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INTERACTION OF USP1 PROTEIN AND PH DOMAIN OF BCR PROTEIN AND ITS ROLE IN BCR-ABL EVASION FROM PROTEASOMAL DEGRADATION

S.V. Antonenko, G.D. Telegeev
Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine
antonenkoimbg@gmail.com

Introduction. Chronic myelogenous leukemia (CML) is characterized by the appearance of the cytogenetic marker of the Philadelphia chromosome (Ph), which is the result of translocation between 9 and 22 chromosomes. The product of this aberration is the hybrid oncoprotein Bcr-Abl. According to the preliminary results of the mass spectrometric analysis, 23 proteins were identified as potential candidates for interaction with the Ph domain of Bcr-Abl oncoprotein. The main function of ubiquitin specific protease 1 (USP1) protein is deubiquitination of cell proteins. As a result of deubiquitination, USP1 protein can prevent proteasomal degradation of Bcr-Abl oncoprotein in a cell and, consequently, contribute to its accumulation and progression of the disease.

Aim. To create the pCMVHA-*USP1* genetic construct for eukaryotic protein expression and to determine the interaction of USP1 protein with the Ph domain of the Bcr-Abl oncoprotein.

Materials and Methods. The amplification of the coding sequence of *USP1* gene was performed using primers *USP1* fwd AATTGCCTGGTGTCATACCTAGTG and *USP1* rev GAGAGACCAATAATATCCAGTAGC, pCMV-XL5-*USP1* was used from the plasmid bank of the Department of Molecular Genetics of the IMBG NASU as a template. The resulting sequence of *USP1* gene was ligated to pUC18 vector at Sma1 site. *USP1* was subcloned to pCMVHA vector using Kpn1 and Sall restriction endonucleases.

Co-transfection of pCMVHA-*USP1* and pmCitrineC1-PH plasmids into HEK 293T cells was performed using 3:1 ratio of µl PEI : µg DNA. Transfection mixture was added to HEK293T cells and grown for 24 hours at a temperature of +37 °C and 5% CO_2. Co-immunoprecipitation of HEK293T cells was done using Sepharose G and anti-His antibodies (Sigma, USA). The results were visualized by Western blot analysis using anti-USP1 antibodies (Thermo Fisher Scientific, USA), anti-His (Sigma, USA).

Results and Discussion. Coding sequence of *USP1* gene was amplified by PCR and ligated to the pUC18 vector. Genetic construct pCMVHA-*USP1* for eukaryotic protein expression was created by sub-cloning the sequence of *USP1* gene. Co-transfection of pCMVHA-*USP1* and pmCitrineC1-PH vectors in HEK293T cells and co-immunoprecipitation assay revealed the interaction between USP1 protein and Ph domain of the Bcr-Abl oncoprotein.

Conclusions. Genetic constructs pUC18-*USP1* and pCMVHA-*USP1* have been created. For the first time, the interaction between USP1 protein and PH domain of the Bcr-Abl oncoprotein was shown. The results obtained are important for the understanding of signaling in this pathology and may be the basis for the development of new alternative methods of CML treatment.

ADSORPTIVE CARBON DRESSING FOR THE TREATMENT OF RAT MODEL WOUND AFTER CISPLATIN ADMINISTRATION AND GUERIN'S CARCINOMA GRAFTING

O.Yu. Babchenko, L.A. Sakhno, V.G. Nikolaev
R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine
helgadoctor@yahoo.com

Introduction. The metabolic intoxication and high systemic toxicity of cytostatic therapy affect the dynamics and character of the postoperative wounds healing in cancer patients, and, in particular, in those with purulent-inflammatory complications. In such situations, the use of adsorptive carbon dressing (ACD) that can absorb a number of toxic components from wound content and bind microbial cells seems relevant.

Aim. To study the effect of ACD on the healing rate of full-thickness cutaneous wound in rats after cisplatin (CP) administration, and on a complicated wound process in Guerin's carcinoma-bearing rats after a CP course followed by tumor resection.

