V.G., Sinitsky M.Y., Asanov M.A.* Polymorphisms of GSTM1, GSTT1, GSTP1 genes and chromosomal aberrations in lung cancer patients. J Cancer Res Clin Oncol. 2017 Nov; 143(11): 2235-2243. doi: 10.1007/s00432-017-2486-3.

3. *Altunkol A., Savaş M., Dilmeç F., Utangaç M.M., Abat D., Gümüş K., Karlıdağ İ., Yeni E.* Detection of CYP1A1 and GSTP1 gene polymorphisms in bladder cancer patients in a Turkish population using a polymerase chain reaction-restriction fragment length polymorphism method. Turk J Urol. 2018 Mar; 44(2): 125-131. doi: 10.5152/tud.2018.23571.

4. *Masood N., Mubashar A., Yasmin A.* Epidemiological factors related to GSTM1 and GSTT1 genes deletion in colon and rectum cancers: A case-control study. Cancer Biomark. 2015; 15(5): 583-9. doi: 10.3233/CBM-150498.

5. *Chen Z.H., Xian J.F., Luo L.P.* Association between GSTM1, GSTT1, and GSTP1 polymorphisms and gastric cancer risk, and their interactions with environmental factors. Genet Mol Res. 2017 Feb 8; 16(1). doi: 10.4238/gmr16018877.

6. *Sharma A., Das B.C., Sehgal A., Mehrotra R., Kar P., Sardana S., Phukan R., Mahanta J., Purkayastha J., Saxena S., Kapur S., Chatterjee I., Sharma J.K.* GSTM1 and GSTT1 polymorphism and susceptibility to esophageal cancer in high- and low-risk regions of India. Tumour Biol. 2013 Oct; 34(5): 3249-57. doi: 10.1007/s13277-013-0897-6.

7. *Zhang J., Wu Y., Hu X., Wang B., Wang L., Zhang S., Cao J., Wang Z.* GSTT1, GSTP1, and GSTM1 genetic variants are associated with survival in previously untreated metastatic breast cancer. Oncotarget. 2017 Nov 14; 8(62): 105905-105914. doi: 10.18632/oncotarget.22450.

8. *Turner S., Francis B., Wani N., Vijverberg S., Pino-Yanes M., Mukhopadhyay S., Tavendale R., Palmer C., Burchard E.G., Merid S.K., Melén E., Maitland-van der Zee A.H.* The Pharmacogenomics In Childhood Asthma Consortium OBO. The Pharmacogenomics In Childhood Asthma Consortium OBO. Variants in genes coding for glutathione S-transferases and asthma outcomes in children. Pharmacogenomics. 2018 Jun 1; 19(8): 707-713. doi: 10.2217/pgs-2018-0027.

9. *Gataa I., Emile G., Loriot M.A., Goldwasser F., Alexandre J.* Association between high antitumor activity of oxaliplatin and cyclophosphamide and constitutional GSTM1 homozygous deletion in an advanced ovarian cancer patient. Chemotherapy. 2013; 59(4): 290-3. doi: 10.1159/000357517.

ANALYSIS OF DRAS1 GENE EXPRESSION IN CLOSELY RELATED SPECIES OF DROSOPHILA VIRILIS GROUP

E.A. Sivoplyas[1], M.D. Maksimchuk[1], A.M. Kulikov[2]

Federal State Budgetary Educational Institution of Higher Education «Moscow State University of Education» (MSPU), Moscow, Russia[1]
Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Russia[2]

The *Dras1* gene is the most well known proto-oncogene. Most studies of carcinogenesis showed the participation of Ras family genes in the formation of malignant tumors. The *Dras1* gene is an orthologist of human genes (*N-ras, K-ras* and *H-ras*) and belongs to the highly conservative gene: the nucleotide sequence is little variable from yeast to human. These genes were first discovered in rat sarcoma cells which led to the name of this group. *RAS* genes are involved in the regulation of cell division, normal and in pathologies. Mutation of these genes is found in nearly 15% of all cases of tumors of human tumor formation. Most often in the cells of large tumors point mutations activate proto-oncogenes of the RAS family.

Keywords: Differential expression, developmental stages, micro RNA, proto-oncogen *Dras1*, *Drosophila melanogaster, Drosophila virilis*, transgenic structures.

Introduction

The *Dras1*gene and the protein produced by it belong to the ras superfamily, which includes well-studied orthologists in human *HRas, KRas* and *NRas*. Their participation in signal transmission from cell receptors to the MAR-kinase cascade is shown. In addition to this function, proteins in this family affect the state of the actin cytoskeleton, malignant transformation and other processes. However, data on the regulation of the gene's own transcriptional activity are very little known. The involvement of miRNAs in regulating gene expression activity of orthologous ras gene has previously been shown to man. This process includes the participation of 17 microRNAs. For Drosophila, the influence of miRNAs on the expression of oncogenes previously was not shown. For invertebrates, data were obtained only for Caenorhabditis elegans.

Material and Methods

1. Production of transgenic structures bearing the GFP reporter gene under the Heat Shock promoter, containing a 3'- untranslated region of the *Dras1* gene with the presence / absence of the analyzed conservative sequence.
2. Getting transgenic flies.
3. Analysis of the degree of GFP reporter gene expression using a confocal laser microscope.
4. Expression score.

Results

Sites of binding of miRNA (mir-313, mir-92a, mir-312) with the investigated gene *Dras1* in the region 3`-UTR were found.

Ttransgenic construct containing the GFP gene and the 3'UTR of the *Dras1* gene in *D. melanogaster* with the presence / absence of the analyzed conservative sequence was made.

Transgenic flies were obtained

The degree of gene expression was analyzed.

Conclusion

For the first time, transgenic structures based on The R-element with a reporter gene containing and not containing the region of binding of micro RNA were obtained.

This work is supported by RFBR, grant № 16-34-00840

REFERENCES

1. *Ezer S.T., Sahar D., Salzberg A., Lev Z.* Differential expression during embryogenesis of three genes clasnered in the Ras1 region of Drosophila melanogaster. Dev Dyn. 1994 Oct; 201(2): 179-90. doi: 10.1002/aja.1002010208.
2. *Valencia A., Chardin P., Wittinghofer A., Sander C.* The ras protein family: evolutionary tree and role of conserved amino acids. Biochemistry. 1991 May 14; 30(19): 4637-48.
3. *Spicer G.S., Bell C.D.* Molecular phylogeny of the Drosophila virilis species group (Diptera: Drosophilidae): inferred from mitochondrial 12S and 16S ribosomal RNA genes. Annals of the Entomological Society of America. 2002; 95(2): 156-161.

MONITORING OF EGFR MUTATIONS IN THE PLASMA DNA OF PATIENTS WITH NON-SMALL CELL LUNG CANCER

V.A. Shamanin[1,2], E.E. Pisareva[1], E.V. Gornostaeva[2], P.A. Gervas[3], N.I. Gutkina[1], S.P. Kovalenko[1,2]

Federal Research Center of Fundamental and Translational Medicine, Novosibirsk, Russia[1]
BioLink Ltd, Novosibirsk, Russia[2]
Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[3]

Several EGFR mutations were monitored in circulating free plasma DNA of 14 non-small cell lung cancer patients being under Tyrosine kinase inhibitors treatment. Allele-specific PCR (AS-PCR) and deep sequencing on IonTorrent platform were used for the analysis. AS-PCR and NGS analysis were in agreement if plasma DNA contained at least 5% of DNA with mutation. However, some discrepancies between two techniques were observed in samples with low content of DNA with mutation. Increase of DNA with T790M mutation in plasma was demonstrated for several patients before the signs of clinical progression. Monitoring of NSCLC patients can be used for the adjustment of the therapy on the basis of T790M mutation appearance in plasma DNA. Advantages and disadvantages of AS-PCR and NGS for plasma DNA mutations analysis are discussed.

Keywords: Lung cancer, EGFR gene, mutations, monitoring.

Introduction

Mutation analysis in non-small cell lung cancer (NSCLC) tumors has already become a routine clinical practice. There are several somatic mutations in EGFR gene which are strongly connected with the response of the tumor to tyrosine kinase inhibitor (TKI) - based treatment [1]. EGFR mutations in tumor cells can be analyzed with the use of formalin-fixed paraffin embedded tumor samples or with the use of plasma circulating free DNA (cfDNA). An obvious advantage of cfDNA analysis is the possibility to monitor the appearance of mutations connected with tumor resistance to TKI in disease progression. T790M mutation is associated with the resistance to Gefitinib/ Erlotinib at least in 50% of cases [2]. New tyrosine kinase inhibitor Osimertinib can overcome T790M-connected resistance of tumor [3], so monitoring of early appearance of T790M mutation in lung cancer patients has definite practical interest.

There are three main approaches to analyze mutations in cfDNA: AS-PCR, ddPCR and Deep sequencing [4]. In this study we compared suitability of deep sequencing on IonTorrent platform and AS-PCR analysis of cfDNA for the monitoring of T790M mutation in NSCLC patients being under treatment by Iressa.

Material and Methods

Patients

The study enrolled 14 NSCLC patients with confirmed EGFR mutations in FFPE samples. Besides, this study included 12 healthy control individuals who donated blood for cfDNA analysis.

Plasma collection and cfDNA isolation

Whole blood was collected in EDTA–containing tubes and centrifuged at 2000g for 10 minutes at 4 °C. Plasma samples were aliquoted to 1,5 ml tubes and stored at -70 °C until use. Isolation of cfDNAfrom 3-5 ml of plasma was performed by PME free-circulating DNA Extraction Kit (Analytik Jena, Germany).

AS-PCR, Library Preparation and NGS

Four exons (exons 18-21) of the EGFR gene with predominant amounts of known mutations were analyzed by AS-PCR and by targeted sequencing. Each exon was covered by a single amplicon (122–169 bp). One multiplex PCR reactions was used to amplify the 4 selected amplicons. The resulting amplicons were analyzed by AS-PCR and by NGS. AS-PCR analysis was optimized for each mutation; NGS with the coverage not less than 5.000 reads was performed on PGM IonTorrent machine according to the manufacturer's instructions. Reads were mapped to the human genome reference GRCh37/hg19 using BWA-MEM [5] version 0.7.5.

Results

Sensitivity of the assay in NGS and AS-PCR

Mutant allele frequencies (MAF) were analyzed by NGS in wild-type DNA and in cfDNA of 12 healthy individuals. The median MAF varied from 0 to 0.77% in wild type DNA and from 0 to 0.44% in healthy individuals as calculated on the basis of reads amounts. There were no statistically significant differences between the baseline MAF in wild type genome DNA and cfDNA from healthy individuals. According to this data the sensitivity of the assay was 0.77%. AS-PCR data were validated with the control DNA and in the majority of cases the provided sensitivity of the assay was in range of 1%.

Testing of clinical samples of plasma cfDNA

87 plasma samples from 14 patients with NSCLC were analyzed. Four patients had mutation EGFR T790M associated with resistance to TKI before the start of the therapy. Monitoring of cfDNA in plasma of 14 patients with NSCLC revealed various dynamic profiles of EGFR mutations associated with sensitivity and resistance to TKI. We analyzed the period of 18 months for the majority of patients with a 2-month interval. At least 5 patients were found where NGS and AS-PCR data were in agreement. In all those cases the dynamics of mutations were different. However, at least in two cases an obvious increase of DNA fragments with T790M mutation was observed well before clinical symptoms appearance.

Conclusion

This study highlights the utility of cfDNA for analysis EGFR mutations in blood of NSCLC patients being treated by TKI. The level of sensitivity appeared to be comparable between NGS and AS-PCR.

This study was supported by Russian Science Foundation grant № 15-14-10004.

REFERENCES

1. *Suda K., Mitsudomi T.* Role of EGFR mutations in lung cancers: prognosis and tumor chemosensitivity. Arch Toxicol. 2015; 89(8): 1227-40. doi: 10.1007/s00204-015-1524-7.

2. *Sakai K., Horiike A., Irwin D.L., Kudo K., Fujita Y., Tanimoto A., Sakatani T., Saito R., Kaburaki K., Yanagitani N., Ohyanagi F., Nishio M., Nishio K.* Detection of epidermal growth factor receptor T790M mutation in plasma DNA from patients refractory to epidermal growth factor receptor tyrosine kinase inhibitor. Cancer Sci. 2013 Sep; 104(9): 1198-204. doi: 10.1111/cas.12211.

3. *Li W., Qiu T., Guo L., Ling Y., Gao Y., Ying J., He J.* Primary and acquired EGFR T790M-mutant NSCLC patients identified by routine mutation testing show different characteristics but may both respond to osimertinib treatment. Cancer Lett. 2018 Jun 1; 423: 9-15. doi: 10.1016/j.canlet.2018.03.005.

4. *Francis G., Stein S.* Circulating Cell-Free Tumour DNA in the Management of Cancer. Int J Mol Sci. 2015 Jun 19; 16(6): 14122-42. doi: 10.3390/ijms160614122.

5. *Li H., Durbin R.* Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009 Jul 15; 25(14): 1754-60. doi: 10.1093/bioinformatics/btp324.

EFFECT OF BIODEGRADABLE SCAFFOLDS FOR TARGETED IMMUNOTHERAPY ON DIFFERENTIATION OF MACROPHAGES

Ye. Shapovalova[1], E.G. Churina[1,2], Yu.G. Kzhyshkowska[1,3]

Tomsk State University, Tomsk, Russia[1]
Siberian State Medical University, Tomsk, Russia[2]
Ruprecht-Karls University of Heidelberg, Mannheim, Germany[3]

The influence of polycaprolactone's scaffolds on the mechanisms of differentiation of primary monocytic human macrophages *in vitro* was studied.

Keywords: polycaprolactone, bone implants, bioresorbable scaffold.

Relevance

Traditional methods of treatment of malignant tumors include surgery, radiation and chemotherapy, which have reached the limit of their technical perfection to-date, and their further development will not lead to a significant improvement in the situation. The most actual of alternative therapies is personalized targeted immunotherapy, the realization of which requires targeted drug delivery. Polymeric delivery systems allow transporting drugs during the optimal period of time to a local site of an organ or tissue. The biodegradable polycaprolactone is used in medicine as a suture material and as a thermal bioresorbable implant depot (filler), so it can be used as capsule shells and for drugs. However, polymers have such a disadvantage as hydrophobicity, which prevents

adhesion of cells and their directional migration. Thus, a modification of the surface properties of polycaprolactone is necessary.

The aim of the study is to evaluate the influence of scaffolds based on polycaprolactone with a surface modified by plasma on the differentiation's mechanisms of primary monocyte macrophages *in vitro* [1]. Depending on the surface treatment time (0, 30, 60, 120, 240 seconds), the samples designated as № 1, 2, 3, 4, 5.

The studies were performed on human monocytes isolated from the buffy coat of a healthy human donor. The cells were isolated by magnetic separation on a double ficoll gradient of different densities using magnetic assays conjugated to antibodies to the CD14+ [2].The samples were placed on the bottom of a 24-well plate, isolated monocytes were added at a concentration of 1×10^6 in 1 ml. For each coating 3 experimental groups were formed: control (non-stimulated), with the addition of IFNγ (M1 activation) and with IL-4 (M2 activation). To assess the functional phenotype and the direction of differentiation of macrophages after 6 days of incubation, the cells were stained. In study antibodies: CD68 (mouse monoclonal), Stabilin-1 (RS1, rabbit polyclonal), CD206 (rabbit polyclonal) were used. As secondary antibodies Alexa488-conjugated anti-mouse and Cy3-conjugated anti-rabbit antibodies were used. The combinations of antibodies were verified: CD68/RS1 and CD68/CD206. The analysis of double positive cells was performed using a Carl Zeiss laser scanning microscope at a 100-fold magnification.

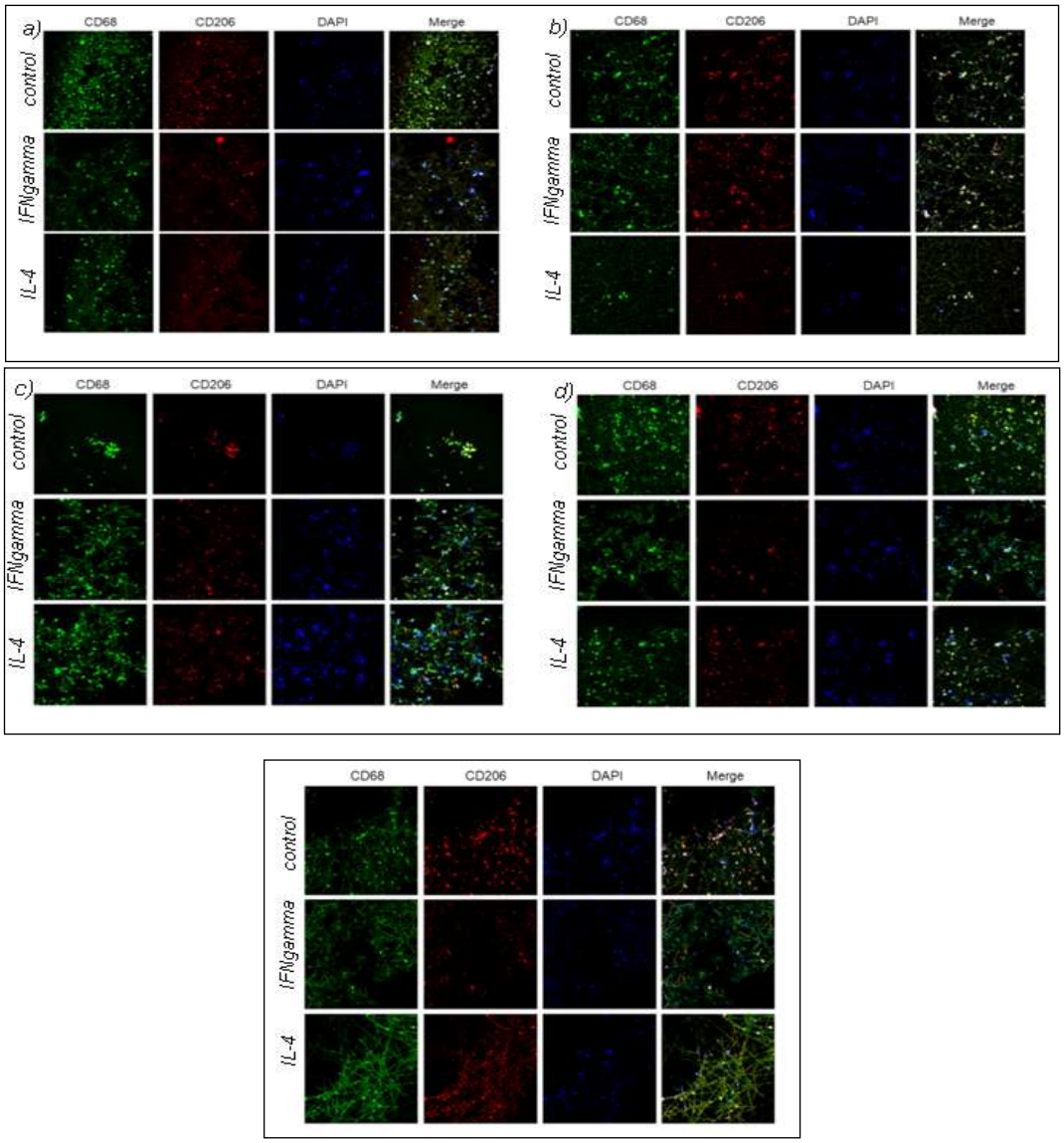


Figure 1. Analysis of CD68/CD206 phenotype macrophage on the sample surface: a – 1, b – 2, c – 3, d – 4, e – 5.

The analysis showed that in all groups after 6 days of cultivation all cells were M2 macrophages phenotype: CD68+/stabilin-1+ excluding group with sample 2. In this case, non stimulated cells

(M0 macrophages) and with the addition of IFNγ (M1 macrophages) had the phenotype CD68+/ stabilin-1. Study of CD206-positive cells showed that after the 6 days of incubation all cells in groups had a double positive color of CD68 +/CD206+ – subpopulation of M2 macrophages. Thus, macrophages cultured on scaffolds express on their surface scavenger receptors with the function of regulation of the intensity of the immune response and acquire a functional phenotype of anti-inflammatory macrophages M2 with immunosuppressive properties. This allows us to conclude that scaffolds have tolerogenic and anti-inflammatory properties. Samples 4 and 5 have the strongest stimulating effect on the expression of stabilin-1 in all three subpopulations of macrophages.

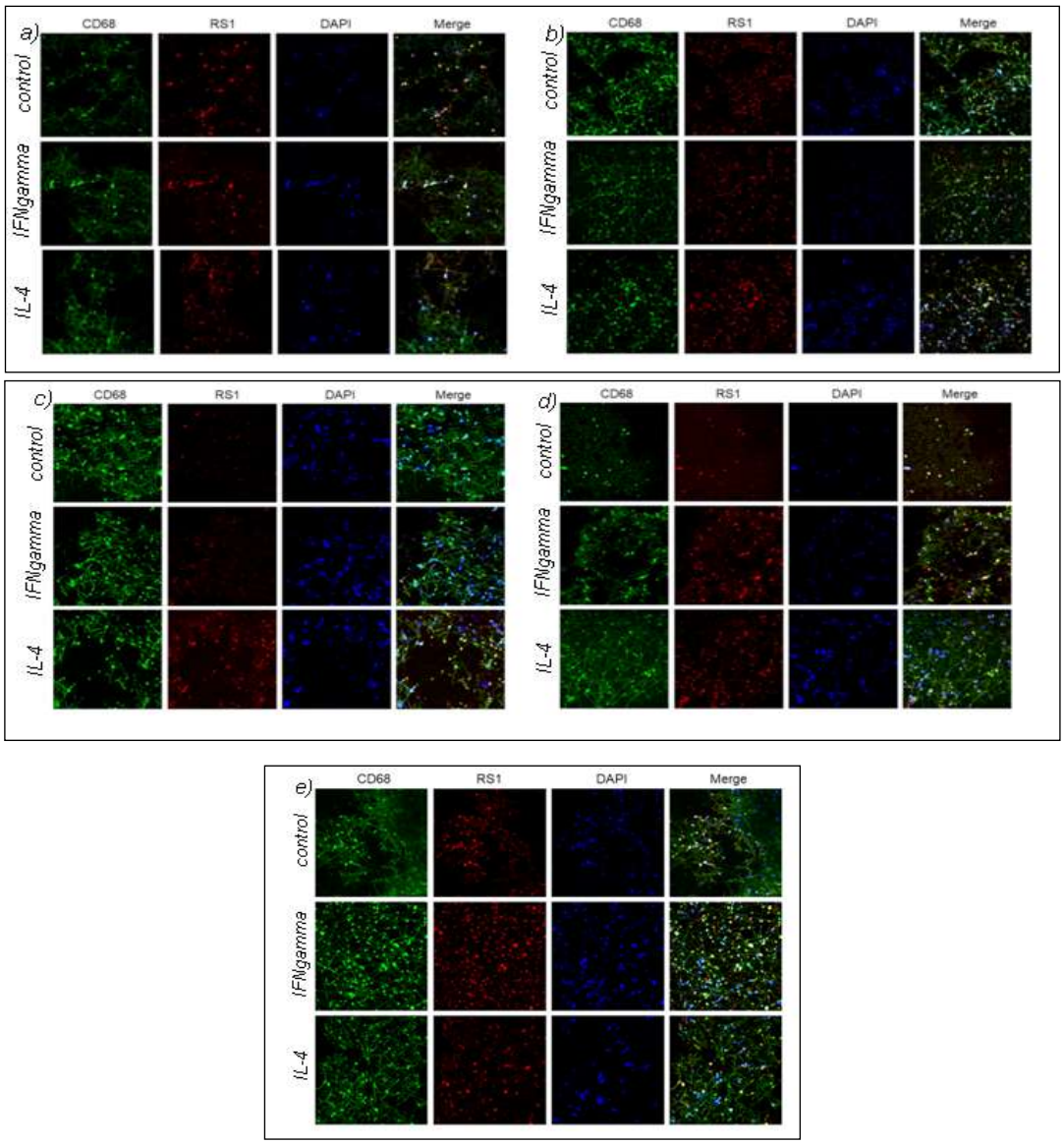


Figure 2. Analysis of CD68/RS1 phenotype macrophage on the sample surface: a – 1, b – 2, c – 3, d – 4, e – 5.

REFERENCES

1. *Kudryavtseva V., Stankevich K., Kibler E., Golovkin A., Mishanin A., Bolbasov E., Tverdokhlebov S.* The deposition of thin titanium-nitrogen coatings on the surface of PCL-based scaffolds for vascular tissue engineering. Applied Physics Letters. 2018; 112(15): 153705. doi: 10.1063/1.5017580.

2. *Gratchev A., Kzhyshkowska J., Köthe K., Muller-Molinet I., Kannookadan S., Utikal J., Goerdt S.* Mphi1 and Mphi2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. Immunobiology. 2006; 211(6-8): 473-86. doi: 10.1016/j.imbio.2006.05.017.

ASSOCIATION OF PROTEASOMAL AND CALPAIN ACTIVITIES AND CONTENTS OF LOCOMOTOR PROTEINS WITH LYMPHOGENOUS METASTASES IN LUMINAL AND TRIPLE NEGATIVE BREAST CANCER

E.E. Shashova[1,2], N.A. Tarabanovskaya[1], E.M. Slonimskaya[1,2], G.V. Kakurina[1], E.S. Kolegova[1], I.V. Kondakova[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Siberian State Medical University, Tomsk, Russia[2]

The aim of the work was to study the association of contents of actin binding proteins (cofilin, gelsolin, Ser45 β-catenin), chymotrypsin-like (ChTL) and caspase-like (CL) activities of proteasomes and calpain activity (CA) in tissues of primary tumors with lymphogenous metastasis of breast cancer. ChTL, CL activity of proteasomes and CA were increased in luminal and TNC breast cancer and lymphogenous metastases tissue compared to adjacent tissues. The thymosin-4-β level was significantly higher in luminal A and TNC metastases compared to the primary BC tissue. The cofilin level was significantly higher in metastases of luminal A and B molecular subtypes compared to the primary BC tissue. There were shown the associations of the content of actin binding proteins (cofilin, gelsolin) and Ser45-β-catenin) and intracellular proteases in primary tumors and lymphogenous metastases, which were the same for different molecular subtypes of BC. Probably, the actin binding proteins, proteasomes and calpains play an important role in tumor dissemination of breast cancers.

Introduction

Breast cancer represents a highly heterogeneous disease comprised by several subtypes with distinct histological features, underlying molecular etiology and clinical behaviors. Various molecular subtypes of breast cancer differ in pathogenesis, in response to chemotherapy, in pathways of metastatic spread [1, 2]. These processes depend on the adequate functioning of protein multiplicity in the cell, which is regulated by proteases, e.g. proteasomes and calpains. We have previously demonstrated increased activity of proteasomes in malignant tumors, in particular, in breast cancer (BC) [3, 4]. However, this study did not take into account the molecular subtype of breast cancer. Proteasomes can cleave cytosceletal proteins, actin-binding proteins and transcriptional factors, which regulate cell locomotion and thus participate in the metastasis [5-7]. Some proteins involved in cell locomotion also serve as substrates for calpains [8, 9]. In this context, analysis of the relationship between activities of proteasome, calpains and locomotor proteins in tumor and metastases is of specific interest. The aim of the study was to investigate the association of actin binding proteins (cofilin, gelsolin, arp-3, thymosin-4-β, Ser45 β-catenin), proteasomes and calpains activities in tissues of primary tumors and lymphogenous metastases of BC.

Experimental

We conducted a prospective analysis of 40 patients with histologically confirmed primary breast cancer in stage $T_{1-3}N_{1-2}M_0$ who had not received neoadjuvant treatment. Fifteen patients (37.5 %) had luminal A breast cancer, 15 patients (37.5 %) had luminal B breast cancer, and 10 patients (25 %) had triple- negative breast cancer.

Samples of tumors, adjacent tissues and lymphogenous metastases were the material for the study. Proteasome chimotrypsin-like (ChTL) and caspase-like (CL) activities, calpain activity (CA) were determined by hydrolysis of fluorogenic oligopeptides Suc-LLVY-AMC and Z-LLGlu-AMC, Suc-LLVY-AMC respectively. The levels of p45 Serβ-catenin, Arp3 protein, and gelsolin in cytokeratin 18 positive cells were studied by flow cytometry. The levels of cofilin and thymosin-4-β were determined by Western blotting.

Statistical treatment of the results was performed using the Statistica 7.0 software package.

Results

ChTL, CL activity of proteasomes and CA were increased in samples of luminal and TNC breast cancers and in lymphogenous metastases compared to adjacent tissues. The thymosin-4-β level was significantly higher in luminal A and TNC metastases compared to the primary BC tissues. The cofilin level was significantly higher in metastases of luminal A and B molecular subtypes compared to the primary BC tissues. There were shown the associations of actin binding proteins (cofilin, gelsolin,

Ser45 β-catenin) and intracellular systems of proteolysis in primary tumors and lymphogenous metastases, which were the same for different molecular subtypes of BC (Figure 1).

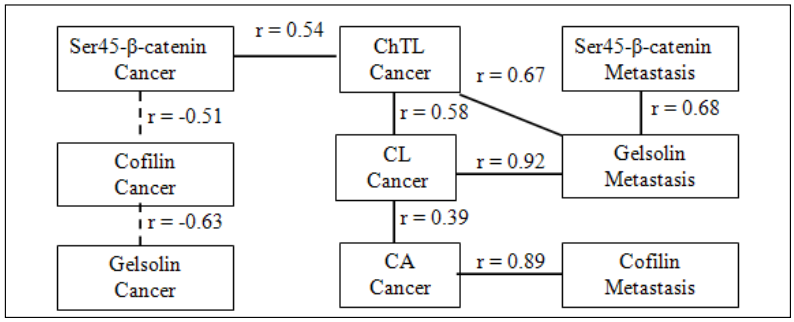


Figure 1. Correlation between actin binding proteins and intracellular systems of proteolysis in primary tumors and lymphogenous metastases

The level of p45-Ser-β-catenin correlated with increased ChTL activity of proteasomes in primary tumors. The levels of p45-Ser-β-catenin and gelsolin in lymphogenous metastases correlated with increased ChTL and CL proteasomal activities in primary tumors. The cofilin level in lymphogenous metastases correlated with CA of cancer tissue (Figure 1). These associations can be associated with destruction of adhesion intercellular contacts and promote the exit of cells into regional lymph nodes.

Conclusion

Probably, the actin binding proteins proteasomes and calpains play an important role in tumor dissemination of breast cancers.

Keywords: proteosome, calpain, metastasis, breast cancer.

REFERENCES

1. *Molnár I.A., Molnár B.Á., Vízkeleti L., Fekete K., Tamás J., Deák P., Szundi C, Székely B., Moldvay J., Vári-Kakas S., Szász M.A., Kulka J., Tőkés A.M.* Breast carcinoma subtypes show different patterns of metastatic behavior. Virchows Arch. 2017; 470(3): 275 283. doi: 10.1007/s00428-017-2065-7.

2. *Neophytou C., Boutsikos P., Papageorgis P.* Molecular Mechanisms and Emerging Therapeutic Targets of Triple-Negative Breast Cancer Metastasis. Front Oncol. 2018 Feb 22; 8: 31. doi: 10.3389/fonc.2018.00031.

3. *Kondakova I.V., Spirina L.V., Koval V.D., Shashova E.E., Choinzonov E.L., Ivanova E.V., Kolomiets L.A., Chernyshova A.L., Slonimskaya E.M., Usynin E.A., Afanasev S.G.* Chymotrypsin-like activity and subunit composition of proteasomes in human cancers. Molecular Biology. 2014; 48(3): 384 389.

4. *Shashova E.E., Kolegova E.S., Zavyalov A.A., Slonimskaya E.M., Kondakova I.V.* Changes in the Activity of Proteasomes and Calpains in Metastases of Human Lung Cancer and Breast Cancer. Bull Exp Biol Med. 2017 Aug; 163(4): 486 489. doi: 10.1007/s10517-017-3834-7.

5. *Kondakova I.V., Yunusova N.V., Spirina L.V., Kolomiets L.A., Villert A.B.* Association between intracellular proteinase activities and the content of locomotor proteins in tissues of primary tumors and metastases of ovarian cancer. Bioorg Khim. 2014 Nov-Dec; 40(6): 735 42.

6. *Liu F., Zhou J., Zhou P., Chen W., Guo F.* The ubiquitin ligase CHIP inactivates NF-κB signaling and impairs the ability of migration and invasion in gastric cancer cells. Int J Oncol. 2015 May; 46(5): 2096 106. doi: 10.3892/ijo.2015.2893.

7. *Yoo Y., Ho H.J., Wang C., Guan J.L.* Tyrosine phosphorylation of cofilin at y68 by v-Src leads to its degradation through ubiquitin-proteasome pathway. Oncogene. 2010 Jan 14; 29(2): 263 72. doi: 10.1038/onc.2009.319.

8. *Storr S.J., Carragher N.O., Frame M.C., Parr T., Martin S.G.* The calpain system and cancer. Nat Rev Cancer. 2011 May; 11(5): 364 74. doi: 10.1038/nrc3050.

9. *Sundaramoorthy P., Sim J.J., Jang Y.S., Mishra S.K., Jeong K.Y., Mander P., Chul O.B., Shim W.S., Oh S.H., Nam K.Y., Kim H.M.* Modulation of intracellular calcium levels by calcium lactate affects colon cancer cell motility through calcium-dependent calpain. PLoS One. 2015 Jan 28; 10(1): e0116984. doi: 10.1371/journal.pone.0116984.

ANALYSIS OF SPFH PROTEINS IN EXOSOMES PRODUCED BY NON-SMALL CELL LUNG CANCER CELLS

G.O. Skryabin, A.V. Komelkov, S.A. Galetsky, M.E. Akselrod, E.M. Tchevkina

N.N. Blokhin National Medical Research Center of Oncology, Moscow, Russia

Stomatin, flotillin-1 and flotillin-2 belong to the SPFH family of membrane lipid raft proteins, and their members are supposedly involved in the biogenesis of exosomes as well as in the sorting of their protein cargo. In this study we showed for the first time the presence of Stomatin in exosomes, produced by cultured non-small cell lung cancer (NSCLC) cells as well as in exosomes from blood plasma of NSCLC patients. We also revealed the correlation between the levels of flotillin-1 and flotillin-2 in studied exosomes samples.

Keywords: exosomes, stomatin, flotillin-1, flotillin-2.

Background

The growing pool of data points to the significance of raft-forming proteins in biogenesis and secretion of exosomes [1-4], although not all of them has been enough studied. Production of exosomal Flotillin-1 has been repeatedly shown for tumor cells of various origins [5, 6]. In a number of cases this protein along with tetraspanins CD9, CD63 and CD81 was used as an exosome marker [7]. Unlike flotillin-1, little is known about the participation of flotillin-2 in biogenesis and secretion of exosomes. Notably, the suppression of one of the homologues (flotillin-1 or -2) in PC3 prostate cancer cells led to the decrease in the level of the other homolog and to the change in the protein composition of exosomes, which indicates the presence of mutual regulation of flotillins and points on their involvement in the Cargo sorting [8]. It is also known that the Flotillin-1 and Flotillin-2 often form oligomers, and these dimers, apparently, can determine the functional activity of Flotillins on plasma membrane [9]. Stomatin is a major protein of the erythrocyte membrane. Despite the similarity with other lipid raft proteins, the presence of the Stomatin and stomatin-like proteins in exosomes has not been studied to date, except for a few studies of exosomes secreted by red blood cells [10,11], K562 cells (erythroleukemia), Daudi cells (B-cell lymphoma), and by reticulocytes from patients' blood. The authors suggest that these proteins within the lipid rafts participate in the sorting of other proteins secreted by exosomes [12].

Material and Methods

Exosomes from conditioned media of NSCLC cells were isolated using differential ultracentrifugation method. Briefly, cells were grown to subconfluent state in DMEM media supplemented with 10% exosome-depleted FBS. Culture media was removed few times to collect in sum 300 ml of conditioned media for each cell line. Media was centrifugated 10 min. at 300xg to remove cells, then centrifugated at 2000xg 10 min.to remove debris, after which supernatant was centrifugated at 10 000xg (30 min) and 20 000xg (30min) to remove large vesicles. Supernatant was used for isolation of exosomes. After ultracentrifugation (110 000xg 90 min) pellet was washed with PBS and second round of ultracentrifugation (110 000xg 90 min) was performed. The pellet was diluted in 100mkl of PBS and transferred to low binding Eppendorf tubes (DNA LoBind Tube 0.5mL). Protein concentration was measured using Breddferd assay. Equal amount (10 mkg) of exosomal proteins was loaded to the SDS-PAAG followed by immunoblotting using antibodies: anti Flot-1 (BD 610821), anti-Flot-2 (BD 610384) anti-Stom (Santa Cruz Biotechnology sc-134554) and anti-CD9 (Cell Signaling 13174S). Corresponding cells were lysed and cellular proteins were analyzed in the same way and used as controls. Samples of peripheral blood from 15 patients with a verified diagnosis NSCLC were collected before the treatment. For isolation of vesicles 15 ml of purified plasma was diluted 1:3 with PBS, exosomes were isolated similarly excepting the time of first ultracentrifugation which was 3 hours. Exosomes were validated by TEM and NTA (Nanoparticle Tracking Analysis) and by the presence of CD9 exosomes protein marker.

Results

The analysis of Flotillins showed that the levels of Flotillin-1 and Flotillin-2 correlate with each other both in cellular exosomes and in exosomes from blood plasma. Thus, according to the densitometric analysis, the distribution of the of Flotillin-1 levels between the samples corresponds to the distribution of Flotillin-2 levels in the same samples. The results obtained here are in agreement with the literature data, according to which Flotillins-1 and -2 are presented in a ratio of 1: 1 on cellular membranes. The maximum level of both Flotillins was detected in H1299 cell line, which is derived from lung carcinoma metastasis and is characterized by the most aggressive phenotype. Stomatin was detected in exosomes from all NSCLC cells studied. In A549 line Stomatin was detected at a very low level (both in exosomes and in cells), while H1299 line was characterized by the maximum level of Stomatin. Notably, exosomes from H1299 are characterized by a much higher content of Stomatin than the parental cells. A high level of Stomatin was detected in exosomes of blood plasma of patients. Interestingly, the level of Stomatin did not correlate with the level of the Flotillins in the same samples.

Conclusion

The obtained data for the first time testifies the presence of Stomatin in exosomes produced by NSCLC cells, as well as in the exosomal fraction of NSCLC patients' blood plasma. A correlation was found between the levels of exosomal flotillin-1 and -2 proteins. It could be suggested that both Flotillins equally contribute to the formation of ILV (intraluminal vesicles, intracellular precursors of exosomes) on the membrane of late endosomes by forming oligomers in the sites of

future exosome formation. In this case, the found correlation between these proteins in exosomes testifies to the lipid raft-dependent formation of exosomes.

REFERENCES

1. *Kajimoto T., Okada T., Miya S., Zhang L., Nakamura S.* Ongoing activation of sphingosine 1-phosphate receptors mediates maturation of exosomal multivesicular endosomes. Nat Commun. 2013; 4: 2712. doi: 10.1038/ncomms3712.
2. *Soung Y.H., Nguyen T., Cao H., Lee J., Chung J.* Emerging roles of exosomes in cancer invasion and metastasis. BMB Rep. 2016 Jan; 49(1): 18-25. doi: 10.5483/BMBRep.2016.49.1.239.
3. *Yuyama K., Sun H., Mitsutake S., Igarashi Y.* Sphingolipid-modulated exosome secretion promotes clearance of amyloid by microglia. J Biol Chem. 2012 Mar 30; 287(14): 10977-89. doi: 10.1074/jbc.M111.324616.
4. *Trajkovic K., Hsu C., Chiantia S., Rajendran L., Wenzel D., Wieland F., Schwille P., Brügger B., Simons M.* Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science. 2008 Feb 29; 319(5867): 1244-7. doi: 10.1126/science.1153124.
5. *Keerthikumar S., Gangoda L., Liem M., Fonseka P., Atukorala I., Ozcitti C., Mechler A., Adda C.G., Ang C.S., Mathivanan S.* Proteogenomic analysis reveals exosomes are more oncogenicthan ectosomes. Oncotarget. 2015 Jun 20; 6(17): 15375-96.
6. *Minciacchi V.R., Freeman M.R., Di Vizio D.* Extracellular vesicles in cancer: exosomes, microvesicles and the emerging role of large oncosomes. Semin Cell Dev Biol. 2015 Apr; 40: 41-51. doi: 10.1016/j.semcdb.2015.02.010.
7. *Demory Beckler M., Higginbotham J.N., Franklin J.L., Ham A.J., Halvey P.J., Imasuen I.E., Whitwell C., Li M., Liebler D.C., Coffey R.J.* Proteomic analysis of exosomes from mutant KRAS coloncancer cells identifies intercellular transfer of mutant KRAS. Mol Cell Proteomics. 2013 Feb; 12(2): 343-55. doi: 10.1074/mcp.M112.022806.
8. *Phuyal S., Hessvik N.P., Skotland T., Sandvig K., Llorente A.* Regulation of exosome release by glycosphingolipids and flotillins. FEBS J. 2014 May; 281(9): 2214-27. doi: 10.1111/febs.12775.
9. *Otto G.P., Nichols B.J.* The roles of flotillin microdomains - endocytosis and beyond. J Cell Sci. 2011 Dec 1; 124(Pt 23): 3933-40. doi: 10.1242/jcs.092015.
10. *Salzer U., Prohaska R.* Stomatin, flotillin-1, and flotillin-2 are major integral proteins of erythrocyte lipid rafts. Blood. 2001 Feb 15; 97(4): 1141-3.
11. *Salzer U., Hinterdorfer P., Hunger U., Borken C., Prohaska R.* Ca(++)-dependent vesicle release from erythrocytes involves-stomatin-specific lipid rafts, synexin (annexin VII), and sorcin. Blood. 2002 Apr 1; 99(7): 2569-77.
12. *de Gassart A., Geminard C., Fevrier B., Raposo G., Vidal M.* Lipid raft-associated protein sorting in exosomes. Blood. 2003 Dec 15; 102(13): 4336-44.

EPSTEIN-BARR VIRUS BIOMARKERS FOR NASOPHARYNGEAL CARCINOMA IN RUSSIA

K.V. Smirnova, N.B. Senuyta, V.N. Kondratova, V.A. Lichtenstein, V.E. Gurtsevitch

FSBI «N.N.Blokhin National Medical Research Center of Oncology» of the Ministry of Health of the Russian Federation, Moscow, Russia

The Epstein–Barr virus (EBV) plays a key role in the development of undifferentiated nasopharyngeal carcinoma (uNPC). The aim of this study was to compare the clinical significance of EBV serological markers and plasma EBV DNA levels for uNPC patients in a non-endemic region, Russia. The results obtained indicate that both viral capsid antigen/immunoglobulin A (VCA/IgA) antibodies and plasma EBV DNA copies can effectively be used for nasopharyngeal carcinoma (NPC) diagnosis. Besides, plasma EBV DNA load was found to be a more sensitive marker of uNPC than VCA/IgA antibody titres, as it reflected the effect of the therapy in stages of remission and relapse of the disease more precisely. Our study, for the first time, demonstrates that the simultaneous use of plasma EBV DNA loads and VCA/IgA antibody levels are indispensable markers for uNPC in non-endemic regions: a serological marker can be more effectively used for NPC screening, but EBV DNA copies are better for monitoring the disease.