Materials and Methods. CP was administered to intact inbred rats at a dose of 1 mg/kg bw intravenously every other day (total dose 5 mg/kg). In two days, the animals were inflicted to full-thickness cutaneous wounds of 2.5 cm^2 in size in the interscapular region. Gauze dressings were used in control and ACD in experimental group immediately after surgery. The rats were housed in separate cages. In the next group of animals, Guerin's carcinoma was subcutaneously transplanted into the interscapular region. Three CP injections before and two ones after the surgical intervention for removing the tumor were administered. Closure and state of wounds were assessed via daily planimetry and photo-monitoring.

Results and Discussion. The healing terms of wounds in intact rats increased after the course of intravenous administration of CP not less than in 1.3 times (16.7 ± 0.5 *vs* 22.9 ± 3.0 days). Transient weight loss was observed on the background of CP. After 1–3 bandages in experimental animals, an "artificial black crust" was formed and remained on the wound until it was partially/completely epithelized. The wound surface area in CP-injected rats in 6 and 14 days after the wound infliction was respectively 1.7 and 4.1 times less under ACD than gauze dressings. The healing time for the wounds under ACD was 17.2 ± 1.5 *vs* 22.0 ± 3.6 days in control. A characteristic difference in the dynamics of the surface area changes of wounds after tumor resection is the absence of an increase in their size for the first three days under ACD, which is inherent for wounds after using gauze dressings. After three days, the average wound surface area in control animals (gauze dressing) was 1.21 times higher as compared with the initial one and vice versa 1.42 times lower under ACD. Visually, within this period, control animals showed swelling of the wound edges and hemorrhages, which were practically absent in experimental ones. Since the fifth day, the wounds in most rats in the experimental group were partially or completely covered with a dry black crust, signs of inflammation were absent. In 7–11 days after the tumor resection, in most rats in both groups tumor

Conclusions. Combined *BRCA1, CHEK2, TP53* genes mutations determine disease course in patients with BC, regardless of treatment standard used. It is necessary to analyze combinations of mutations to predict the disease course and create a personalized treatment strategy. We support the use of multigene panel testing in diseases cases with hereditary predisposition to BC.

TUMORS AND BLOOD PLASMA OF ANIMALS WITH WALKER-256 CARCINOSARCOMA

N.Yu. Lukianova[1], Yu.V. Lozovska[1], L.A. Naleskina[1], I.N. Todor[1], I.M. Andrusyshyna[2], A.P. Burlaka[1], V.F. Chekhun[1]

[1]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine
[2]State Institution "Yu.I. Kundiev Institute of Occupational Health", NAMS of Ukraine, Kyiv, Ukraine

Introduction. Today it is known that lactoferrin (LF) influences in many ways the most important processes in the body in normal state and under carcinogenesis. However, the morphologic structure of tumors, the profile of different metabolic units at the tumor and organism levels, namely, homeostasis of essential elements (EE), enzymes of pro/antioxidant balance, and energy metabolism parameters, have not been fully understood.

Aim. To investigate the modifying effect of exogenous LF on the architectonics of tumors in the *in vivo* system, the content of EE (Cu, Zn, Mg, Fe, Ca), energy metabolism indices (glucose, lactate) and the changes in the activity of metal-containing enzymes (ceruloplasmin (CP), myeloperoxidase (MPO)) in blood plasma (BP) and tumor tissue (TT).

Materials and Methods. Analysis of morpho-structural features of TT, including its vasculature, was performed on rats with Walker-256 carcinosarcoma exposed to LF in the doses of 1 and 10 mg/kg of body weight. The EE content was determined by inductively coupled plasma atomic emission spectroscopy, CP activity was evaluated by electron paramagnetic resonance (EPR), and MPO — by a unified biochemical method.

Results. It was shown that exogenous LF at the doses of 1 and 10 mg/kg b.w. inhibited tumor growth by 44% ($p < 0.05$) compared to the control. Morphologically upon LF treatment tumor cells are characterized by segregation, pycnotic changes, hyperchromatosis of the nuclei, the phenomena of necrobiosis and necrosis, and as a consequence — reduction of the tumor lesion. The most pronounced changes were observed in tumor vasculature: dilation, thinning of vascular walls, hemorrhage. In the TT, LF caused a decrease in the content of Ca (1.8 times), Fe (1.2 times), Zn (1.4 times), with a more pronounced effect when applying a dose of 10 mg/kg b.w. At the same time, this dose changed the bioenergetic phenotype of tumors by reducing the content of glucose and lactate by 1.2–1.4 times. In animal BP, the treatment with LF caused a decrease in the content of Ca and Fe, but had opposite effect on the content of Zn; also, in LF-treated animals increased CP activity (1.2 times) and MPO (1.3 times) in BP were registered.

Conclusions. The analysis of the obtained data allowed establishing the mechanisms that lead to inhibition of tumor growth and changes in the tumor vasculature upon the action of LF at the mentioned doses. It is proved that EE and the state of some enzymes of the pro/antioxidant system play a key role in these processes.

ON THE PROBLEM OF THE DEFINITION OF BIOCHEMICAL PREDICTORS OF NON-MALIGNANT CELLS IN ONCOLOGICAL PATIENTS

L.I. Makovetska, E.A. Domina, M.O. Druzhyna
R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine
tsigun@ukr.net

Introduction. The irradiation of healthy tissues during the radiation therapy due to the primary cancer can be the cause of not only radiation complications, but also secondary cancers. The majority of secondary cancers develop in the tissues that are characterized by high radiosensitivity. Although these tissues do not differ by their structure from the normal tissues, they nevertheless display some metabolic changes. A question rises as to which biochemical changes occur in normal tissues of cancer patients and how they influence the mechanism of radiosensitivity formation according to the dose of irradiation as compared to the healthy control. Therefore, the determination of predictors of radiosensitivity in normal cells of cancer patients is of a high importance for decreasing the complications of radiotherapy.

Aim. To determine the intensity of free radical processes in the blood of donors depending upon the doze of irradiation in the conditions *in vitro*.

Materials and Methods. Blood samples of donors were irradiated in the range of 0.5–3.0 Gy. The intensity of free radical processes was assessed by the content of malonic dialdehyde (MDA) and the prooxidant — antioxidant ratio (PAR) analyzing hydrogen peroxide induced chemiluminescence.

Results and Discussion. For the first time, the linear relationship between *in vitro* test irradiation and radiation-induced biochemical changes in PAR and MDA levels has been demonstrated in the peripheral blood of conditionally healthy persons. The interindividual variability of the values of these indicators was detected. It has been shown that the level of MDA increased to 172% with an increase in radiation dose to 3.0 Gy. At the same time, the changes in the PAR are approximated by the linear equation of two types: with an increase (up to 136%) of the values and a decrease (up to 57%), which may indicate a different protective potential of human peripheral blood cells and the effectiveness of its implementation and thus individual radiosensitivity.

Conclusions. The results obtained at the first stage of the research are the basis for determining the biochemical predictors of radiosensitivity of non-malignant cells of cancer patients, who undergo radiotherapy.

METHYLATION STATUS IN PROMOTER REGION OF *RUNX3* GENE AMONG WOMAN WITH BREAST CANCER DEPENDING OF AGE

N. Medvedieva[1], O. Lobanova[2], Z. Rossokha[1], V. Vershyhora[1], L. Ivanets[1], V. Cheshuk[2], R. Vereshchako[2], N.G. Gorovenko[3]

[1]State Institution "Reference Centre for Molecular Diagnostic of Ministry of Health of Ukraine", Kyiv, Ukraine
[2]Bogomolets National Medical University, Kyiv, Ukraine
[3]P.L. Shupyk National Academy of Postgraduate Education, Kyiv, Ukraine
refcentre2013@ukr.net