Keywords: Epstein–Barr virus, undifferentiated nasopharyngeal carcinoma; plasma DNA EBV; EBV serology.

Introduction

Epstein-Barr virus (EBV), a member of the human herpesviruses family, has unique biological properties. Over 90% of the world's population is infected with EBV, usually without clinical manifestations in carriers of the virus. Furthermore, the virus is recognized as an etiologic agent for a number of benign and malignant diseases [1], one of which is nasopharyngeal carcinoma (NPC). NPC is a malignant tumour, and EBV has been found to play a key role in its development. In the nasopharynx, the virus stimulates the pathological process in which pre-cancerous lesions progress to the development of cancer [2].

The global incidence of NPC is characterized by geographic and ethnic variability [3]. Its prevalence is highest in the southern provinces of China and South-East Asia (25–30 cases per 100 000 people per year), and is less prevalent in the North African Arabs and the indigenous people

of Greenland and Alaska [4]. In the territory of the former Soviet Union, including Russia, where in 2013 the prevalence of NPC was recorded to affect 0.14% of men and 0.06% of women [5].

Unfortunately until present time we haven't specific virus markers in NPC patients from non-endemic regions. Based on the foregoing, the aim of this study was to perform a comparative assessment of the clinical value of two EBV serological markers, the level of VCA/IgG and VCA/IgA antibody titres and plasma EBV DNA load, in NPC patients in Russia, a non-endemic region.

Material and Methods

A total of 45 patients with diagnosis of undifferentiated nasopharyngeal carcinoma (uNPC) proven on the WHO criteria [6], and 52 patients with other tumours of the oral cavity (OTOC), in which EBV has not been shown to play a role in pathogenesis, and 19 blood donors (BDs) were included in this study. The OTOC were represented by cancers of the mucous membrane of the tongue, floor of the mouth, cheek, retro molar area, lower jaw and palate.

The indirect immunofluorescence staining was performed as previously described [7, 8]. Antibody titres are expressed as their geometric mean value. The amount of viral DNA copies in 1 ml of plasma was determined by real-time PCR with specific primers for the 76 bp fragment in the BamHIW region of viral DNA [9]. Statistical significance of the distribution differences of various measurements was evaluated with the Kruskal–Wallis test. The obtained P-values were considered significant at $P<0.05$.

Results

Serological responses to EBV in uNPC patients and patients without EBV associated with other tumours of the oral cavity (OTOC) and blood donors (BDs) showed that antibodies to VCA/IgG were present in 100% of uNPC patients and BDs. In uNPC patients, the percentage of VCA/IgA antibodies was also high, and was observed in 93.8% (30/32) of uNPC patients before treatment, in 96.8% (30/31) of patients following treatment who were in remission and in 100% (9/9) of patients following treatment who had relapsed; however, the presence of VCA/IgA antibodies was negative in BDs. Within the 57 OTOC patients, the percentage of seropositive samples was rather lower. Thus both types of EBV-specific antibodies follow the same pattern of clinical characteristics of uNPC patients, and VCA/IgA and VCA/IgG antibodies revealed nearly the same level of sensitivity. The antibody response to EBV in OTOC patients also differed between the pre- and post-treatment samples.

Furtheremore, we observed a high concentration of viral DNA in the plasma samples of uNPC patients prior to treatment (median 5594 copies ml^{-1}, IQR –559–32 740 copies ml^{-1}), which dropped to background levels in plasma samples of patients in remission (median 11 copies ml^{-1}, IQR–0 –448.0 copies ml^{-1}) and increased sharply in plasma samples of patients with tumour recurrence or distant metastasis (median 332 177 copies ml^{-1}, IQR–79 492–2 209 558 copies ml^{-1}). The plasma EBV DNA concentration in OTOC patients prior to and following treatment did not exceed the background values (median 22 copies ml^{-1}, IQR–0–83 vs median 0 copies ml^{-1}, IQR–0–133, respectively).

Importantly, both types of EBV-specific antibody levels (VCA/IgG and VCA/IgA) followed the varying concentrations of EBV DNA in the plasma of uNPC patients. The reduction of plasma EBV DNA load in patients in remission, and its increase in patients who had relapsed, was accompanied, respectively, by the weakening and strengthening of the anti-EBV serological response.

Conclusions

Thus, our study carried out in a non-endemic region of Russia clearly demonstrated that plasma EBV DNA load and IgA antibody levels are indispensable uNPC markers, combination of which can be effectively used in this region not only for the diagnosis of uNPC, but also to evaluate the effect of therapy and prognosis of the disease. However, an IgA/VCA marker does not, and the plasma EBV DNA load has limited use for assessing a patient's clinical status.

The reported study was funded by RFBR according to the research project № 18-015-00505.

REFERENCES

1. *Young L.S., Rickinson A.B.* Epstein-Barr virus: 40 years on. Nat. Rev. Cancer. 2004; 4: 757–68. doi: 10.1038/nrc1452.

2. *Gu A.D., Zeng M.S., Qian C.N.* The criteria to confirm the role of Epstein-Barr virus in nasopharyngeal carcinoma initiation. Int J Mol Sci. 2012 Oct 23; 13(10): 13737-47. doi: 10.3390/ijms131013737.

3. *Chang C.M., Yu K.J., Mbulaiteye S.M., Hildesheim A., Bhatia K.* The extent of genetic diversity of Epstein-Barr virus and its geographic and disease patterns: a need for reappraisal. Virus Res. 2009 Aug; 143(2): 209-21. doi: 10.1016/j.virusres.2009.07.005.

4. *Yu M.C., Yuan J.M.* Epidemiology of nasopharyngeal carcinoma. Semin Cancer Biol. 2002 Dec; 12(6): 421-9.

5. *Davydov M.I., Axel E.M.* Statistics of malignant tumours in Russia and CIS countries in 2013. Journal of N.N. Blokhin Russian Cancer Research Center, Ministry of Health. Moscow: Publishing Group of the Russian Cancer Center. 2015.

6. *Barnes L., Eweson J.W., Reichart P., Sidransky D.* Pathology and Genetics Head and Neck Tumors. Lyon: IARC Press. 2005. 433.

7. *Gurtsevitch V., Ruiz R., Stepina V., Plachov I., Le Riverend E., Glazkova T., Lavoué M.F., Paches A., Aliev B., Mazurenko N.* Epstein-Barr viral serology in nasopharyngeal carcinoma patients in the USSR and Cuba, and its value for differential diagnosis of the disease. Int. J. Cancer. 1986; 37: 375–81.
8. *Henle G., Henle W.* Epstein-Barr virus-specific IgA serum antibodies as an outstanding feature of nasopharyngeal carcinoma. Int. J. Cancer. 1976. 17: 1–7.
9. *Lo Y.M., Leung S.F., Chan L.Y., Chan A.T., Lo K.W., Johnson P.J., Huang D.P.* Kinetics of plasma Epstein-Barr virus DNA during radiation therapy for nasopharyngeal carcinoma. Cancer Res. 2000; 60: 2351–55.

GERMLINE *BRCA1* AND *BRCA2* MUTATIONS IN CASTRATION-RESISTANT PROSTATE CANCER, ASSOCIATION WITH EXPRESSION OF AKT/M-TOR SIGNALING COMPONENTS

L.V. Spirina[1,2], I.V. Kondakova[1], A.K. Gorbunov[1], E.A. Usynin[1], E.M. Slonimskaya[1,2]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Siberian State Medical University, Tomsk, Russia[2]

The association of *BRCA* mutations with activation of AKT/m-TOR signaling pathway is not fully understood. The purpose was to study the AKT/m-TOR pathway component expression in patients with castration-resistant prostate cancer (CRPC) according to the presence of *BRCA1/2* mutations. Forty patients with prostate cancer, 15 patients with castration-resistant prostate cancer and 20 patients with benign hyperplasia were enrolled in the study. The expression of AKT, c-Raf, GSK-3, PDK1, and m-TOR, 70-S64, E-BP1 was determined by real-time PCR. The *BRCA1/2* mutation was determined in allele-specific PCR in real time. Activation of AKT/m-TOR signaling pathway was found in prostate cancers. The activity of this pathway was decreased in CRPCa tissues. Germline *BRCA* mutations in CRPCa were revealed in up to 33% of patients. *BRCA1/2* carriers had an increased duration of ADT high level of PSA and elevated level of AKT expression compared to non-carriers.

Keywords: *BRCA* **mutations; prostate cancer; AKT/m-TOR signaling pathway.**

Introduction

Despite the development of novel effective therapeutic strategies, prostate cancer remains a lethal disease with a high biological and molecular heterogeneity. Germline DNA damage repair gene mutation is found in >10% of metastatic or advanced prostate cancer [1, 2]. To date, germline mutations in the *BRCA* gene represent one of the main risk factors for developing prostate cancer [3]. Cells deficient in functional *BRCA1/2* become unable to repair DSBs, resulting in chromosomal instability, cell cycle arrest and apoptosis, underlining the increased cancer-susceptibility in *BRCA* mutation carriers.

Metastatic castration-resistant prostate cancer (CRPCa) is the lethal form of the disease. Targeted sequencing can identify clinically informative molecular abnormalities in CRPC, which can be found in 25-30% of CRPCa [4].

It is found the high prevalence of activating AKT/m-TOR pathway alterations in prostate cancer [5]. The association of *BRCA* mutations with activation of AKT/m-TOR signaling pathway is not fully understood. The purpose was to study the AKT/m-TOR pathway component expression in CRPCa patients according to the presence of *BRCA1/2* mutations.

Material and Methods

40 patients with advanced prostate cancers, 15 patients with castration resistant prostate cancers and 20 patients with benign hyperplasia were enrolled in the investigation. The expression of AKT, c-Raf, GSK-3, PDK1, and m-TOR, 70-S64, E-BP1 was determined by real-time PCR. The *BRCA1/2* mutation was determined in allele-specific PCR in real time.

Results

We demonstrated that activation of the AKT/m-TOR signaling cascade was detected in prostate cancers. The high levels of AKT and m-TOR expression were revealed. On the contrary, the 4E-BP1 expression was decreased in castration-resistant prostate cancer patients.

The *BRCA1-5382insC* mutation was detected in 3 patients (20%), *BRCA1-4153delA* – in 5 patients (33%), *BRCA1-185delAG* – in 2 patients (13%), *BRCA1-T300G* – in 2 patients (13%) and *BRCA2-6174del* – in 4 patients (27%). Giving the paucity of *BRCA1/2* mutation incidence in patients with CRPCa, we combined all positive for *BRCA1/2* mutation patients in one group.

It is important to be aware that *BRCA1/2* carriers with CRPCa had a higher PSA level and ADT duration compared to non carriers. No differences were seen between carriers and non carriers in a Gleason score. Castro et al. reported that patients with prostate cancer who were positive for a *BRCA1/2* mutation had higher Gleason scores (≥ 8) and PSA level [6]. Our results also raise the question of whether adjuvant treatments may be beneficial for *BRCA* carriers. Because androgen receptor–maintained activity has been shown to increase chromosomal instability [7], an increased duration of ADT may possibly benefit patients with DNA repair defects.

The high prevalence of activating AKT/mTOR pathway alterations was found in prostate cancer [5, 8]. AKT was also involved in *BRCA1*-deficiency mediated tumorigenesis [9] and this fact was also confirmed in the study. AKT expression in prostate cancers was higher in *BRCA1/2* mutation carriers compared to non carriers.

Conclusion

Activation of AKT/m-TOR signaling pathway was found in prostate cancers. The activity of this pathway was decreased in CRPCa tissues. Germline *BRCA* mutations in CRPCa were revealed in up to 33% of patients. *BRCA1/2* carriers had an increased duration of ADT, a high level of PSA and elevated level of AKT expression compared to non-carriers.

REFERENCES

1. *Antonarakis E.S., Lu C., Luber B., Liang C., Wang H., Chen Y., Silberstein J.L., Piana D., Lai Z., Chen Y., Isaacs W.B., Luo J.* Germline DNA-repair Gene Mutations and Outcomes in Men with Metastatic Castration-resistantProstate Cancer Receiving First-line Abiraterone and Enzalutamide. Eur Urol. 2018 Feb 10. pii: S0302-2838(18)30089-7. doi: 10.1016/j.eururo.2018.01.035.

2. *Mateo J., Cheng H.H., Beltran H., Dolling D., Xu W., Pritchard C.C., Mossop H., Rescigno P., Perez-Lopez R., Sailer V., Kolinsky M., Balasopoulou A., Bertan C., Nanus D.M., Tagawa S.T., Thorne H., Montgomery B., Carreira S., Sandhu S., Rubin M.A., Nelson P.S., de Bono J.S.* Clinical Outcome of Prostate Cancer Patients with Germline DNA Repair Mutations: RetrospectiveAnalysis from an International Study. Eur Urol. 2018 May; 73(5): 687-693. doi: 10.1016/j.eururo.2018.01.010.

3. *Modena A., Iacovelli R., Scarpa A., Brunelli M., Ciccarese C., Fantinel E., Bimbatti D., Massari F., Martignoni G., Tortora G.* Investigating *BRCA* Mutations: A Breakthrough in Precision Medicine of Castration-Resistant Prostate Cancer. Target Oncol. 2016 Oct; 11(5): 569-577. doi: 10.1007/s11523-016-0450-9.

4. *Robinson D., Van Allen E.M., Wu Y.M., Schultz N., Lonigro R.J., Mosquera J.M.* Integrative clinical genomics of advanced prostate cancer. Cell. 2015 May 21; 161(5): 1215-1228. doi: 10.1016/j.cell.2015.05.001.

5. *Bitting R.L., Armstrong A.J.* Targeting the PI3K/Akt/mTOR pathway in castration-resistant prostate cancer. Endocr Relat Cancer. 2013 May 20; 20(3): R83-99. doi: 10.1530/ERC-12-0394.

6. *Castro E., Goh C., Leongamornlert D., Saunders E., Tymrakiewicz M., Dadaev T., Govindasami K., Guy M., Ellis S., Frost D., Bancroft E., Cole T., Tischkowitz M., Kennedy M.J., Eason J., Brewer C., Evans D.G., Davidson R., Eccles D., Porteous M.E., Douglas F., Adlard J., Donaldson A., Antoniou A.C., Kote-Jarai Z., Easton D.F., Olmos D., Eeles R.* Effect of *BRCA* Mutations on Metastatic Relapse and Cause-specific Survival After Radical Treatment for Localised Prostate Cancer. Eur Urol. 2015 Aug; 68(2): 186-93. doi: 10.1016/j.eururo.2014.10.022.

7. *Chiu Y.T., Liu J., Tang K., Wong Y.C., Khanna K.K., Ling M.T.* Inactivation of ATM/ATR DNA damage checkpoint promotes androgen induced chromosomal instability in prostate epithelial cells. PLoS One. 2012; 7(12): e51108. doi: 10.1371/journal.pone.0051108..

8. *Spirina L.V., Gorbunov A.K., Kondakova I.V., Slonimskaya E.M., Usynin E.A.* Transcription factor brn-3α and prostate cancer, relation with hormone receptors and akt/m-tor signaling pathway activation. Siberian Journal of Oncology. 2018; 17(1): 26-31. doi: 10.21294/1814-4861-2018-17-1-26-31. [In Russian]

9. *Xiang T., Jia Y., Sherris D., Li S., Wang H., Lu D., Yang Q.* Targeting the Akt/mTOR pathway in *BRCA1*-deficient cancers. Oncogene. 30(21): 2443-50. doi: 10.1038/onc.2010.603.

THE SIGNIFICANCE OF THE IMMUNE SYSTEM FOR THE EFFICIENT CANCER THERAPY

M.N. Stakheyeva[1,2], D.V. Eidenzon[3], M.R. Patysheva[1], N.V. Cherdyntseva[1,2]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University, Tomsk, Russia[2]
NovoSpark Corporation, Waterloo, Canada[3]

During the last decade it was shown that immune effectors contribute to efficiency of cancer therapy. As a result, the functional state of the host immune system has been considered to have a major prognostic and predictive impact on an efficiency of cancer therapy. The aim of this study was to find out whether the united state of the immune system is associated with the efficiency of the neoadjuvant chemotherapy in breast cancer patients. One hundred six patients diagnosed with primary local breast cancer (I-III stages) were enrolled in the investigation. The cancer treatment obligatory included the appropriate courses of neoadjuvant chemotherapy. The response to neoadjuvant chemotherapy was used as criterion of the efficiency of the cytostatic therapy. The immune system state as an integral unit was estimated by NovoSpark Visualization approach. The favorable immune system states in BC patients were associated with effective NAC: 68 %

of patients from this group responded to cytostatic treatment in comparison with 45 % in women with unfavorable immune system states ($\chi 2=3,67$, $p=0,05$). Thus, the efficiency of the cytostatic therapy is associated with the immune system state in BC patients.

Keywords: breast cancer, chemotherapy efficiency, cytostatic therapy, immune system.

For a long time, anticancer therapies were believed to work either by killing cancer cells or by inducing a permanent arrest in their cell cycle [1]. But during the last decade, it is becoming clear that many of the available anticancer drugs mediate therapeutic effects by eliciting de novo or reactivating pre-existing tumor-specific immune responses suppressed by an established malignant tumor [2].

In clinical and experimental studies it was shown that immune effectors contribute to efficiency of cancer therapeutics [1, 2, 3]. As a result, the functional state of the host immune system has been considered to have a major prognostic and predictive impact on the fate of cancer patients treated with conventional or targeted chemotherapies [2, 3].

In general, cancer therapy efficiency was associated with functioning of cytotoxic mechanisms of immune system, such as activity of cytotoxic T-cells, NK and antibodies as mediators of antibody-dependent cell cytotoxicity [3]. But the outcome of chemotherapy can be influenced by the host immune system at multiple levels. This fact supports the idea that immune works in a coordinated fashion against numerous threats from the external or internal environment. Moreover, in our previous studies, we demonstrated that immune response to tumor can be presented as united strategy [4]. We have found that the immune system state can be split on at least two different ones. One state, which we called as favorable, was observed in BC patients without recurrence, and another state – unfavorable - was associated with relapses in BC patients within 3 year follow-up [4]. The aim of this study was to find out whether the united state of the immune system is associated with the efficiency of the neoadjuvant chemotherapy in breast cancer patients.

Material and Methods

One hundred six patients diagnosed with primary local breast cancer were enrolled in the investigation. The patient pathological stages ranged from I to III. Patients with diagnosed metastatic breast cancer (i.e. IV stage) were not enrolled. The cancer treatment obligatory included the appropriate courses of neoadjuvant chemotherapy, adjuvant chemo-, radio- and hormonal therapies according to the pathological stages and routine predictive criteria. The standard chemotherapies regimens predominantly used in the study are FAC, CAF. The response to neoadjuvant chemotherapy according to the WHO was used as criterion of the efficiency of the cytostatic therapy. The therapy was considered effective, when complete or partial responses were achieved in BC patients, and it was ineffective, when stable or progressive disease were observed after 2 cycles of the neoadjuvant treatment.

In order to test the immune system state, venous blood was obtained from the patients before cancer treatment. To characterize the immune system state as an integral unit, we estimated separate parameters of the innate and adaptive arms of immune system in the BC patients and then we integrated the obtained values into a single characteristic as a visual image using NovoSpark Visualization approach [4]. Presenting an immune system state in an integral visual image of NovoSpark Visualizer software for all 106 BC patients, we identified two groups of patients: with favorable (associated with disease-free survival) or unfavorable (associated with recurrences) of immune system state.

All the patients gave written informed consent to participate in this investigation. The study was approved by Ethics Committee of Tomsk Cancer Research Institute and performed according to the guidelines of Declaration of Helsinki.

Statistical analysis was performed using Statistica version 6.1 (StatSoft Inc). Differences between groups were evaluated using the $\chi 2$ test.

Results

In the group, including all BC patients, the half of them (53 women of 106) responded to NAC, but other half did not. The complete and particular responses were observed in four patients and forty nine patients, respectively. Forty two cases of stable diseases and eleven cases of progressive diseases consisted of the group of BC patients without therapeutic response.

Then we classified all BC patients depending on their immune system states with using our original approach. Only twenty two BC patients (21 %) had favorable immune system states prior cancer, but eighty four women (79 %) were characterized as having unfavorable immune system states. The favorable immune system states in BC patients were associated with effective NAC:

68 % of patients from this group responded to cytostatic treatment in comparison with 45 % in women with unfavorable immune system states (Table, $\chi 2=3,67$, $p=0,05$).

Conclusion

The efficiency of the cytostatic therapy is associated with the immune system state in BC patients. Responses to NAC were observed in 68% in patients with favorable immune system states in comparison with 45 % in women with unfavorable ones. Assessment of the immune system status is necessary to select an appropriate cytostatic therapy.

This study was supported by the Russian Foundation for Basic Research (№ 17-29-06037).

REFERENCES

1. *Hannani D., Sistigu A., Kepp O., Galluzzi L., Kroemer G., Zitvogel L.* Prerequisites for the antitumor vaccine-like effect of chemotherapy and radiotherapy. Cancer J. 2011 Sep-Oct; 17(5): 351-8. doi: 10.1097/PPO.0b013e3182325d4d.

2. *Zitvogel L., Galluzzi L., Smyth M.J., Kroemer G.* Mechanism of Action of Conventional and Targeted Anticancer Therapies: Reinstating Immunosurveillance. Immunity. 2013 Jul 25; 39(1): 74-88. doi: 10.1016/j.immuni.2013.06.014.

3. *Stakheyeva M., Yunusova N., Patysheva M., Mitrofanova I., Faltin V., Tuzikov S., Slonimskaya E.* Chemotherapy changes cytotoxic activity of NK-cells in cancer patients. Proceedings of the International Conference on Physics of Cancer: Interdisciplinary Problems and Clinical Applications (PC IPCA'17). Book series: AIP Conference Proceedings. 23-26 may 2017. 1882: 020072-1-020072-4. doi: 10.1063/1.5001651.

4. *Stakheyeva M., Eidenzon D., Cherdyntseva N., Slonimskaya E., Cherdyntsev E.* Multidimensional Visualization for the Immune System State Presentation in Breast Cancer Patients. AIP Conference Proceedings. 1688: 080003 (2015). doi: 10.1063/1.4936066.

OPTIMIZATION OF DENDRITIC CELL MATURATION PROTOCOL TO ASSESS THE SPECIFIC ACTIVITY OF ANTICANCER DNA-VACCINES IN EX VIVO SYSTEM

E.V. Starostina, E.A. Borobova, L.I. Karpenko

State Research Center of Virology and Biotechnology «Vector», Koltsovo, Novosibirsk, Russia

Treatment of advanced stages of cancer using surgery, chemotherapy, radiation therapy or hormone therapy remains challenging and may not be effective, so it is important to design and improve new treatment methods. One of the promising methods is immune therapy, in particular, the development of anti-cancer vaccines, including genetically engineered DNA-vaccines that induce specific T-cell immune response against the tumor [1]. Lately, to estimate the immune response induced by T-cell vaccine, researchers use the method based on the ability of a vaccine to cause formation of effector T-cells in *ex vivo* system [2]. Generation of mature dendritic cells is a crucial step of that method. The goal of this study is improving the protocol of generation of mature dendritic cells (DC) to study immunogenicity of DNA-vaccines against **oncological diseases** *ex vivo* using PBMC from healthy donors [3]. There are various ways of generating mature dendritic cells, differing in the reagents used [4]. Unlike mature DC, immature DC are capable of endocytosis, therefore they can process and present antigen on their surface. So, immature DCs are used for antigen uptake. After loading with antigen, DCs are cultivated with maturing factors [1]. During the maturation, immature DC lose specific markers such as CD11c, CD83 and costimulatory signals CD86 and CD80 as well as start producing cytokines [5,6]. To obtain mature DC we tried several protocols. First, we obtained mononuclear cells that adhere to cultural plastic from human peripheral blood, then cultivated it with GM-CSF (50 ng/ml) and IL-4 (100 ng/ml) for two days [7]. After the cultivation immature DC were magnetically transfected with DNA-vaccines; maturation factors were added and cells were cultivated for two more days. We used three different maturation cytokine cocktails: 1) only TNFα (25 ng/ml) [6]; 2) TNFα (25 ng/ml), IFN-γ (3000 units/ml) and polyI:C (25 μg/ml); 3) TNFα (50 ng/ml), IFN-γ (3000 units/ml), polyI:C (25 μg/ml) and IL-1b (25 ng/ml) [8,9]. Using each cocktail, we obtained mature DC expressing costimulatory signals CD86, CD80, and markers CD11c and CD83. However, we obtained DC with more number of maturation markers when third maturation cocktail was used. The system *ex vivo* was approved for evaluation of the efficacy of DNA vaccines against melanoma pMEL-TCI-A0201 and pMEL-TCI in our laboratory [3]. DC obtained using the third cocktail, transfected with pMEL-TCI-A0201 and pMEL-TCI, provided more effective activation of specific cytotoxic T-lymphocytes.

Keywords: DNA-vaccine, dendritic cells, oncological diseases, cytokine cocktail.

REFERENCES

1. *Skalova K., Mollova K., Michalek J.* Human myeloid dendritic cells for cancer therapy: does maturation matter? Vaccine. 2010 Jul; 28(32): 5153-60. doi: 10.1016/j.vaccine.2010.05.042

2. *Sennikov S.V., Kulikova E.V., Obleuhova I.A., Shevchenko Yu. A.* Technologies of cellular antitumor immune response induction in vitro. Geny i kletki. 2015; 10(2): 16-22.

3. *Borobova E.A., Antonets D.V., Starostina E.V., Smirnova O. Yu., Scherbakov D.N., Volkova O. Yu., Oreshkova S.F., Karpenko L.I., Ilyichev A.A., Bazhan S.I.* Candidate DNA vaccines against melanoma: design and evaluation of expression of target genes in eukaryotic cells. Vestnik NGU. Seriya: Biologiya, klinicheskaya medicina. 2012; 10(5): 23-30.

4. *Nazarkina Zh., Laktionov P.* Preparation of dendritic cells for cancer immunotherapy. Biomed Khim. 2015 Jan-Feb; 61(1): 30-40.

5. *Decker W., Xing D., Shpall E.* Dendritic cell immunotherapy for the treatment of neoplastic disease. Biol Blood Marrow Transplant. 2006 Feb; 12(2): 113-25.

6. *Ueno H., Klechevsky E., Morita R., Aspord C., Cao T., Matsui T., Di Pucchio T., Connolly J., Fay J.W., Pascual V., Palucka A.K., Banchereau J.* Dendritic cell subsets in health and disease. Immunol. Rev.2007; 219: 118-142. doi: 10.1111/j.1600-065-X.2007.00551.x.

7. *Grinevich Yu., Hranovskaya N.* Dendritic cells and prospects of their use in immunotherapy of patients with malignant neoplasms. Zhurn. AMN Ukraïni. 2003; 9(4): 736–753.

8. *Mailliard R.B., Wankowicz-Kalinska A., Cai Q., Wesa A., Hilkens C.M., Kapsenberg M.L., Kirkwood J.M., Storkus W.J., Kalinski P.* Alpha-type-1 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity. Cancer Res. 2004; 64: 5934-5937. doi: 10.1158/0008-5472.CAN-04-1261.

9. *Vopenkova K., Mollova K., Buresova I., Michalek J.* Complex evaluation of human monocyte-derived dendritic cells for cancer immunotherapy. J Cell Mol Med. 2012 Nov; 16(11): 2827-37. doi: 10.1111/j.1582-4934.2012.01614.x.

HEPARAN SULFATE AS POTENTIAL MICROENVIRONMENT BIOMARKERS FOR GLIOBLASTOMA

A.V. Suhovskih[1,4], G.M. Kazanskaya[1,2], A.Y. Tsidulko[1], A.M. Volkov[2], R.S. Kiselev[2,3], V.V. Kobozev[2], A.S. Gaytan[3,5], S.V. Aidagulova[3], A.L. Krivoshapkin[3,5], E.V. Grigorieva[1,2,4]

Institute of Molecular Biology and Biophysics FRC FTM, Novosibirsk, Russia[1]
Meshalkin National Medical Research Center, Novosibirsk, Russia[2]
Novosibirsk State Medical University, Novosibirsk, Russia[3]
Novosibirsk State University, Novosibirsk, Russia[4]
European Medical Centre, Moscow, Russia[5]

Introduction

Protein molecules are used as molecular markers for diagnostics and prognosis of oncological diseases, while the value of glycosylated markers remains underestimated. Glycosylated molecules are important participants of malignant transformation and contribute to creation preferable microenvironment for tumour cells. One of the main protein-carbohydrate components of tumour microenvironment are heparansulfate proteoglycans (HSPGs), which are involved in maintaining of the extracellular matrix structure and cell-cell interactions. HSPG core proteins have been proposed as diagnostic and prognostic markers of malignant tumours. However, the carbohydrate chains of these complex molecules called heparan sulfate (HS) also contribute significantly to the functional activity of PG molecule. During malignant transformation, composition and structure of HS in different cancers are changed but the functional meanings of the changes remain unclear. The purpose of this study was to investigate whether heparan sulfate chains of HSPGs have a clinical significance for diagnosis and prognosis of human brain tumours.

Material and Methods

The expression and distribution of carbohydrate chains of heparan sulfate in glioblastomas was studied by immunohistochemical analysis using specific antibodies to carbohydrate HS epitopes. The expression of HSPG core proteins was studied using real-time RT-PCR. The overall and relapse-free survivals of glioblastoma patients were determined by the Kaplan-Meier survival analysis.

Results

The HS expression was detected in 50-55% of glioblastoma tumours, while almost no HS content was detected in paratumourous brain tissues. It was shown that high heparan sulfate content correlated with low relapse-free survival of patients with glioblastoma according Kaplan-Meier curves. The increase in HS content in glioblastoma tissues did not result in the activation of HS biosynthesis but was associated with up-regulation of expression of HSPGs core proteins (syndecan-1, glypican-1 and perlecan/HSPG2). The expression of perlecan/HSPG2 (but not syndecan-1 or glypican-1) core protein was also associated with low-survival rates of glioblastoma patients according Kaplan-Meier curves. Additionally, heparanase (an enzyme that degradates carbohydrate chain of heparan sulfate) seems to contribute to the increased HS content in poor-prognosis gliobastoma tumours.

Thus, perlecan/HSPG2 core protein as well as heparan sulfate polysaccharide chains are associated with relapse-free survival of the patients with glioblastoma.

Conclusion

The heparan sulfate accumulation and up-regulation of perlecan/HSPG2 expression in glioblastoma tissues were revealed for the first time as prognostic factors for glioblastoma progression and might be used as additional targets for anti-glioblastoma therapy.

Keywords: proteoglycan, heparan sulfate, perlecan/HSPG2, glioblastoma, biomarker.

This work has been supported by a Russian Science Foundation (RSF grant N16-15-10243).

DISRUPTION OF BRAIN EXTRACELLULAR MATRIX BY ANTI-GLIOMA CHEMOTHERAPEUTIC DRUGS PROMOTES INVASION OF GLIOBLASTOMA CELLS IN *EX VIVO* MODEL

A.Y. Tsidulko[1], T.M. Pankova[1], E.V. Grigorieva[1,2]

Institute of Molecular Biology and Biophysics FRC FTM, Novosibirsk, Russia[1]
Novosibirsk State University, Novosibirsk, Russia[2]

Introduction

Glioblastoma (GBM) is the most aggressive malignant brain tumor. Despite numerous studies and new treatment approaches, progress in this field remains insufficient. One of the most dangerous glioblastoma features is its' active invasion into the surrounding normal brain tissue. Survival and invasion of the tumor cells seem to depend not only on their characteristics but also on the extracellular matrix (ECM) of surrounding tissue. During anti-glioma treatment, temozolomide (TMZ) is used as the first-line adjuvant chemotherapy, and dexamethasone (DXM) is often administered as an anti-edema drug. Both the glioblastoma cells and the surrounding normal brain are exposed to the drugs, and effects of the drugs on the normal brain tissue are poorly studied. We hypothesized that the disruption of normal brain ECM by TMZ and/or DXM may lead to formation of the favorable microenvironment for tumor cell proliferation, thus promoting tumor relapse. The purpose of the study was to investigate the effects of the TMZ and DXM on normal brain tissue ECM and its ability to affect glioblastoma cell invasion and proliferation in *ex vivo* organotypic model.

Methods

The *ex vivo* organotypic hippocampus culture (OHC) model was used as it represents the real 3D structure of brain tissue and thus can be used to study ECM. As brain ECM is mainly consists of proteoglycans (PGs), we analysed the expression of a wide panel of PGs (syndecan-1, glypican-1, perlecan, versican, brevican, NG2, decorin, biglican and lumican) in OHCs before and after treatment with DXM and TMZ using RT-PCR. U87 glioblastoma cells were co-cultured with treated and non-treated OHCs, the tumor cells proliferation and invasion were assessed using confocal microscopy.

Results

Treatment of OHCs with DXM led to significant changes in PGs expression. Interestingly, low and high concentrations of DXM resulted in different changes in the expression of individual proteoglycans compared with the control organotypic culture - low concentrations (0.01-0.5μM) increased expression levels of glypican-1 and versican core proteins (2-3-fold and 6-10-fold, respectively), whereas high concentrations (50-200 μM) suppressed syndecan-1 and biglycan expression (2-3-fold each). The results demonstrate complex attenuation of PGs expression in normal rat brain by different DXM concentrations. To investigate the ability of treated and non-treated OHCs to affect proliferation and invasion of U87 glioblastoma cells, the cells were co-cultured with OHCs. U87 cells did not demonstrate any significant invasion into control non-treated OHC. Moreover, cell clones were not abundant and were bounded by OHCs cells. U87 cells co-cultured with DXM-treated OHCs were widely disseminated on the OHCs surface but still did not show significant invasion. Co-culture of U87 cells with OHCs treated with TMZ alone or in combination with DXM led to increased proliferation of U87 cells and their deep invasion into the OHCs.

Conclusions

The data obtained showed that exposure of normal brain tissue to temozolomide and dexamethasone significantly affected the normal brain ECM, creating the appropriate microenvironment for tumor cells proliferation and invasion, thereby promoting tumor relapses.

Keywords: glioblastoma, chemotherapy, extracellular matrix, invasion, proteoglycan.

This work has been supported by a Russian Science Foundation (RSF grant № 16-15-10243). Tsidulko A.Y. was supported by scholarship of Russian Federation President for young scientists (SP-5435.2018.4). Microscopy was performed at the Microscopy Center of the Institute of Cytology and Genetics, SB RAS, Russia

CNA LANDSCAPE OF BREAST TUMOR, CONNECTION WITH THE EFFICIENCY OF NEOADJUVANT CHEMOTHERAPY

M.M. Tsyganov[1], M.K. Ibragimova[1,2], I.V. Deryusheva[1], E.M. Slonimskaya[1,3], N.V. Litviakov[1,2]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University, Tomsk, Russia[2]
Siberian State Medical University, Tomsk, Russian[3]

The research involved 80 patients diagnosed with breast cancer (BC). Biopsy samples were collected before treatment. We studied the tumor tissue using the CytoScan HD Array (Affymetrix, USA) microarray to evaluate the CNA landscape. We studied the frequency of segmental and numerical CNA occurrence and their association with the efficiency of neoadjuvant chemotherapy (NAC).

Introduction

Deletions and amplifications of chromosome regions and individual chromosomes are called Copy Number Aberrations (CNA). These types of cytogenetic disorders may affect the expression of genes; generally, in cases of deletions, the expression of genes located in the deleted regions is decreased, and it is increased in the cases of amplifications [1, 2]. CNA are especially widespread in solid tumors of various localizations, namely in breast tumors[3]. CNA is most frequently observed in the 1q, 8q, and 16q regions of a breast tumor.

Only a few studies have reported an association between CNA and NAC in BC. Thus, the study of the CNA landscape of breast tumor before treatment and evaluation of the association between CAN and NAC response is of great importance.

Material and Methods

We examined 80 patients with histologically verified breast cancer (BC) in IIA – IIIB (T1-3-N0-3M0) stages. The patients were in the age range of 28–68 years (median age: 48.2±2.4). The patients received 2-4 courses of chemotherapy according to the FAC, CAX regimens or taxotere monotherapy in the neoadjuvant mode. The efficiency of preoperative chemotherapy was evaluated according to the WHO [3] criteria using findinga of ultrasound imaging and/or mammography. According to the international recommendations, we formed the groups of the patients whose tumors stabilized or progressed after the preoperative chemotherapy (NAC no-response group), and the patients with partial regression (positive response group) [4]. Biopsy samples taken before treatment were used as the material for the study. We extracted DNA from breast tumor tissues using QIAamp DNA mini Kit (Qiagen, Germany). Microarray analysis was carried out using the CytoScanTM HD Array high-density microchips manufactured by Affymetrix (USA).

Results

At the first stage, we analyzed the frequency of segmental chromosome anomalies for each chromosome in each patient. The study showed that the highest frequency of the amplifications (more than 60.0% of the patients) was detected on the long arm of the 1 chromosome in the following locuses: 1q32.1, 1q32.2, 1q32.3, 1q42.13, 1q42.2, 1q43. The biggest frequency of deletions (more than in 58.0% of the patients) was found in these locuses: 16q21, 16q23.2, 16q23.3, 17p13.1, 17p12. We also found the locuses with the complete absence of segmental chromosome anomalies – the absence of the amplifications is the case for the *4p13, 13p13-13p11.1, 14p13-11.1,*

14q11.1, 15p13-11.1, 15q11.1, 21p13-11.1, 21q11.1, 22p13-11.1 locuses; the deletions are absent in the *1q23.2, 1q25.3, 8q23.2, 8q23.3, 8q24.11, 13p13, 13p12, 13p11.2, 13p11.1, 14p13, 14p12, 14p11.2, 14p11.1, 14q11.1, 15p13, 15p12, 15p11.2, 15p11.1, 15q11.1, 21p13, 21p12, 21p11.2, 21p11.1, 21q11.1, 22p13, 22p12, 22p11.2, 22p11.1* locuses. We also found the regions with neither deletions nor amplifications: *13p13-13p11.1, 14p13-11.1, 14q11.1, 15p13-11.1, 15q11.1, 21p13-11.1, 21q11.1, 22p13-11.1.* Then all patients were divided into two groups depending on their response to NAC: group 1 – patients with no response to NAC, group 2 - patients with objective response to NAC (partial or complete regression of tumor after treatment). The frequency of CNA occurrence in patients with stable disease or cancer progression is shown in Figure 1.

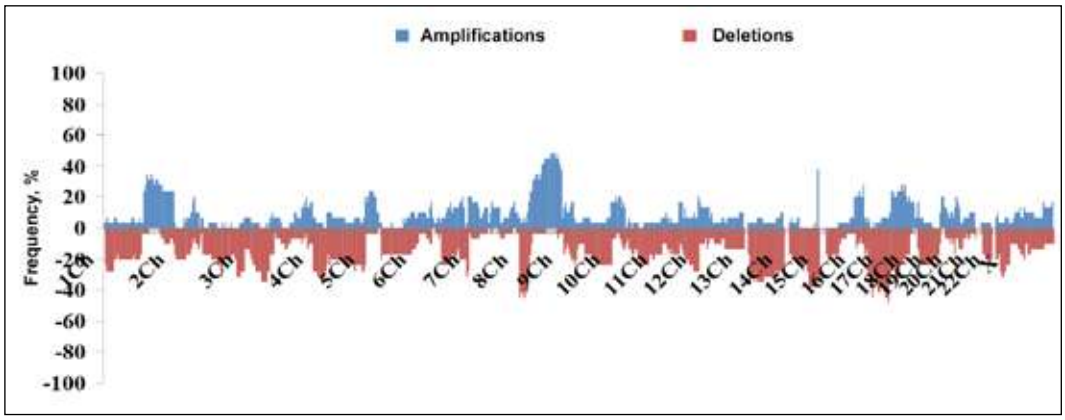


Figure 1. CNA occurrence frequency in the patients with stabilizing or progressing. Note: the horizontal axis – the cytobands and chromosomes from 1 to X; the vertical axis – the frequencies of deletions (below the X-axis) and amplifications (above the X-axis) for each cytoband

The locuses with simultaneous absence of CNA are: *13p13-11.1, 14p13-11.1, 14q11.1, 15p13-11.1, 15q11.1, 21p13, 21p12, 21p11.2, 21p11.1, 21q11.1, 22p13-11.1.* The maximum frequency of amplification occurrence (48.3% or more) was detected in the *8q22.3, 8q23.2, 8q23.3, 8q24.13* regions. It should be noted that the highlighted locuses with high frequency of amplifications occurrence showed the absence of deleted regions. The biggest amount of deletions (44.8-48.3% and more) in this group was detected in the *8p23.3, 8p21.3, 8p21.2, 16q21, 17p13.1, 17p12, 17p11.2* cytobands. The frequency of CNA occurrence in patients with partial or complete regression of tumor is shown in Figure 2.

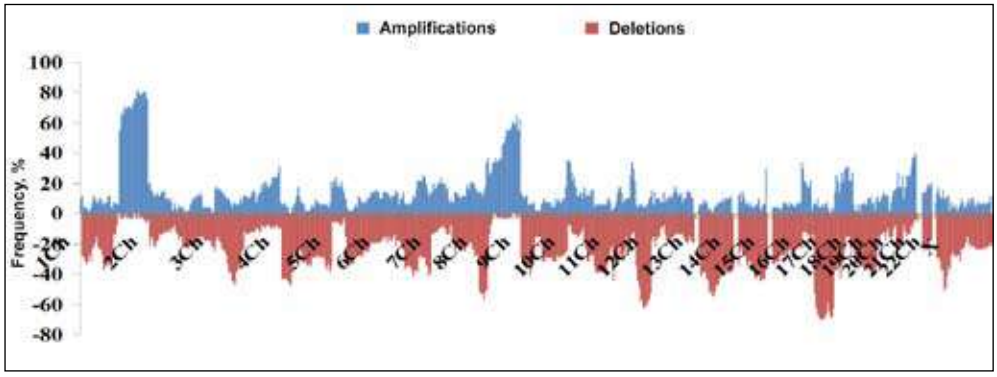


Figure 2. The frequency of CNA occurrence in patients with partial or complete regression of tumor. Note: the horizontal axis – the cytobands and chromosomes from 1 to X; the vertical axis – the frequencies of deletions (below the X-axis) and the amplifications (above the X-axis) for each cytoband

The maximum frequency of amplification occurrence (60.0% and more) was detected in the long arm of the 1 chromosome *1q21.3-44* and in the cytobands. The maximum number of deletions (60.0% and more) in this group was detected in the *11q22.3-23.3, 16q12.2, 16q21-24.2, 17p13.3-11.2* locuses.

The analysis found the cytobands, in which the difference in frequencies of chromosome anomaly occurrence between the groups with or without objective response to NAC reached a maximum value of 35% and more. The biggest difference in the frequency of amplification occurrence between the groups was shown on the long arm of the 1 chromosome *1q23.1-44*, and the biggest difference

in the frequency of deletion occurrence between the groups was in the *11q22.1-23.2, 16q22.2, 16q22.3, 16q23.1, 18p11.21* regions. We also calculated the numerical chromosome anomalies.

Conclusions

The data obtained may be used to predict the efficiency of NAC. The patients with amplifications on the long arm of the 1 chromosome and/or deletions in certain cytobands of the 11, 16, 18 chromosomes are more likely to respond to NAC.

The study is funded by the Russian Science Foundation № 17-15-01203 «Metastatic breast tumor clones»

REFERENCES

1. *Huang N., Shah P.K., Li C.* Lessons from a decade of integrating cancer copy number alterations with gene expression profiles. Brief Bioinform. 2012 May; 13(3): 305-16. doi: 10.1093/bib/bbr056.

2. *Xu Y., Duanmu H., Chang Z., Zhang S., Li Z., Li Z., Liu Y., Li K., Qiu F., Li X.* The application of gene co-expression network reconstruction based on CNVs and gene expression microarray data in breast cancer. Mol Biol Rep. 2012 Feb; 39(2): 1627-37. doi: 10.1007/s11033-011-0902-3.

3. *Hayward J.L., Carbone P.P., Heuson J.C., Kumaoka S., Segaloff A., Rubens R.D.* Assessment of response to therapy in advanced breast cancer: a project of the Programme on Clinical Oncology of the International Union Against Cancer, Geneva, Switzerland. Cancer. 1977 Mar; 39(3): 1289-94.

4. *Kaufmann M., von Minckwitz G., Mamounas E.P., Cameron D., Carey L.A., Cristofanilli M., Denkert C., Eiermann W., Gnant M., Harris J.R., Karn T., Liedtke C., Mauri D., Rouzier R., Ruckhaeberle E., Semiglazov V., Symmans W.F., Tutt A., Pusztai L.* Recommendations from an international consensus conference on the current status and future of neoadjuvant systemic therapy in primary breast cancer. Ann Surg Oncol. 2012 May; 19(5): 1508-16. doi: 10.1245/s10434-011-2108-2.

THE EFFECT OF ALKYLOXYBENZENES ON THE THERMAL DENATURATION OF PROTEINS OF THE COLON CARCINOMA CELL LINE HT-29

E.V. Tsypandina, E.K. Rumyantsev, V.M. Nikolaev, S.D. Efremova, F.V. Vinokurova, S.I. Sofronova

Yakutian Scientific Center of Complex Medical Problems, Yakutsk, Russia

Using a cellular thermal shift assay (CESTA), we showed that alkyloxybenzenes (AOB) have intracellular target proteins in mammalian cells with which they can be bound to. Data were obtained on the change in the properties of proteins under the influence of AOB in physiological concentrations.

Keywords: alkyloxybenzenes, 4-hexylresorcinol, 5-heptylresorcinol, cells of the colon carcinoma cell line large HT-29, the cellular thermal shift assay.

Alkyloxybenzenes (AOB) are mainly synthesized by microorganisms [1], so their concentration increases with infectious diseases in the blood, as well as in intestinal contents in dysbacteriosis. Microorganisms increase the synthesis of AOB under stress, which increases the resistance of these cells. A number of researchers believe that the mechanism of protection of microorganisms AOB is their ability to change the native functions of enzymes [3]. Cells of the colon cancer show resistance to stress, probably as a result of contact with AOB, which are synthesized by the microflora of the colon.

Material and Methods

The material for study was the HT-29 cell lines (colon carcioma). In the work we used the techniques: CESTA [2], polyacrilamide gel electrophoresis (PAGE), staining with silver nitrate. Chemically synthesized AOBs were used: 4-hexylresorcinol, 5-heptylresorcinol.

Results

Figure 1 shows that at a temperature ranging from 55 °C to 65°C, the number of proteins unrelated to the ligand (lanes under number 1) is slightly more (a protein with a molecular mass in the range of 50 and 37 kDa) compared to that after AOB treatment. It should be noted that after heating until 75 °C, this effect is not detected, while proteins treated with AOB are seen. It can also be noted that proteins incubated with 0.2 μM 5-heptylresorcinol compete in the intensity of the bands with a control at a temperature ranging from 55 to 65°C, and an increase in the intensity of staining is observed with increasing temperature.

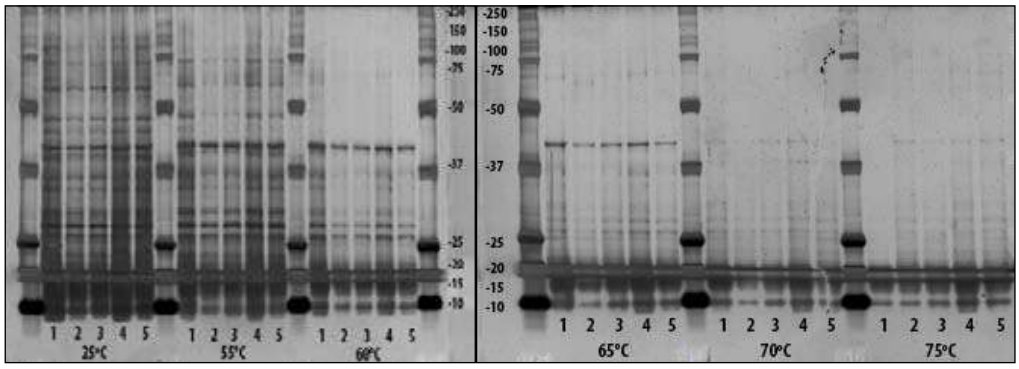


Figure 1. CESTA cells of the HT-29 cell line treated with alkyloxybenzenes

Note. Method of processing: 1 - without processing AOB; 2 - 0.2 μM treatment with 4-hexylresorcinol; 3 - treatment with 2 μM 4-hexylresorcinol; 4 - treatment with 0.2 μM 5-heptylresorcinol; 5 - treatment with 2 μM 5-heptylresorcinol.

Thus, it was found that at physiological concentrations of 0.2 μM and 2 μM, 4-hexylresorcinol approximately stabilized the cellular proteins of colon carcinoma with a molecular weight above 15 kDa, while, for a protein with a molecular weight from 10 kDa to 15 kDa treated with 2 μM 4-hexylresorcinol, greater stabilization was observed compared to proteins stabilized with 0.2 μM 4-hexylresorcinol.

Conclusion

Using thermal denaturation (CESTA) for colon carcinoma cell lines HT-29, it was shown that alkyloxybenzenes have intracellular targets in mammalian cells with which they can be bound to.

4-hexylresorcinol and 5-heptylresorcinol in physiological concentrations are ligands of membrane and / or intracellular proteins of HT-29 cells - human colon carcinoma.

REFERENCES

1. *Gryazev IV; Davydova O.K., Deryabin, D.G., Gennadievna S.T., Sviridov A.P.* The predicted and experimental detected antioxidant activity of alkyloxybenzenes. Questions of biological, medical and pharmaceutical chemistry. 2013; 8: 1-7. [in Russian]

2. *Jafari R., Almqvist H., Axelsson H., Ignatushchenko M., Lundbäck T., Nordlund P., Martinez Molina D.* The cellular thermal shift assay for evaluating drug target interactions in cells. Nat Protoc. 2014; 9(9): 2100-22. doi: 10.1038 / nprot.2014.138.

3. *Stasiuk M., Kozubek A.* Biological activity of phenolic lipids. Cell Mol.Life. Sci. 2010; 67: 841-60. doi: 10.1007 / s00018-009 -0193-1.

PROTEASES IN EXOSOMES IN COLORECTAL CANCER

E.A. Tugutova[1], N.V. Yunusova[1,2], M.R. Patysheva[1], S.G. Afanasiev[1], A.A. Tsydenova[1], I.V. Kondakova[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Siberian State Medical University, Tomsk, Russia[2]

In this work we studied the tetraspanin-associated and tetraspanin-non-associated proteases of blood plasma exosomes. Exosomes were isolated from colorectal cancer and control patients. Differences in the level of proteases, depending on the stage of the disease and on the presence of a metabolic syndrome, were revealed. Further researches are needed to study the role of these proteases in the development and progression of cancer.

Keywords: exosomes, ADAM10, ADAM17, 20S proteasomes, colorectal cancer.

Colorectal cancer (CRC) is the third most common malignancy in most countries of the world. At present, extracellular vesicles, including exosomes (30-100 nm) are of great importance in the processes of tumor invasion and metastasis. Enzymes constitute up to 32% of the protein composition by exosomes. Proteases, enzymes of the hydrolase class play an essential role in the functional activity of extracellular vesicles [1]. Exosomal proteases play an important role in regulation of signaling from growth factor and adhesion receptors as well as in regulation of cell

motility and protein folding. The aim of the study was to evaluate the level of ADAM 10, ADAM17 and 20S proteasomes in exosomes in colorectal cancer patients in association with clinical and histopathological parameters.

Blood samples from control patients (CPs) (n=10, 44.3±3.1 years) and colorectal cancer patients (CRCPs) with stage T2-4N0-2M0-1 (n=60, 58.6±1.6 years) were obtained from the Cancer Research Institute of Tomsk National Research Medical Center. The control group included 10 patients who underwent videocolonoscopy. No colorectal cancer as well as other malignant neoplasms was detected in these patients. Blood plasma exosomes were isolated using ultrafiltration with ultracentrifugation [2, 3]. The obtained exosomes were characterized using transmission electron microscopy and flow cytometry for the content of the most common exosomal membrane proteins [4]. The level of tetraspanin-associated (ADAM10 and ADAM17) and tetraspanin-non-associated (20S proteasome) was evaluated by flow cytometry and western blot analysis. Clinical and histopathological parameters of patients are presented in the Table 1. The study was approved by the Local Ethics Committee of the Cancer Research Institute of Tomsk National Research Medical Center. All patients were fully informed of the purpose and nature of the treatment and provided an informed written consent.

Table 1

Clinical and histopathological parameters of colorectal patients

Parameters	N (%)
Sex:	
Male	29 (48.3)
Female	31 (51.7)
Age	
≤59	18 (30)
>59	42 (70)
Stage	
Nonmetastatic	
Stage II	27 (45)
Stage III	25 (41.6)
Metastatic	8 (13.4)
Tumor grade	
G1-G2	53 (88.3)
G3	7 (11.7)
Presence of metabolic syndrome	
Yes	33 (55)
No	27 (45)

The ADAM10-/ADAM17- population predominated in plasma exosomes of CRCPs, and the level of ADAM10-positive exosomes was significantly higher in exosomes of CPs compared to that of CRCPs. No differences in the level of exosomal 20S proteasomes between subpopulations of ADAM10/ADAM17 exosomes were found. Simultaneous decrease in ADAM10+/ADAM17- subpopulation of exosomes and exosomal 20S proteasomes was observed in patients with metastatic CRC compared with patients with non-metastatic CRC. Colorectal cancer is associated with metabolic syndrome [5]. The level of ADAM17-positive exosomes was significantly lower in exosomes of CRCPs with metabolic syndrome than in exosomes of CRCPs without metabolic syndrome (3.97±0.71 (%) versus 13.04±1.34 (%), respectively ($p<0.05$). The 20S proteasome level in plasma exosomes was lower in CRCPs with metabolic syndrome than in CRCPs without metabolic disorders (1.98±0.25 (r.u.) and 2.92±0.42 (r.u.), respectively, ($p<0.05$).

Further studies of subpopulation composition of exosomes CRCPs are need for elucidating the role of tetraspanin-associated and tetraspanine-non-associated exosomal proteases in cancer development and progression.

The reported research was funded by Russian Foundation for Basic Research and the government of the Tomsk region of the Russian Federation, grant № 18-415-703006.

REFERENCES

1. *Yunusova N.V., Spirina L.V., Kondakova I.V., Kolomiets A.L., Chernyshova A.L., Koval' V.D., Nedosekov V.V., Savenkova O.V.* Relationship between the expression levels of PAPP-A metalloproteinase and growth and transcriptional factors in endometrial cancer. Biology Bulletin. 2013; 40(3): 253-259.

2. *Tamkovich S.N., Yunusova N.V., Stakheeva M.N., Somov A.K., Frolova A.Y., Kirushina N.A., Afanasyev S.G., Grigoryeva A.E., Laktionov P.P., Kondakova I.V.* Isolation and characterization of exosomes from blood plasma of breast cancer and colorectal cancer patients. Biomed Khim. 2017 Mar; 63(2): 165-169. doi: 10.18097/PBMC20176302165.

3. *Yunusova N.V., Tamkovich S.N., Stakheeva M.N., Afanas'ev S.G., Frolova A.Y., Kondakova I.V.* The characterization of exosome from blood plasma of patients with colorectal cancer [Internet]. Physics of cancer: interdisciplinary problems and clinical applications. Tomsk, May 22-25, 2016. AIP Conference Proceedings, 020070; 2016. URL: http://aip.scitation.org/doi/abs/10.1063/1.4960289

4. *Tamkovich S.N., Yunusova N.V., Somov A.K., Afanas'ev S.G., Kakurina G.V., Kolegova E.S., Tugutova E.A., Laktionov P.P., Kondakova I.V.* Comparative subpopulation analysis of plasma exosomes from cancer patients. Biochemistry. Suppl. Series B: Biomedical Chemistry. 2018; 12(2): 151–155. doi: 10.1134/S1990750818020130.

5. *Yunusova N.V., Kondakova I.V., Kolomiets L.A., Afanas'ev S.G., Chernyshova A.L., Shatokhina O.V., Frolova A.E., Zhou Z., Wang W.* Serum adipokines and their receptors in endometrial and colon cancer patients: relationship with tumor invasion and metastasis. Voprosy Onkologii. 2015; 61(4): 619-623.

STUDY OF EXTRACELLULAR DNA IN BLOOD AND ASCITES OF RATS WITH TRANSPLANTED OVARIAN CANCER

I.N. Vasilyeva[1], A.L. Semenov[1], G.V. Tochilnikov[1], V.G. Bespalov[1,2]

N.N. Petrov National Medical Research Center of Oncology, St.-Petersburg, Russia[1]
ITMO University, St.-Petersburg, Russia[2]

The first results of the study of extracellular DNA in blood plasma of female rats with transplanted ovarian cancer indicate the possibility of its using for the diagnosis of the pathological process, monitoring and evaluation of the effectiveness of treatment. The developed model is planned to be used to study the antitumor activity of new drugs.

Keywords: extracellular DNA, blood plasma, transplanted ovarian cancer.

Currently, active research of extracellular DNA (exDNA) is being conducted for the development of minimally invasive methods of diagnosis as a replacement or supplement of tissue biopsy. It is believed that DNA is circulated in the body mainly into the composition of apoptotic bodies, and apoptosis is a major mechanism for DNA releasing in circulation [1]. Tissue diagnostics is a snapshot of the molecular status of a tumor at any time point. The study of DNA circulating in biological fluids allows overcoming the limitations associated with the heterogeneity and plasticity of a tumor and reflects the dynamics of individual tumor biology [2].

The aim of the work is to evaluate the dynamics of changes in the content of extracellular DNA in plasma and ascites during the development of tumor in the model of transplanted ascitic ovarian cancer in female rats.

Material and Methods

The study was conducted on 12 week Wistar female rats (n = 53) weighing 210 ± 35 g purchased from the animal breeding farm Rappolovo (Leningrad region). The rats were kept in standard vivarium conditions in accordance with the national standard of the Russian Federation GOST R 53434-2009 "Principles of Good Laboratory Practice". The work was approved by the local ethics committee of the N.N. Petrov National Medical Research Center of Oncology. The strain of ovarian cancer (OC) was obtained from N.N. Blokhin National Medical Research Center of Oncology. Ovarian cancer was pre-transplanted intraperitoneally to 4 rats, ascites was taken on the 7th day from one rat and 42 rats were inoculated with 0.5 ml of ascitic fluid diluted with saline and containing 1×10^7 cells. Day of transplantation was taken as the 0th. Rats were randomized to 7 groups, 6 rats per group: intact control; 1 day (24 hours), 2 days (48 hours), 3 days, 4 days, 7 days, and 9 days after OC transplantation. Under ether anesthesia blood and ascites fluid were taken correspondingly from heart and abdomen the rats into tubes containing EDTA. The plasma was separated by centrifugation for 10 min, 810 g and 4 °C in the bucket rotor, and centrifuged twice at 2200 g for complete removal of the cell debris. The exDNA in the blood plasma and ascites was determined by Cell Death Detection ELISA PLUS (Roche) [3]. The experimental results were statistically processed in the program GraphPad Prism 6 using Student's t test for unrelated samples. The arithmetic mean and standard error (M ± m) were calculated with the calculation of p.

Results

It was used strain of OC generated by inoculation OC from rat subjected to transplacental exposure of a carcinogen. The initial histological type of the tumor is metastatic papillary

adenocarcinoma, at present it is an ascites tumor [4]. We used an adequate model of transplantable OC that was developed in the abdomen as well as ovarian cancer at later stages in humans. With the development of transplantable ascitic OC in rats, it was found that the level of exDNA in blood plasma did not change significantly during 9 days after its transplantation, but it showed a tendency to decrease by 3-7 days after transplantation, followed by a tendency to increase by the 9th day (Table 1). At the 7-9th days after OC transplantation ascites was developed in the peritoneal cavity of rats. The level of exDNA in ascites was significantly higher than in the blood plasma of both control rats (0th day) and rats with OC from 1 to 9 days after transplantation.

Table 1

Content of extracellular DNA of blood plasma and ascite of female Wistar rats with inoculated ovary tumor

Samples	Day after transplantation	ExDNA, ng/μL
Blood plasma	0	39.53±13.71
	1	34.57±14.04
	2	33.32±11.44
	3	18.87±4.02
	4	35.08±3.11
	7	12.78±3.52
	9	44.8±15.02
Ascites	9	204.26±31.61*

* - Significant differences compared with the 0th day (p=0.0007) and with the 9th day (p=0.0021).

Conclusion

The determination of the exDNA in the model of transplanted OC in female rats appears to be a promising method that requires further investigation. The developed model is supposed to be used for evaluation of effect of cytotoxic drugs. The received data generally confirm the assumption of decreasing level of exDNA during carcinogenesis which in this case occurs in the development of the transplanted tumor.

REFERENCES

1. *Podgornaya O.I., Vasilyeva I.N., Bespalov V.G.* Heterochromatic tandem repeats in the extracellular DNA. Adv Exp Med Biol. 2016; 924: 85-9. doi: 10.1007/978-3-319-42044-8_16.

2. *Holdenrieder S.* Circulating nucleosomes: a new addition to the personalized medicine toolkit. Per Med. 2014 Aug; 11(6): 565-568. doi: 10.2217/pme.14.47.

3. *Holdenrieder S., Stieber P.* Clinical use of circulating nucleosomes. Crit Rev Clin Lab Sci. 2009; 46(1): 1-24. doi: 10.1080/10408360802485875.

4. *Bespalov V.G., Alvovsky I.K., Tochilnikov G.V., Stukov A.N., Vyshinskaya E.A., Semenov A.L., Vasilyeva I.N., Belyaeva O.A., Kireeva G.S., Senchik K.Y., Zhilinskaya N.T., Von J.D., Krasilnikova L.A., Alexandrov V.A., Khromov-Borisov N.N., Baranenko D.A., Belyaev A.M.* Experimental study of antitumor activity and effects on leukocyte count of intraperitoneal administration and hyperthermic intraperitoneal chemoperfusion (HIPEC) with dioxadet in a rat model of ovarian cancer. Int J Hyperthermia. 2017 Sep; 18: 1-6. doi: 10.1080/02656736.2017.1375161.

INFLUENCE OF NATURAL DNA-BINDING COMPOUNDS WITH CANCER PREVENTIVE ACTIVITY ON CHROMATIN STRUCTURE AND INTERFERON SIGNALING

O.A. Vlasova[1], V.P. Maksimova[1], T.I. Fetisov[1], A. Safina[2], E.A. Lesovaya[1,3], G.A. Belitsky[1], K. Gurova[2], K.I. Kirsanov[1,4], M.G. Yakubovskaya[1]

Blokhin NMRCO, Department of Chemical Carcinogenesis, Moscow, Russia[1]
Roswell Park Cancer Institute, Department of Cell Stress Biology, Buffalo, USA[2]
Ryazan State Medical University, Ryazan, Russia[3]
RUDN University, Moscow, Russia[4]

Chromatin-destabilizing effects of the natural DNA-binding small molecules Curcumin, Quercetin, Resveratrol, Genistein, Fisetin, Coumarin, Apigenin, EGCG, Thymoquinon and Naringenin on chromatin structure were analyzed by histone H1 and chaperone of histone SSRP translocation in cell nuclear. Using live cell fluorescent imaging we demonstrated that all the natural DNA-binding compounds studied were able to cause the appearance of cell fractions with high

histone H1 amount localized in nucleolus. Visible changes in location of SSRP in cells treated with all the phytonutrients was also observed by live cell fluorescent imaging and for the number of compounds its translocation from nucleoles was confirmed by Western Blotting. It should be noted that dynamics of the phytonutrients influence on H1 and SSRP translocation varied greatly. We revealed activation of INF signaling by Genistein, Curcumin, Kaempferol, Sanguinarin, Naringenin, Resveratrol, Fisetin and Quercetin using flow cytometry of the HeLa-TI cells with ISRE-mCherry reporter.

Keywords: Histone H1, SSRP, Chromatin structure, Interferon signaling, phytonutrients.

Introduction

Recently, the whole number of natural anticancer compounds has been selected using different animal models of chemical carcinogenesis [1]. Many of these compounds are DNA-binding agents. Cancer preventive effects of the phytonutrients Curcumin, Quercetin, Resveratrol, Genistein, Fisetin, Coumarin, Apigenin, EGCG, Thymoquinon and Naringenin are considered to involve activation of p53-mediated apoptosis and inhibition of NF-kappaB, PI3K, Wnt signaling pathways. However, molecular mechanism of this influence is not understood. We propose that their effects include interference with the normal modes of DNA packaging process via influence on linker histone H1 association with chromatin and c-trapping of FACT (facilitating chromatin transcription) - a heterodimeric protein complex. Previously we described this mechanism for a new promising anticancer drug Curaxin CBL0137, which simultaneously interfere with several signal transduction pathways. CBL0137 binds DNA through intercalation of carbazole moiety between base pairs and symmetrical side chains of the molecule protrude into the major groove of DNA, while carbazole N-side chain fills minor groove. It was confirmed by CD using a cholesteric liquid-crystalline dispersion (CLD) of DNA-ligand complexes. We have shown that the interaction of CBL0137 with DNA duplex leads to disruption of nucleosomes followed by negative DNA supercoiling, stabilization of alternative DNA structures and disorders in the function of topoisomerase I and II, H1, H2B, PARP and FACT [2]. Using methodological approaches developed in the course of our CBL0137 research, we have performed a comparative analysis of the molecular effects of several non-mutagenic DNA-binding phytonutrients with confirmed anticancer activity. Moreover, in our previous studies we demonstrated that minor groove binding ligands prevent PARP1 interaction with DNA and by this way activate retrotransposon expression [3, 4]. Taking into consideration that many natural DNA-binding compounds could interact with DNA helix minor groove, we propose that this mechanism may be also realized by these compounds. As interferon (INF) signaling is induced by retrotransposon expression, its enhancement may be observed in cells treated with natural DNA-binding compounds.

Material and Methods

Live cell fluorescent imaging: two cell lines expressing mCherry-tagged histone H1 (or H2B) were treated with non-toxic dose of the compounds. Estimation of the effect by live cell fluorescent imaging of the cells were both before and after the treatment with different doses of phytonutrients to analyze dose-dependence.

To study the effects of DNA-binding natural ligands on localization of the histone chaperone FACT subunits two different tests were applied. The method of live cell imaging by fluorescent microscopy visualize translocations of GFP tagged FACT subunits in cell lines: (1) HeLa SSRP1-GFP H2B-mCherry, (2) HT1080 SPT16-GFP H2B-mCherry. By means of Western Blotting, we analyzed the redistribution of these protein subunits between the pellet (chromatin binding fraction) and soluble fraction of nucleoplasm.

To analyze influence of natural DNA-binding compounds on INF-signaling, we employed a reporter assay that detected an IFN response through the activation of a consensus IFN-sensitive response element (ISRE), which drove the expression of mCherry. We performed flow cytometry using cell line HeLa-TI with ISRE-mCherry reporter after the cell treatment with non-toxic dose of the compounds. Obtained data were analyzed by WinList 3D program. Curaxin, a new prospective drug with anticancer and tumor preventive activity, was used as a positive control, as previously its effect on the INF-signaling was demonstrated.

Results

Using live cell imaging by fluorescent microscopy, we revealed a significant difference in histone H1 localization among cell population treated with all the phytonutrients and the control group. In particular, there was an increase in the proportion of cells, in which histone H1 was translocated to nucleoli. Effects of Genistein, Resveratrol, Curcumin, Fisetin increased during 24 hours, and

Quercetin, Coumarin, EGCG, Thymoquinon and Naringenin influenced on histone H1 localization faster with maximum effects observed during 6 hour treatment.

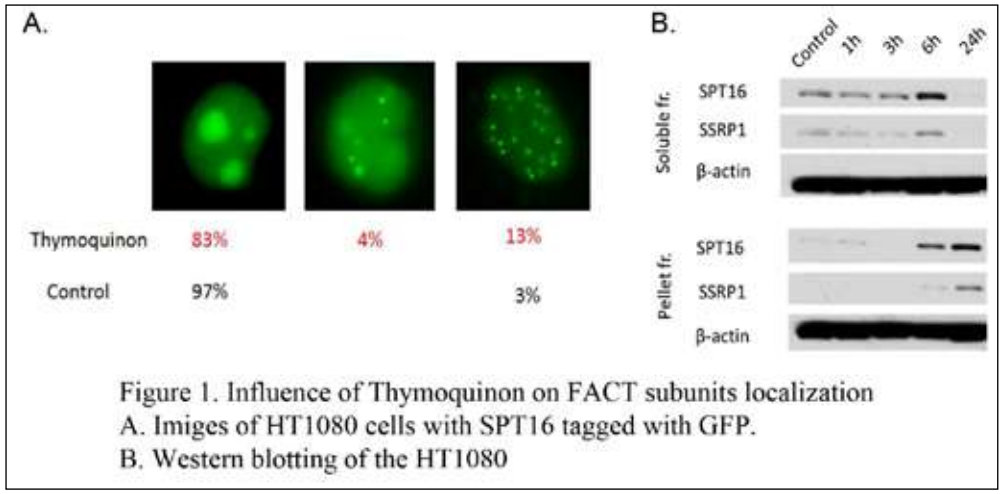


Figure 1. Influence of Thymoquinon on FACT subunits localization
A. Imiges of HT1080 cells with SPT16 tagged with GFP.
B. Western blotting of the HT1080

Several compounds (Resveratrol, Thymoquinon, Curcumin, Sanguinarine, EGCG and Fisetin) caused a lower change of the SSRP1 localization, when cells were treated with the maximum non-toxic doses during 24 hours (Figure 1, A). The redistribution of FACT subunits in time-dependent manner was demonstrated by Western Blotting for Sanguinarine, EGCG and Thymoquinon (Figure 1, B; Figure 2).

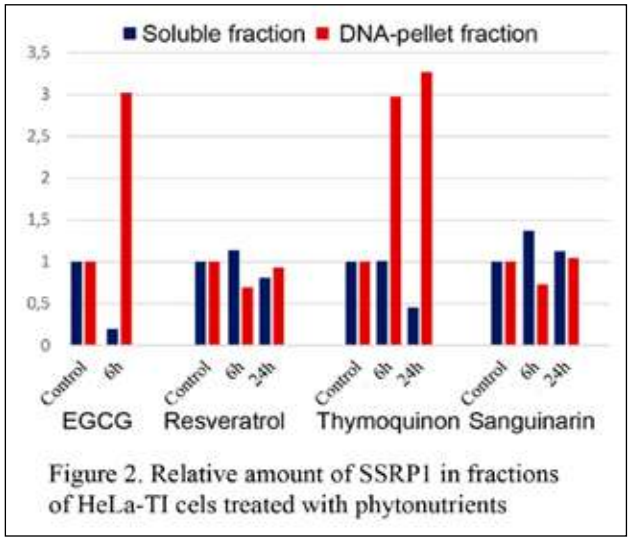


Figure 2. Relative amount of SSRP1 in fractions of HeLa-TI cels treated with phytonutrients

Recently it has been shown that DNA depletion of 2-nd and 4-th isoforms of histone H1 is followed by retrotransposons expression and activation of INF-signaling. We revealed activation of INF signaling after 48-hour cell treatment with the maximum non-toxic dose of Genistein, Curcumin, Kaempferol, Sanguinarin, Naringenin, Resveratrol, Fisetin and Quercetin HeLa-TI with ISRE-mCherry reporter. The level of INF-signaling activity increased up to 20-, 17-, 6-, 13-, 14-, 22-, 27- and 14-folds, correspondingly.

Conclusion

Thus, weak chromatin-destabilizing effects were shown for a number of natural DNA-binding compounds, although dynamics of their influence may vary. We also demonstrated that a number of compounds may induce Interferon signaling.

This work was supported by Russian Scientific Foundation (№ 17-15-01526).

REFERENCES

1. *Pratheeshkumar P., Sreekala C., Zhang Z., Budhraja A., Ding S., Son Y.O., Wang X., Hitron A., Hyun-Jung K., Wang L., Lee J.C., Shi X.* Cancer prevention with promising natural products: mechanisms of action and molecular targets. Anticancer Agents Med Chem. 2012 Dec; 12(10): 1159-84.

2. *Safina A., Cheney P., Pal M., Brodsky L., Ivanov A., Kirsanov K., Lesovaya E., Naberezhnov D., Nesher E., Koman I., Wang D., Wang J., Yakubovskaya M., Winkler D., Gurova K.* FACT is a sensor of DNA torsional stress in eukaryotic cells. Nucleic Acids Res. 2017 Feb 28; 45(4): 1925-1945. doi: 10.1093/nar/gkw1366.

3. *Kirsanov K.I.[1], Kotova E., Makhov P., Golovine K., Lesovaya E.A., Kolenko V.M., Yakubovskaya M.G., Tulin A.V.* Minor grove binding ligands disrupt PARP-1 activation pathways. Oncotarget. 2014 Jan 30; 5(2): 428-37.

4. *Leonova K., Safina A., Nesher E., Sandlesh P., Pratt R., Burkhart C., Lipchick B., Gitlin I., Frangou C., Koman I., Wang J., Kirsanov K., Yakubovskaya M.G., Gudkov A.V., Gurova K.* TRAIN (Transcription of Repeats Activates INterferon) in response to chromatin destabilization induced by small molecules in mammalian cells. Elife. 2018 Feb 5; 7. pii: e30842. doi: 10.7554/eLife.30842.

IMMUNO-ONCOLOGY – A PARADIGM SHIFT IN ANTI-CANCER RESEARCH

E. Vorontsova

Thermo Fisher Scientific, Life Science Solutions, Novosibirsk, Russia

Immuno-oncology is a research approach which harnesses the body's own immune system to fight cancer. Rather than targeting the cancer cell directly, immuno-oncology - also known as cancer immunotherapy - studies ways to turn the host's body against it. It's also important to make the distinction that immunotherapy can be used to treat multiple diseases that stimulate the immune response, which not only includes cancer but cardiovascular, infectious and neurodegenerative diseases as well.

Cancers originate within the bodies of their victims. But contrary to popular misconception, this does not render a cancer invisible to its host's immune system. In fact, many precancerous events do get destroyed by the immune system early on. The ones that last long enough to become cancers do so because they evade and manipulate the host's tissues for long enough to establish themselves. By studying the immune response to cancer, researchers are discovering new therapeutic targets like immune checkpoint inhibitors targeting PD-L1 and CTLA-4 [1], Chimeric antigen receptor (CAR) [2,3].

Older cancer treatments, such as radiation, drugs that target blood vessel growth, and chemotherapy, attack the processes that cancers use to sustain themselves. Unfortunately, these processes are also used by healthy bodies for regular functions, so cancer treatment is difficult to endure. Immuno-oncology research represents a potentially massive step forward for future treatments that are more targeted: the ability to induce an immune response specific to a patient host's own cancer, with the same swift efficacy and comparative safety that a body can bring to bear against harmful invaders.

The next generation of targets in immuno-oncology will need to measure and integrate the complexity of host, tumor, and environment interaction. The measurement of DNA, RNA, and proteins simultaneously with the same sample to obtain a more complete understanding of the immune system response to disease [4, 5].

Thermo Fisher Scientific strives to provide the most innovative technologies to support efforts of scientists in the field of immuno-oncology research with tools for cell-, protein-, and gene-level analysis.

Keywords: cancer immunology, target therapy.

REFERENCES

1. *Srinivasan P., Wu X., Basu M., Rossi C., Sandler A.D.* PD-L1 checkpoint inhibition and anti-CTLA-4 whole tumor cell vaccination counter adaptive immune resistance: A mouse neuroblastoma model that mimics human disease. PLoS Med. 2018 Jan 29; 15(1): e1002497. doi: 10.1371/journal.pmed.1002497.

2. *Grupp S.A., Kalos M., Barrett D., Aplenc R., Porter D.L., Rheingold S.R., Teachey D.T., Chew A., Hauck B., Wright J.F., Milone M.C., Levine B.L., June C.H.* Chimeric antigen receptor–modified T cells for acute lymphoid leukemia. N Engl J Med. 2013 Apr 18; 368(16): 1509-1518. doi: 10.1056/NEJMoa1215134.

3. *Corrigan-Curay J., Kiem H.P., Baltimore D., O'Reilly M., Brentjens R.J., Cooper L., Forman S., Gottschalk S., Greenberg P., Junghans R., Heslop H., Jensen M., Mackall C., June C., Press O., Powell D., Ribas A., Rosenberg S., Sadelain M., Till B., Patterson A.P., Jambou R.C., Rosenthal E., Gargiulo L., Montgomery M., Kohn D.B.* Mol Ther. 2014 Sep; 22(9): 1564-74. doi: 10.1038/mt.2014.148.

4. *Seton-Rogers S.* Immunotherapy: Two antigens are better than one. Nat Rev Cancer. 2016 Mar; 16(3): 128-9. doi: 10.1038/nrc.2016.17.

5. *Batlevi C.L., Matsuki E., Brentjens R.J., Younes A.* Novel immunotherapies in lymphoid malignancies. Nat Rev Clin Oncol 13:25–40. PLoS Med. 2018 Jan; 15(1): e1002497.

DEVELOPMENT AND CHARACTERIZATION OF CV6-168, A NOVEL AND SELECTIVE DUTPASE INHIBITOR THAT ENHANCES THE ANTITUMOUR EFFICACY OF TS-TARGETED THERAPIES

P.M. Wilson[1], K.A. Mulligan[1], M.J. LaBonte[2], K. McLaughlin[1], V. Prise[1], P.G. Johnston[1], R.D. Ladner[1]

CV6 Therapeutics (NI) Ltd, Health Sciences Building (HSB), Centre for Cancer Research and Cell Biology, Northern Ireland, United Kingdom[1]
QUB, Northern Ireland, United Kingdom[2]

Background

Thymidylate synthase (TS) inhibitors are standard-of-care therapies for the majority of high incidence cancers. Deoxyuridine triphosphate nucleotidohydrolase (dUTPase) is a gatekeeper enzyme that protects cancer cell DNA from the misincorporation of uracil that would otherwise occur during treatment with TS-targeted therapies such as 5-FU, capecitabine, S-1, pemetrexed and methotrexate. Uracil misincorporation induced by inhibition of dUTPase causes extensive and irreparable DNA damage during treatment with TS-targeted therapies and strongly enhances cancer cell death. dUTPase is highly-expressed across many cancer types and is associated with resistance to TS-targeted therapies. Here we report the development and characterization of CV6-168, a novel and selective inhibitor of dUTPase that significantly enhances the efficacy of TS-targeted therapies against a broad spectrum of human cancer models.

Methods

The mode of inhibition of dUTPase by CV6-168 was determined against recombination human dUTPase in a fluorescence-based assay developed in our laboratory. In vitro cell growth inhibition was determined by CellTiter-Glo in 20 human cancer cell lines spanning 13 cancer types. Cell viability was measured by clonogenic cell survival assay in 48 human cancer cell lines spanning 18 cancer types. DNA damage was assessed by flow cytometry by measuring H2A.X and ATM activation. Nucleotide pools were measured using the aforementioned fluorescence-based assay. The in vivo antitumor activity of CV6-168 was evaluated in xenograft models.

Results

CV6-168 inhibited dUTPase in a competitive and reversible mode with a Ki of 251 nmol/L. CV6-168 exhibited no intrinsic cytotoxicity as a single agent but significantly enhanced cancer cell death in clonogenic assays when combined with FUdR (5-FU metabolite) in all cell lines across all cancer types tested. CV6-168 significantly enhanced the growth inhibition induced by FUdR in 21 out of 22 cancer cell lines analysed. The mean sensitization factor was 23.7 and median 11.7 (median range 1.5119). Mechanistic analyses demonstrated that this enhancement was accompanied with a significant increase in dUTP pool expansion and DNA damage. CV6-168 significantly increased the antitumour activity of 5-FU in both the HCT116 and LoVo colon xenograft cancer models with no evidence of increased toxicity.

Conclusions

CV6-168 is a potent and selective dUTPase inhibitor that significantly enhances the anticancer activity of 5-FU in both in vitro and in vivo models. CV6-168 represents a promising new therapeutic agent with the potential to significantly improve the clinical efficacy of TS-targeted therapies across multiple cancer types. CV6-168 will be evaluated in an upcoming Phase I clinical trial.

EXTRANUCLEAR AZASTEROIDS AS POTENT ANTIPROLIFERATIVE AGENTS

M.A. Yastrebova[1], M.V. Gudkova[1], A.M. Scherbakov[1], A.S. Komendantova[2], A.V. Komkov[2], E.I. Chernoburova[2], Y.A. Volkova[2], I.V. Zavarzin[2]

N.N. Blokhin National Medical Research Center of Oncology, Moscow, Russia[1]
N.D. Zelinsky Institute of Organic Chemistry, Moscow, Russia[2]

Hormone-dependent cancers (HDC), including those of the breast and prostate, are of the most commonly diagnosed cancers in women/men and the second most common cause of cancer deaths in the world. Typical medicines used to treat breast/prostate cancers are derived from sex steroid hormones (Figure 1), playing role of the primary modulators in steroidogenesis of malignant growths. Usually, these drugs are effective if the cancer complies, but lack of compliance can lead to the development of multidrug resistance resulting in unsuccessful chemotherapies. Therefore, there is a need in the development of new, more potent and selective cytotoxic drugs for prostate and breast cancer.

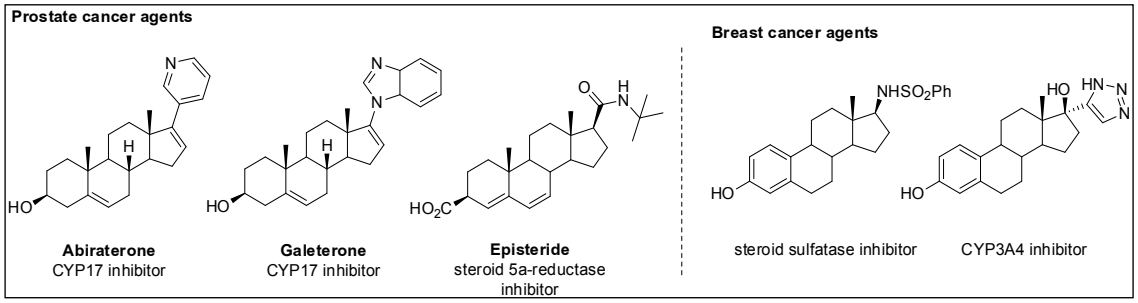
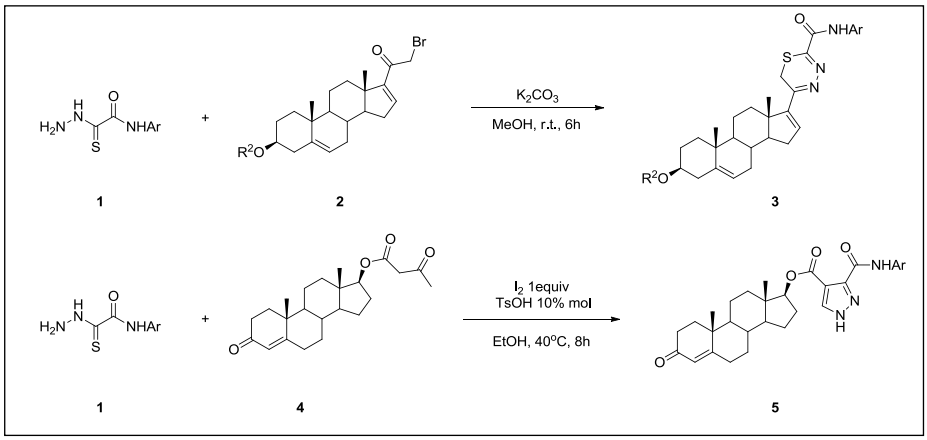


Figure 1. Examples of steroid-based cytotoxic drugs and agents.

Heterosteroids of the estrane and androstane series containing the aza-substituent on rings A and D are among privileged scaffolds for the anti-HDC drug discovery (Figure 1). These compounds demonstrate activity as steroid sulfatase inhibitors for breast cancer, 5α-reductase inhibitors for the treatment of benign prostatic hyperplasia, and 17α-hydroxylase-C17,20-lyase (CYP 17) inhibitors for advanced prostate cancer therapy [1]. A number of steroids containing imidazole, indazole, pyrazole, thiazole, or triazole pendants at 17-position demonstrated inhibition against prostate cancer cells at the nanomolar level [2, 3]. Abiraterone – 17-(pyridin-3-yl)androsta-5,16-dien-3-ol (the first steroidal CYP17 inhibitor approved by the FDA in 2011) - became one of the top pharmaceutical products by U.S. retail sales in 2014, while galeterone - 17-(1H-benzimidazol-1-yl)androsta-5,16-dien-3β-ol, another CYP17 inhibitor with AR antagonistic and ablative activities, - reached phase III clinical trials. Recently, we have reported the synthesis and antiproliferative activity of steroidal pyridazines, thiadiazoles, and 4,5-disubstituted pyrimidines [4, 5]. This study was focused on the synthesis of unique derivatives of androstene series containing *N*-aryl-6H-1,3,4-thiadiazine-2-carboxamide and *N*-aryl-1H-pyrazole-3-carboxamide motifs linked to the D ring of the steroid core and the evaluation of their inhibitory activity against prostate cancer cells.

Material and Methods

Steroidal 6H-1,3,4-thiadiazines 3 were synthesized by condensation of oxamic acid thiohydrazides 1 with 21-bromo-pregna-5,16-diene-20-ones 2 under mild reaction conditions (Scheme 1). Steroidal 1H-pyrazoles 5 were obtained from acetoacetic acid-(3-oxo-androst-4-en-17β-yl ester) (4) by treatment with oxamic acid thiohydrazides 1 in the presence of molecular iodine. All compounds were isolated by column chromatography. Their structure was proved by ^{1}H, ^{13}C NMR spectra.



Scheme 1. Synthesis of novel heterosteroids

Results

Our interest in the preparation of structurally diverse heterosteroids created a need for a facile flexible strategy, in which a common intermediate can be used in a conjunctive fashion to form an array of *N*-heterocycles attached or fused to the steroid core. Hence, we turned to oxamic acid thiohydrazides, as simple "versatile agents" for the modification of steroids bearing a carbonyl group.

Based on our previous results, we accomplished the efficient synthesis of novel heterosteroids possessing a six-membered *N*-heterocycle attached to the D ring of the steroid core starting from readily available materials. Thus, the synthesis of derivatives of the androstene series 3 and 5 containing D-ring linked *N*-aryl-6H-1,3,4-thiadiazine-2-carboxamide and *N*-aryl-1H-pyrazole-3-carboxamide motifs was accomplished starting from preliminary modified natural hormones pregnane and androstene 2 and 4 by the condensations with oxamic acid thiohydrazides 1 (Scheme 1).

Evaluation of the antiproliferative activity of synthesized compounds was carried out on AR-negative DU145 and AR-positive 22Rv1 prostate cancer cell lines. The compounds demonstrated selectivity to the hormone-dependent prostate cancer cells. Series **5** showed activity towards 22Rv1 cells at concentrations above 25 μM, which is comparable to that of androgen receptor inhibitor bicalutamide. Series **3** was significantly more active than bicalutamide and caused cytotoxic effects at concentrations of about 3 μM. The overall potency of steroidal compounds **3** was much higher than that of chemotherapeutic drug cisplatin.

Conclusions

In general, the series of extranuclear azasteroids can be considered for the development of new drug candidates against hormone-dependent prostate cancers.

The biology experiments of the research were supported by RFBR 18-015-00422, the chemistry part was supported by RFBR projects 17-53-45127 and 18-33-00913.

Keywords: hormone-dependent cancers, heterosteroids, steroidal 6H-1,3,4-thiadiazines, steroidal 1H-pyrazoles, androgen receptor.

REFERENCES

1. *Gupta A., Kumar B.S., Negi A.S.* Current status on development of steroids as anticancer agents. J Steroid Biochem Mol Biol. 2013 Sep; 137: 242-70. doi: 10.1016/j.jsbmb.2013.05.011.

2. *Gauthier S., Martel C., Labrie F.* Steroid derivatives as pure antagonists of the androgen receptor. J Steroid Biochem Mol Biol. 2012 Oct; 132(1-2): 93-104. doi: 10.1016/j.jsbmb.2012.02.006.

3. *Iványi Z., Szabó N., Wölfling J., Szécsi M., Julesz J., Schneider G.* Novel series of 17β-pyrazolylandrosta-5,16-diene derivatives and their inhibitory effect on 17α-hydroxylase/C17,20-lyase, Steroids. 2012. 77: 1152-1159. doi: 10.1016/j.steroids.2012.07.001.

4. *Volkova Y.A., Antonov Y.S., Komkov A.V., Scherbakov A.M., Shashkov A.S., Menchikov L.G., Zavarzin I.V.* Access to steroidal pyridazines via modified thiohydrazides. RSC Advances. 2016. 6: 42863-42868. doi: 10.1016/j.steroids.2010.09.001.

5. *Komkov A.V., Komendantova A.S., Menchikov L.G., Chernoburova E.I., Volkova Y.A., Zavarzin I.V.* A Straightforward Approach toward Multifunctionalized Pyridazines via Imination/Electrocyclization. Organic Letters. 2015. 17: 3734-3737. doi: 10.1016/j.steroids.2010.09.001.

EFFECT OF HUMAN MESENCHYMAL STEM CELLS ON MORPHOLOGY AND MICROENVIRONMENT OF RAT SARCOMA IN EXPERIMENTAL RADIOTHERAPY

V.V. Yuzhakov, L.E. Sevankaeva, A.D. Kaprin, S.A. Ivanov, Yu.S. Romanko, L.N. Bandurko, N.K. Fomina, S.A. Mozerov, I.E. Ingel, N.D. Yakovleva, M.G. Tsyganova, K.S. Korchagina

A. Tsyb Medical Radiological Research Center – branch of the National Medical Research Radiological Center of the Ministry of Health of the Russian Federation, Obninsk, Russia

The effect of human mesenchymal stem cells (MSC) with subsequent local irradiation of rat sarcoma by ^{60}Co γ-rays at a dose of 30 Gy, on animal survival, growth and functional morphology of tumors was studied. The results of studies using markers of proliferation (PCNA), angiogenesis (CD31) and hypoxia (pimonidazole), showed that after the intravenous infusion of MSC, the angiogenesis was activated in growth zones of sarcoma, the proliferation of tumor cells increased in the parenchyma of neoplasms while the fraction of hypoxic cells (HC) decreased more than twice. Transplantation of MSC before the radiotherapy had a stronger effect on radiation inactivation of tumor cells. At the same time, the growth rate of irradiated tumors decreased 1.8 times and the survival rate of animals statistically increased by 31%.

Keywords: mesenchymal stem cells, γ-radiation, M-1 sarcoma, hypoxia, radiosensitization, angiogenesis, PCNA, CD31, pimonidazole.

Introduction

The presence of hypoxia in many types of solid tumors is one of the limiting factors for the complete eradication of tumors with conventional radiation therapy [1]. Approaches that include increasing the delivery of oxygen to tumors are being developed to improve the efficacy of radiotherapy for neoplasias. Particular attention is paid to biological modifiers, as some of them can normalize the vasculature of the tumor, which leads to increased blood flow and oxygenation, thereby potentially increasing the radiosensitiviness of tumor cells [2]. In this respect, attention is drawn to the impact of MSC on transplanted tumors associated with amplified peritumoral angiogenesis and an increase in the proportion of proliferating neoplastic cells [3]. However, literature data regarding the effect of MSC on the microenvironment of malignant tumors under conditions of radiotherapy are scarce.

The aim of this study was to investigate the effect of systemic human MSC transplantation and subsequent irradiation of a connective tissue tumor in rats with ^{60}Co γ-rays on animal survival, tumor growth and morphology.

Material and Methods

The work was performed on rats with sarcoma M-1 implanted under the skin of the hind paw. The tumor-bearing animals were divided into three groups: a control group (n=40) and two experimental groups with 45 rats in each. On the 16th day after implantation of sarcoma, the tumor nodes of the animals in experimental groups were exposed to local gamma radiation at a dose of 30 Gy. Four days before irradiation, the bone marrow-derived human MSC were injected into the tail vein of rats in the second test group at a single dose of 10^6 cells per 100 g of the rat weight. To visualize HC in the tumor tissue prior to irradiation on the 16th day of sarcoma growth, 5 rats from each experimental group were injected intraperitoneally with pimonidazole at a dose of 60 mg/kg body weight 30 minutes before excision of the tumor nodes. The criterion for the efficiency of action of MSC and γ-radiation was a change in tumor growth rate. On the 3rd and 12th days after irradiation, 7-10 rats from each group were withdrawn from the experiment to study the morphology of sarcoma M-1. The survival of the remaining animals was observed for 3 months.

Immunohistochemical studies on serial sections were carried out using biotinylated mouse antibodies to PCNA (PC10, Thermo Fisher Scientific), rabbit antibodies to the vascular endothelium marker CD31 (M-20-R, Santa Cruz) and rabbit antibodies to the marker of hypoxia - pimonidazole (Hypoxyprobe™ -1 Omni Kit). The quantitative density of tumor cells per 1 mm^2 of specimen area (N_{TC}), the PCNA index (I_{PCNA}), the mitotic index (I_{MIT}), and the apoptotic index (I_{AP}) were determined by a standard procedure. Morphometric assessment of the volume fraction of parenchyma with the reaction of nuclei in tumor cells on PCNA (ρ_{PCNA}), the content of necrosis (ρ_{NECR}) and HC zones (ρ_{HC}) was carried out using a computerized analysis of the microscopic images. The relative fraction of the proliferating cells was calculated according to the formula $F_{PCNA} = \rho_{PCNA} \times I_{PCNA} \times K_N$, where $K_N = N_{TC}$ in the experiment / N_{TC} in the control.

Results

Three months after the tumor implantation, 96% of the rats died in the control group, 25% of the rats survived in the 1st experimental group, while the survival of the animals that received MSC before the irradiation increased to 56%, and the tumor growth rate significantly decreased.

On the day of irradiation sarcoma M-1 had a solid type of structure with an intensive reaction of the nuclei of tumor cells on PCNA in growth zones. The central fields of the tumor nodes were occupied by areas of spontaneous necrosis. Judging by the intensity of the reaction to pimonidazole, tumor cells in the state of deep hypoxia were located mainly at the border with necrosis in the form of perinecrotic rims in the width of 40-50 microns. There was no response to the marker of hypoxia in the most vascularized areas of sarcoma. On the 4th day after MSC transplantation, an increase in angiogenesis was evidently observed in the peritumoral zone of specimens stained with CD31. According to the morphometric data, the calculated F_{PCNA} value increased by 20% ($p>0.05$) against the background of MSC administration, whereas ρ_{NECR} and ρ_{HC} decreased almost twofold ($p<0.05$).

Three days after irradiation, in the growth zones of tumor nodes of rats in the 1st experimental group, N_{TC} decreased by 40%, being 18.1±4,1%. When MSC was preliminary administered, N_{TC} and F_{PCNA} in the growth zones of the sarcoma were respectively 1.8 and 2.2 times lower than after γ-radiation only. On the 12th day after irradiation in rats of the 1st test group, ρ_{PCNA} was

0.21±0.03, and F_{PCNA} was 14.5±2.1%. A 1.9 and 2.7-fold decrease in these indices were observed after the administration of MSC prior to irradiation, Histological examination of tumors showed the extensive areas of connective tissue growth with the penetration into the tumor and infiltration of the stroma with numerous macrophages, which, apparently, led to an intensive elimination of dead tumor cells.

Conclusion

For the first time, the radiosensitizing action of MSC infusion before the experimental radiotherapy of transplantable connective tissue tumor with a stronger effect of inactivation of neoplastic cells was recorded. Analysis of the results showed that the enhancement of the radioresponsivity of sarcoma M-1 with MSC transplantation prior to irradiation was caused by stimulation of angiogenesis, decrease in the total fraction of hypoxic cells, and increase in the content of proliferating tumor cells in the irradiated areas.

REFERENCES

1. *Horsman M.R., Overgaard J.* The impact of hypoxia and its modification of the outcome of radiotherapy. J Radiat Res. 2016 Aug; 57(Suppl 1): i90–i98. doi: 10.1093/jrr/rrw007.

2. *Shinohara E.T., Maity A*. Increasing sensitivity to radiotherapy and chemotherapy by using novel biological agents that alter the tumor microenvironment. Curr Mol Med. 2009 Dec; 9(9): 1034-45. doi: 10.2174/156652409789839107.

3. *Yuzhakov V.V., Sevan'kaeva L.E., Konoplyannikov A.G., Bandurko L.N., Konoplyannikov M.A., Ingel I.E., Fomina N.K., Tsyganova M.G., Kal'sina S.Sh*. A morphofunctional study of mesenchymal stem cells' effects on sarcoma M-1. Molecular Medicine. 2015 (1): 39-45. [in Russian].

CONTENTS

Afanasyev A.G. Investigation and analysis of gene structures using time-of-flight mass spectrometry. pplication of the genomic analyzer in scientific and clinical practice 5
Ann D.K. Aspartate couples arginine starvation with mitochondrial dysfunction in breast cancer cells with ASS1 deficiency 5
Aksenenko M.B., Palkina N.V., Komina A.V., Ruksha T.G. Microrna expression in tumor and surrounding non-cancerous melanoma tissue 6
Anisimenko M.S., Paul G.A., Matyash N.A., Gutkina N.I., Gornostaeva E.V., Berdyugina D.A., Garanin A.Yu., Butorina A.V., Kozyakov A.E., Kovalenko S.P. Comprehensive analysis of *BRCA1* gene mutations in siberian breast cancer patients 8
Basharova R.R., Berezina O.V., Perepechaeva M.L., Grishanova A.Yu., Pospelova T.I., Kovynev I.B., Agakishiev M.M., Nechunaeva I.N. Microrna-223 role in the pathogenesis of chronic lymphocytic leukemia 9
Belkovets A.V., Kurilovich S.A., Ragino Y.I., Maksimov V.N., Scherbakova L.V., Voevoda M.I. Polymorphism of genes of inflammatory cytokines (IL1b, IL6) and genes controlling cell cycle or apoptosis (CASP8, TP53) in gastric cancer: a prospective «case-control» study in siberia 11
Bel'skaya L.V., Kosenok V.K. Salivary cytokine levels in lung cancer depending on the tumor size 13
Borobova E.A., Antonets D.V., Starostina E.V., Oreshkova S.F., Shcherbakov D.N., Smirnova O.Yu., Bazhan S.I., Ilyichev A.A., Karpenko L.I., Danilenko E.D., Gamalei S.G., Usova S.V., Bogryantseva M.P. Preclinical studies of the dna vaccine against melanoma 15
Bulgakova O.V., Kussainova A.A., Kausbekova A.Zh., Bersimbaev R.I. Circulating cell-free nucleic acids in blood plasma of lung cancer patients exposed to radon 16
Calaf G.M. Carcinogenicity of environmental substances as parathion and malathion in breast carcinogenesis 18
Chernyavskaya M.A., Atamanov V.V. Increase in the concentration of IL-8 in local fluids, as an additional criterion for the diagnosis of choroidal melanoma 19
Churuksaeva O.N., Kolomiets L.A., Savenkova O.V. The role of KI-67 proliferative index in the prognosis of locally advanced cervical cancer 21
Costa M., Sun H., Chen D., Chen Q., Li F.J., Zhu Y., Jin C. Arsenic Induced Polyadenylation of canonical histone mRNA disrupts chromatinhomeostatsis and is highly carcinogenic 23
Denisov E.V., Gerashchenko T.S., Novikov N.M., Tashireva L.A., Krakhmal N.V., Zavyalova M.V., Gautreau A., Cherdyntseva N.V., Perelmuter V.M. The search for markers of collective cell invasion in breast cancer 24
Dronova T.A., Babyshkina N.N., Patalyak S.V., Cherdyntseva N.V. Prognostic significance of TGF-b-associated proteins in breast cancer patients treated with adjuvant tamoxifen 25
Efremova S.D., Golderova A.S., Nikolaev V.M., Okhlopkova E.D., Alekseev V.A., Vinokurova F.V., Rumyantsev E.K., Tsypandina E.V. Analysis of the level of the serum tumor markers and chemicals in waterbasins of the republic of sakha (yakutia) 26
Enikeev A.D., Komelkov A.V., Akselrod M.E., Tchevkina E.M. Effect of short-term retinoic acid treatment on ERK1/2 and akt activation in lung cancer and neuroblastoma cells 27
Fetisov T.I., Kuzin K.A., Zenkov R.G., Safina A., Lesovaya E.A., Trukhanova L.S., Antoshina E.E., Gorkova T.G., Gurova K., Yakubovskaya M.G., Kirsanov K.I. The novel small molecule CBL0137 (curaxin) exhibits antileukemic activity 29
Gerashchenko T.S., Denisov E.V., Kiselev A.M., Novikov N.M., Tashireva L.A., Pyankov D.V., Cherdyntseva N.V., Perelmuter V.M. RNA sequencing of single cells obtained from immunolabeled tumor sections: the first experience 31
Gomboeva S.B., Ryabov V.V., Demyanov S.V. Oncology disease in patients with acute myocardial infarction with nonobstructive coronary atherosclerosis 33

Huberman E. Drugs to Control Hazardous Viruses Including Some Involved in Human Malignancies 35
Ibragimova M.K., Tsyganov M.M., Deryusheva I.V., Kolomiets L.A., Churuksaeva O.N., Litviakov N.V. HPV-negative cervical cancer: a distinct type of the uterine cervix with poor prognosis 35
Kaigorodova E.V., Tarabanovskaya N.A., Simolina E.I., Perelmuter V.M. Relationship between the level different populations of circulating tumor cells before treatment and effec to neoadjuvant chemotherapy in patients with breast cancer 37
Kakurina G.V., Kolegova E.S., Kondakova I.V., Dobrodeev A.Yu., Kostromitsky D.N., Cheremisina O.V., Choinzonov E.L. MRNA expression of cellular movement proteins and calpains 1 and 2 in metastasis of head and neck squamous cell carcinoma and non-small cell lung cancer 39
Kaplun D.S., Shatova O.P., Zinkovych I.I. Influence of methormin on metabolic activity like the anti-cancer drug 40
Kaplun D.S., Shatova O.P., Zinkovych I.I. Influence of metformin on erythrocyte 42
Kazubskaya T.P., Kozlova V.M., Kondrat'eva T.T., Shishkov R.B., Trofomov Yu.I., Amosenko F.A., Brjezovsky V.J., Polaykov V.G., Lubchenko L.N. Integration molecular testing in diagnosis, treatment and prevention of medullar thyroid cancer in children 44
Kishkina A.Yu., Kolomiets L.A., Yunusova N.V. Clinical and morphological features of endometrial cancer with different structural variants of metabolic syndrome 46
Kistenev Yu.V., Borisov A.V., Knyazkova A.I., Zakharova O.A., Sandykova E.A., Spirina L.V., Gorbunov A.K. Paraffin embedded cancer tissue 2D terahertz imaging and machine learning analysis 47
Kondakova I.V., Yunusova N.V., Spirina L.V., Kakurina G.V., Cheremisina O.V., Kolomiets L.A., Choinzonov E.L. Cellular motility proteins as new molecular markers for early diagnosis of cancer 49
Korchagina K.S., Gamayunov S.V., Yuzhakov V.V., Kaprin A.D., Ivanov S.A., Romanko Yu.S., Dudenkova V.V., Sergeeva E.A., Shakhova N.M., Fomina N.K. Biodistribution of chlorine photosensitizer in tumor microenvironment and morphological changes in human skin cancer after photodynamic therapy 51
Korshunov D.A., Klimov I.A., Ivanov V.V., Kondakova I.V. Study of inhibitors of glycolysis iodoacetate and 2-deoxyglucose as anti-tumor agents in the Lewis lung carcinoma model 53
Kudryavtseva A.V., Lukyanova E.N., Kalinin D.V., Krasnov G.S., Pokrovsky A.V., Golovyuk A.L., Fedorova M.S., Pudova E.A., Pavlov V.S., Kobelyatskaya A.A., Kiseleva M.V., Dmitriev A.A., Snezhkina A.V. Somatic mutations in carotid body tumors 55
Kulbakin D.E., Choinzonov E.L., Mukhamedov M.R., Garbukov E.U., Vasilev R.V., Shiyanova A.A. Reconstructive surgery for oral cavity cancer 56
Kuzmina E.G., Bogatyreva T.I., Terekhova A.Yu., Falaleeva N.A., Mushkarina T.Yu., Konstantinova T.V., Zamulaeva I.A., Zatsarenko S.V. Low count of lYmphocytes and CD4T-cells in peripheral blood of patients with primary Hodgkin'S lymphoma correlates with unfavourable disease prognosis 58
Landolph J.R.Jr., Grondin J., Salazar C.T., Khaksar S., Horton M.A. Insoluble Ni^{+2} compounds induce genotoxicity and gene silencing, disrupting 14 signal transduction pathways, and leading to differential expression of 144 genes and morphological, A. I., and neoplastic transformation of C3H/10T1/2 mouse embryo cells 61
Larionova I.V., Tengfei L., Kiselev A.M., Denisov E.V., Cherdyntseva N.V., Kzhyshkowska J.G. The influence of cisplatin on transcriptional profile of tumor-associated macrophages of breast cancer and colon cancer 62
Lavrentev S.N., Aksenenko M.B., Palkina N.V., Komina A.V., Ruksha T.G. Melanoma cell growth and migration alterations under MIR-204-5p inhibitor application 63
Li W. Targeting Exosome-Mediated Secretion of Heat Shock Protein-90alpha (Hsp90α) by Tumor Cells 65
Link K.H., Jafarov S., Staib L., Kornmann M. Colon- and Rectal Cancer are different Tumor Entities according to Epidemiology, Carcinogenesis, Molecular- and Tumorbiology, Surgery/Multimodal Therapy, Results Quality, and Primary and Secondary Prevention 66
Makarova V.V., Bgatova N.P., Taskaeva Yu.S., Borodin Yu.I. Angiogenesis in experimental hepatocarcinoma-29 67
Maximova V.P., Lizogub O.P., Fedorov D.A., Zhidkova E.M., Lesovaya E.A., Kholodova A.V., Belitsky G.A., Yakubovskaya M.G., Kirsanov K.I. The influence of organic solvents on the epigenetic regulation of gene expression 68
McCutcheon I.E. Malignant peripheral nerve sheath tumors: patterns of recurrence and survival and the role of poly (ADP) ribose polymerase inhibition 70

Navolokin N.A., Bucharskaya A.B., Mudrak D.A., Polukonova N.V., Maslyakova G.N. Morphological changes of the transplanted kidney tumors after administration of flavonoid-containing extract gratiola officinalis L. 71
Neroev V.V., Saakyan S.V., Amiryan A.G., Tsygankov A.Iu., Burdenny A.M., Loginov V.I., Khlgatyan M.R. Association of survival of patients with uveal melanoma with molecular genetic aberrations: long-term follow-up 73
Nikolaev V.M., Vinokurova F.V., Sofronova S.I., Efremova S.D., Fedorova S.A. Investigation of the association of polymorphic variants 481c> t, 590g> a and 857g> a of n-acetyltransferase 2 gene (*NAT2*) with the development of lung cancer in the yakuts 75
Nosareva O.L., Stepovaya E.A., Shakhristova E.V. Glutathione and heat shock proteins 27 and 70 as molecular targets for regulation of apoptosis in jurkat cells 76
Novikov V.A., Vasiljev R.V., Gribova O.V., Choynzonov E.L., Shtin V.I., Surkova P.V., Startseva Zh.A. Intraoperative radiotherapy in combined treatment of sinonasal carcinoma 78
Ochirov M.O., Kolomiets L.A., Chernov V.I. Intraoperative imaging of sentinel lymph nodes in endometrial cancer using Alotech, a new radiopharmaceutical 79
Palkina N.V., Komina A.V., Aksenenko M.B., Moshev A.S., Savchenko A.A., Ruksha T.G. MIR-204-5P inhibitor application affects melanoma cell proliferation rates and immunocompetent cells presence in melanoma metastasis target organ 81
Patysheva M.R., Stakheyeva M.N., Tarabanovskaya N.A., Grigoryeva E.S., Slonimskaya E.M., Kzhyshkowska J., Cherdyntseva N.V. Macrophage polarization markers of blood monocytes in patients with breast cancer 82
Perepechaeva M.L., Vorontsova E.V., Pyatov S.M., Grishanova A.Y. Signal transduction and therapeutic effects of human meningioma embolization 84
Pevzner A.M., Tsyganov M.M., Deryusheva I.V., Ibragimova M.K., Perminova E.E., Kazantseva P.V., Slonimskaya E.M., Litviakov N.V. Expression of *BRCA1* gene in breast tumor: association with disease prognosis 85
Planchon D., Morris E.R., Genest M., Comunale F., Vacher S., Biche I., Denisov E.V., Tashireva L.A., Perelmuter V.M., Linder S., Chavrier Ph., Bodin S., Gauthier-Rouviure C. Up-regulation of flotillins, new marker of metastatic development, induces cell invasion and metastatic development 87
Ponomaryova A.A., Dobrodeev A.Y., Bondar A.A., Cherdyntseva N.V., Zavyalov A.A., Tuzikov S.A., Vlassov V.V., Laktionov P.P., Rykova E.Y. Circulating dna-markers in lung cancer: changes in retrotrasposons methylation status in response therapy and during the post-treatment follow-up 88
Puchinskaya M. Automated image analysis in assessment of e-cadherin down-regulation during epithelial-mesenchymal transition in prostate cancer 89
Rumyantsev E.K., Tsypandina E.V., Nikolaev V.M., Efremova S.D., Vinokurova F.V., Sofronova S.I. The study of delition polymorphism of gstm1 and GSTT1 genes in yakut patients with lung cancer 91
Sivoplyas E.A., Maksimchuk M.D., Kulikov A.M. Analysis of DRAS1 gene expression in closely related species of drosophila virilis group 93
Shamanin V.A., Pisareva E.E., Gornostaeva E.V., Gervas P.A., Gutkina N.I., Kovalenko S.P. Monitoring of egfr mutations in the plasma dna of patients with non-small cell lung cancer 94
Shapovalova Ye., Churina E.G., Kzhyshkowska Yu.G. Effect of biodegradable scaffolds for targeted immunotherapy on differentiation of macrophages 95
Shashova E.E., Tarabanovskaya N.A., Slonimskaya E.M., Kakurina G.V., Kolegova E.S., Kondakova I.V. Association of proteasomal and calpain activities and contents of locomotor proteins with lymphogenous metastases in Luminal and triple negative breast cancer 98
Skryabin G.O., Komelkov A.V., Galetsky S.A., Akselrod M.E., Tchevkina E.M. Analysis of spfh proteins in exosomes produced by non-small cell lung cancer cells 99
Smirnova K.V., Senuyta N.B., Kondratova V.N., Lichtenstein V.A., Gurtsevitch V.E. Epstein-barr virus biomarkers for nasopharyngeal carcinoma in russia 101
Spirina L.V., Kondakova I.V., Gorbunov A.K., Usynin E.A., Slonimskaya E.M. Germline *BRCA1* and *BRCA2* mutations in castration-resistant prostate cancer, association with expression of AKT/m-TOR signaling components 103
Stakheyeva M.N., Eidenzon D.V., Patysheva M.R., Cherdyntseva N.V. The significance of the immune system for the efficient cancer therapy 104
Starostina E.V., Borobova E.A., Karpenko L.I. Optimization of dendritic cell maturation protocol to assess the specific activity of anticancer dna-vaccines in ex vivo system 106

Suhovskih A.V., Kazanskaya G.M., Tsidulko A.Y., Volkov A.M., Kiselev R.S., Kobozev V.V., Gaytan A.S., Aidagulova S.V., Krivoshapkin A.L., Grigorieva E.V. Heparan sulfate as potential microenvironment biomarkers for glioblastoma 107
Tsidulko A.Y., Pankova T.M., Grigorieva E.V. Disruption of brain extracellular matrix by anti-glioma chemotherapeutic drugs promotes invasion of glioblastoma cells in *ex vivo* model 108
Tsyganov M.M., Ibragimova M.K., Deryusheva I.V., Slonimskaya E.M., Litviakov N.V. CNA landscape of breast tumor, connection with the efficiency of neoadjuvant chemotherapy 109
Tsypandina E.V., Rumyantsev E.K., Nikolaev V.M., Efremova S.D., Vinokurova F.V., Sofronova S.I. The effect of alkyloxybenzenes on the thermal denaturation of proteins of the colon carcinoma cell line HT-29 111
Tugutova E.A., Yunusova N.V., Patysheva M.R., Afanasiev S.G., Tsydenova A.A., Kondakova I.V. Proteases in exosomes in colorectal cancer 112
Vasilyeva I.N., Semenov A.L., Tochilnikov G.V., Bespalov V.G. Study of extracellular DNA in blood and ascites of rats with transplanted ovarian cancer 114
Vlasova O.A., Maksimova V.P., Fetisov T.I., Safina A., Lesovaya E.A., Belitsky G.A., Gurova K., Kirsanov K.I., Yakubovskaya M.G. Influence of natural DNA-binding compounds with cancer preventive activity on chromatin structure and interferon signaling 115
Vorontsova E. Immuno-oncology – a paradigm shift in anti-cancer research 118
Wilson P.M., Mulligan K.A., LaBonte M.J., McLaughlin K., Prise V., Johnston P.G., Ladner R.D. Development and characterization of CV6-168, a novel and selective dutpase inhibitor that enhances the antitumour efficacy of TS-targeted therapies 119
Yastrebova M.A., Gudkova M.V., Scherbakov A.M., Komendantova A.S., Komkov A.V., Chernoburova E.I., Volkova Y.A., Zavarzin I.V. Extranuclear azasteroids as potent antiproliferative agents 119
Yuzhakov V.V., Sevankaeva L.E., Kaprin A.D., Ivanov S.A., Romanko Yu.S., Bandurko L.N., Fomina N.K., Mozerov S.A., Ingel I.E., Yakovleva N.D., Tsyganova M.G., Korchagina K.S. Effect of human mesenchymal stem cells on morphology and microenvironment of rat sarcoma in experimental radiotherapy 121

invitrogen

Потрясающе быстрая проточная цитометрия

Проточный цитометр AttuneNxT: когда скорость имеет значение

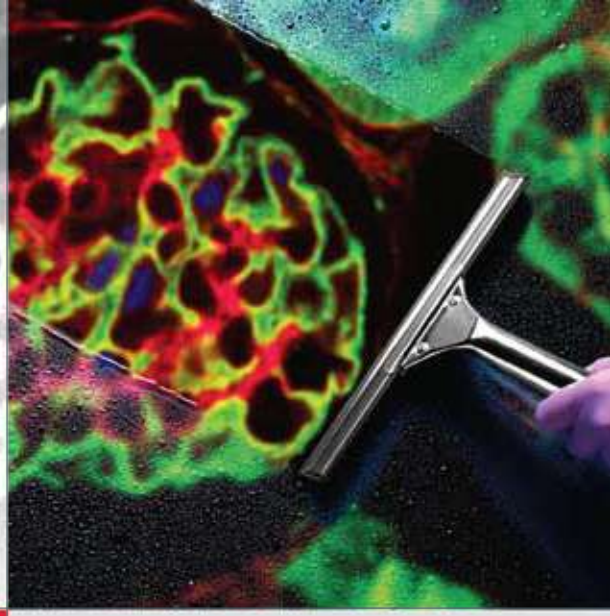
Проточный цитометр Invitrogen™ Attune™ NxT— непревзойденное сочетание качества анализа и скорости. Даже при десятикратной скорости потока — до 1 мл образца в минуту — вы получите не менее качественные данные, чем при низких скоростях. Гибкая модульная конструкция позволяет прибору развиваться вместе с вами. Возможность установки до 4 лазеров и до 14 цветов на выбор, поддержка 96- и 384-луночного форматов — всё это поможет сконфигурировать цитометр AttuneNxT практически под любые условия эксперимента и любой бюджет. И никаких ограничений!

Увлекательная интерактивная экскурсия —на **thermofisher.com/attune**

ThermoFisher SCIENTIFIC

Leica BIOSYSTEMS

PerkinElmer™ For the Better



Оптимизация мультиплексных исследований в патоморфологии

От реагентов до анализа результата

Только для научных исследований

BOND RX, BOND-RXm

Полностью автоматизированные исследовательские протоколы

- Иммуногистохимия (IHC), иммуноцитохимия (ICC) с антителами и системами детекции любых производителей
- *in situ* гибридизация (ISH) с ДНК, РНК, LNA зондами
- Иммунофлуоресценция

MANTRA™, VECTRA®3, VECTRA® POLARIS™

Мультиспектральная визуализация

- Точное разделение сигналов каждого маркера без автофлуоресценции
- Комплексная автоматизация алгоритмов документации и многопараметрового анализа фенотипа клеток и их микроокружения

TSA®, OPAL™

Реагенты

- Усиление сигнала флуоресцентной и хромогенной детекции
- Многоцветное флуоресцентное ИГХ окрашивание до 6 маркеров на одном препарате

Компания «БиоЛайн» — авторизованный дистрибьютор Perkin Elmer и Leica Biosystems в России

BioLine группа компаний

ООО «БиоЛайн»
197101, Россия, Санкт-Петербург
пер. Пинский, д. 3, лит. А
тел.: (812) 320 49 49,
факс: (812) 320 49 40
e-mail: main@bioline.ru
www.bioline.ru

Москва, тел.: (800) 555 49 40
Новосибирск, тел.: (383) 227 09 63
Екатеринбург, тел.: (343) 287 32 49
Владивосток, тел.: (423) 201 18 08
Нижний Новгород, тел.: (831) 278 61 47
Ростов-на-Дону, тел.: (863) 268 99 32
Казань, тел.: (843) 570 66 88
Самара, тел.: (846) 246 06 54

Единый бесплатный номер сервисной службы для всех регионов России: 8 800 333 00 49

РеалБест

Всегда точно, всегда воВРЕМЯ!

ДИАГНОСТИКА ЗАБОЛЕВАНИЙ ЧЕЛОВЕКА МЕТОДОМ РЕАЛ-ТАЙМ ПЦР

Преимущества наборов серии «РеалБест»

- **Лиофилизированные готовые реакционные смеси (ГРС):** снижение затрат труда и времени, высокая стабильность качества исследований
- **Фермент урацил-ДНК-гликозилаза в составе ГРС:** предупреждение внутрилабораторной контаминации
- **Мультиплексный анализ:** увеличение производительности лаборатории
- **Удобство хранения и транспортировки:** хранение всех компонентов набора при одной температуре

Спектр диагностируемых заболеваний

- **ВИЧ-инфекция, вирусные гепатиты A, B, C, D, G**
- **Инфекции и дисбиозы урогенитального тракта**
- **TORCH- и герпесвирусные инфекции**
- **Папилломавирусная инфекция**
- **Природно-очаговые инфекции**
- **Респираторные инфекции**
- **Выявление генетических полиморфизмов и мутаций в генах**

«РеалБест» - организация технологического процесса в крупнейших клинико-диагностических лабораториях России

- Проведение до 10 000 ПЦР-анализов в день
- Валидация качества забора биоматериалов
- Внутрилабораторный контроль качества
- Автоматизация выделения нуклеиновых кислот
- Оригинальные информационные решения

Технология «РеалБест» – минимум усилий для максимального результата!

ВЕКТОР БЕСТ

www.vector-best.ru

АО «Вектор-Бест»
630117, Новосибирск, а/я 492
тел./факс: (383) 227-73-60, 332-81-34
e-mail: vbmarket@vector-best.ru

АЛЬБИОГЕН

illumina AUTHORIZED CHANNEL PARTNER

NovaSeq ™

БЫСТРЫЙ И ПРОСТОЙ: самая производительная NGS-система на сегодняшний день в мире.
АВТОМАТИЧЕСКАЯ КЛАСТЕРИЗАЦИЯ происходит непосредственно в самом приборе.
СКОРОСТЬ РАБОТЫ: 6 Tb данных за 45 часов.
ГИБКОСТЬ: наборы разной производительности. 65 Gb – 6 Tb данных за один запуск прибора.
ЛЕГКИЙ В ЭКСПЛУАТАЦИИ: реагенты в одноразовых картриджах.

- 4–48 геномов
- 40–500 экзома
- 32–400 транскриптома
- 4–48 эпигеномов

HiSeq ™

Высокая производительность для масштабных исследований, задачи любой сложности.

- 12 геномов
- 96 экзомов
- 100 транскриптомов

NextSeq ™

Компактный и производительный. Универсальный. Широкий спектр задач. Секвенатор и сканер биочипов в одном приборе.

- 1 геном
- 9–12 экзомов
- 100 транскриптомов
- Чипы для цитогенетики и PGD (до 12 образцов за сканирование)

MiniSeq ™

Прибор, ориентированный на готовые решения (панели). Максимально простой в обращении и обслуживании. Таргетное секвенирование.

- 2–40 опухолевых образцов
- 2–8 раковых транскриптомов
- Десятки малых геномов
- Онкологические панели (оценка риска развития заболеваний, 12–24 образца)

MiSeq ™

Персональный секвенатор. Идеальное решение для таргетного секвенирования и небольших геномов.

- 4-24 образца целевых клинических панелей
- 384 ампликона
- 3 клинических экзома
- 16 геномов E. Coli
- 6–144 образца HLA
- 24 образца PGS
- 96 генотипов KPC

iSeq ™

ДОСТУПНОСТЬ: самая доступная NGS-система на сегодняшний день. Невысокая цена.
УДОБНЫЙ: самая простая в эксплуатации NGS-система. Практически не требует обслуживания.
ИНТЕГРИРОВАННЫЙ АНАЛИЗ ДАННЫХ: биоинформатическая обработка прямо на приборе.

- 1–8 геномов E.Coli
- 1–48 образцов таргетных панелей

+7 (499) 550-15-25 | www.albiogen.ru

Решения для диагностики и исследований в онкопатологии

Сканирующие микроскопы Pannoramic компании 3DHistech – это уникальные системы получения высокоточных изображений гистологических и цитологических препаратов. Они обладают большой разрешающей способностью и высокой скоростью сканирования. Исследования могут проводиться как в светлом поле, так и с использованием флуоресценции, в том числе многоцветной. Специализированное программное обеспечение позволяет количественно оценить результаты окрашивания препаратов.

Области использования сканеров:

- Диагностика
- Создание архивов и баз данных препаратов
- Научные исследования
- Работа с тканевыми матрицами (TMA)

Дополнительным преимуществом является интеграция с системами для получения тканевых матриц – **Tissue Macro Array (TMA).** Такие системы незаменимы для решения многих исследовательских задач, проведения клинических испытаний, исследования онкомаркеров. TMA-автоматы позволяют собрать в одном гистологическом блоке сотни исследуемых образцов. Далее все они оказываются на одном срезе. Это позволяет обработать их в одинаковых условиях и существенно экономит время и реагенты. Количественная оценка таких препаратов также возможна посредством специализированного ПО.

Сканирующие микроскопы Pannoramic отмечены наградами **International Scanner Contest** за качество и скорость сканирования.

OPTEC
Connecting solutions

Информационная поддержка: **8-800-2000-567**. Звонки по России бесплатно.
Подробная информация о решениях компании ОПТЭК на сайте **www.optecgroup.com**

facebook.com/OPTECGROUP

3 DHISTECH

БГА
БиоГен-Аналитика
ваши задачи - наши решения
www.bga.su

Agena BIOSCIENCE

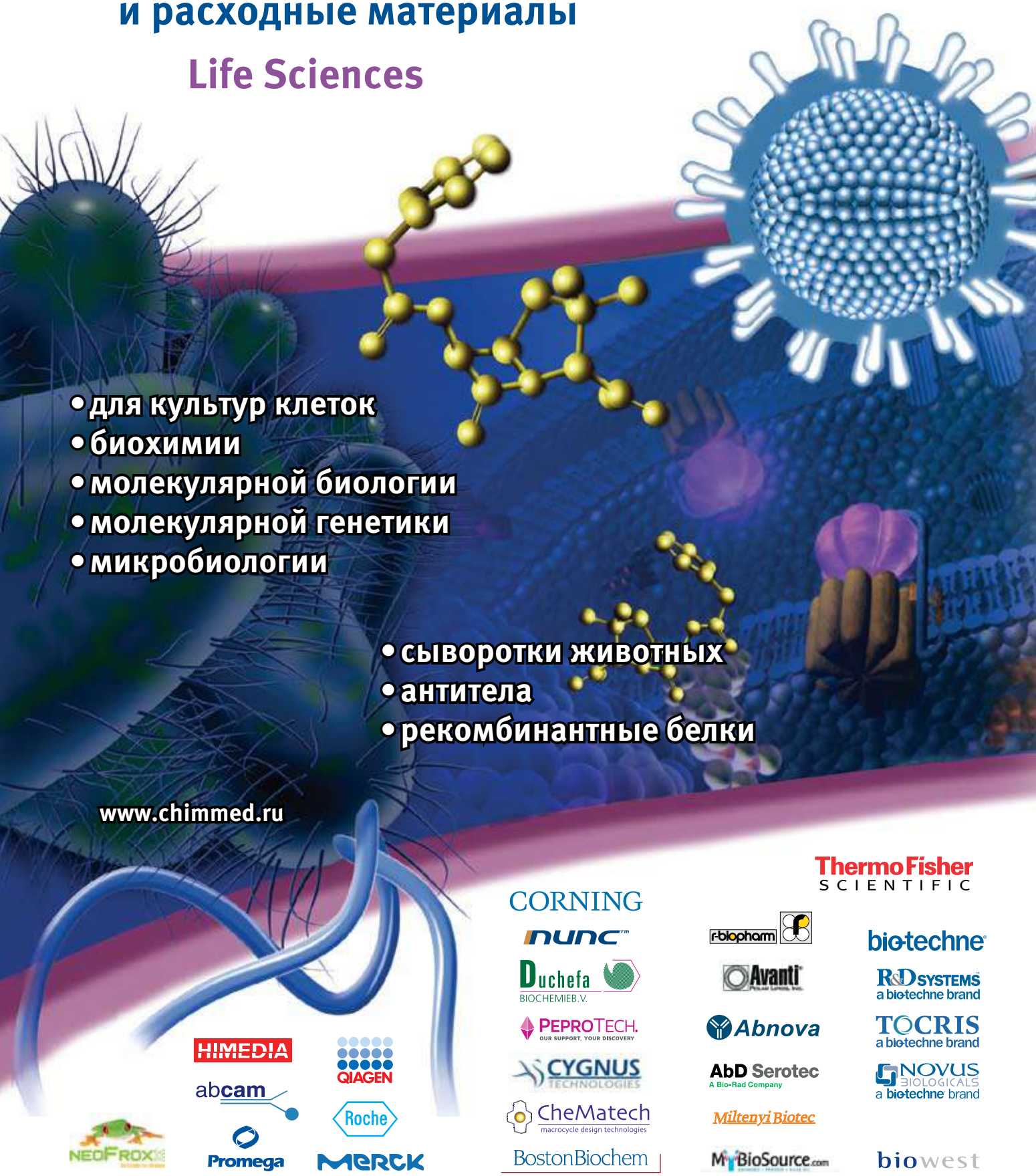
Система MassARRAY для таргетного генетического анализа

- ПРОФИЛИРОВАНИЕ СОМАТИЧЕСКИХ МУТАЦИЙ
- ВЫСОКОЧУВСТВИТЕЛЬНЫЙ КОЛИЧЕСТВЕННЫЙ АНАЛИЗ SNP, CNV
- ОНКОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ И АНАЛИЗЫ
- ИДЕНТИФИКАЦИЯ ОБРАЗЦОВ ID
- КАРДИОПАНЕЛЬ И СЕРДЕЧНО-СОСУДИСТЫЕ ЗАБОЛЕВАНИЯ
- ФАРМАКОГЕНЕТИКА И ТЕСТИРОВАНИЕ НАСЛЕДСТВЕННЫХ ЗАБОЛЕВАНИЙ
- КОЛИЧЕСТВЕННЫЙ АНАЛИЗ МЕТИЛИРОВАННОЙ ДНК
- ГЕНОТИПИРОВАНИЕ ДОНОРОВ С РЕДКИМИ ГРУППАМИ КРОВИ
- СЕЛЬСКОХОЗЯЙСТВЕННАЯ ГЕНОМИКА

Биохимические реактивы и расходные материалы

ХИММЕД

Life Sciences



- для культур клеток
- биохимии
- молекулярной биологии
- молекулярной генетики
- микробиологии

- сыворотки животных
- антитела
- рекомбинантные белки

www.chimmed.ru

ThermoFisher SCIENTIFIC
CORNING
nunc
Duchefa BIOCHEMIE B.V.
PEPROTECH. OUR SUPPORT, YOUR DISCOVERY
CYGNUS TECHNOLOGIES
CheMatech macrocycle design technologies
BostonBiochem
r-biopharm
Avanti
Abnova
AbD Serotec A Bio-Rad Company
Miltenyi Biotec
MyBioSource.com
biotechne
R&D SYSTEMS a biotechne brand
TOCRIS a biotechne brand
NOVUS BIOLOGICALS a biotechne brand
biowest
HIMEDIA
QIAGEN
abcam
Roche
Promega
MERCK
NEOFROX

Москва, 115230, Каширское шоссе, д. 9, корп. 3. Тел.: +7 (495) 728 4192, e-mail: bio@chimmed.ru
Новосибирск, 630090, просп. Академика Лаврентьева, 6/1. Тел.: +7 (383) 335 6108, e-mail: sibir@chimmed.ru

22nd International Charles Heidelberger Symposium on Cancer Research

Tomsk, Russia, Sept. 17-19, 2018

ORGANIZERS

The Ministry Of Science and Higher Education of Russian Federation
Tomsk Region Administration
Tomsk National Research Medical Center, Russian Academy of Sciences
Tomsk State University
The Charles and Patricia Heidelberger Foundation for Cancer Research

GENERAL SPONSORS

ThermoFisher SCIENTIFIC

BioLine группа компаний

ВЕКТОР БЕСТ

MAIN SPONSORS

АЛЬБИОГЕН

OPTEC Connecting solutions

БГА БиоГен-Аналитика ваши задачи - наши решения www.bga.su

RFBR RUSSIAN FOUNDATION FOR BASIC RESEARCH

ХИММЕД