Introduction. Disruption of gene function regulation is associated with an increased risk of cancer. Methylation status of promoter region in gene regulates its expression. The *Runt-related transcription factor 3* (*RUNX3*) gene is a member of the family DNA-binders RUNX transcription factors which are involved into oncogenesis. The *RUNX3* gene is functioned as an oncosuppressor, thus it activates the antioncogene ARF-p21 pathway expression and induces apoptosis. The *RUNX3* gene inactivation has been observed by researchers in various types of cancer caused by promoter gene area hypermethylation and protein structure change with impaired function. Methylation status of promoter region in *RUNX3* gene is less well studied in breast cancer (BC) comparing with *BRCA1/2* genes mutations or methylation.

Aim. To study methylation status in promoter region of *RUNX3* gene among BC patients depending on their age.

Materials and Methods. Molecular genetic analysis of methylation status in promoter region was defined among 60 women with BC. Average patient age was 52.44 ± 1.59 years. There were 17 patients under 40 years (group 1) and 43 patients

over 41 years (group 2). Peripheral blood and/or tumor tissue obtained during surgery were used as biological material. DNA extraction was performed using "Quick-DNA MiniprepKit" (ZymoResearch). DNA sample were frozen until bisulfide conversion via "EZ DNA Methylation-Gold Kit" (Zymo Research). Converted DNA was used for performing PCR-analysis via methyl-specific primers. Availability product analysis of methyl-specific PCR was performed using agarose gel electrophoresis. Statistical analysis included Fisher's test.

Results and Discussion. Hypermethylation in promoter region of *RUNX3* was found in 25 (41.66%) of 60 BC patients BC. 12 (70.58%) of 17 patients of the group 1 and 13 (30.23%) of 43 patients of the group 2 had hypermethylated status in promoter region of investigated gene. We defined significant differences between identified hypermethylated status depending on patients age ($p < 0.05$). Hypermethylation level of genes increases with age. But we specified in our investigation that *RUNX3* gene activity involved in BC development in younger women as one of molecular pathways in disrupted antioncogene processes.

Conclusion. Hypermethylation status in promoter region of *RUNX3* may be an early and/or diagnostic marker of decreasing apoptotic processes and tumor transformation. The direct detection of hypermethylation status in promoter region of *RUNX3* in the tumor focus will allow in the future to establish new pharmacogenetic approaches to the treatment. Thus, clinic interpretation of the gathered results is required for defining strategy of the next researches.

SPECTRUM OF SOMATIC MUTATIONS IN PATIENTS WITH NON-SMALL-CELL LUNG CANCER: USING THE TECHNOLOGY OF NEXT GENERATION SEQUENCING IN THE REPUBLIC OF BELARUS

E.P. Mikhalenko[1], A.N. Shchayuk[1], O.M. Malysheva[1], M.N. Shepetko[2], V.G. Lebetsky[3], V.I. Ltokhin[4], A.V. Kilchevsky[1]

[1]Institute of Genetics and Cytology, NAS of Belarus, Minsk, Republic of Belarus
[2]Belarusian State Medical University, Minsk, Republic of Belarus
[3]City Clinical Anatomopathological Bureau, Minsk, Republic of Belarus
[4]Healthcare Institution "Minsk City Clinical Oncologic Dispensary", Minsk, Republic of Belarus
anna.shchayuk@tut.by

Introduction. Non-small-cell lung cancer (NSCLC) constitutes 85% of all lung cancer types. Due to the development of a personalized approach to the treatment of patients, a study on the molecular genetic characteristics of a tumor, which allows to predict the development and course of the disease and optimize individual antitumor therapy, is of great importance.

Aim. To study somatic mutations in NSCLC patients, who live in the territory of Belarus using the next generation sequencing technique.

Materials and Methods. 101 patients with NSCLC diagnosis receiving a treatment in Minsk City Clinical Oncologic Dispensary from 2012 to 2018 was brought into our research (78 men and 23 women). 50 patients with NSCLC and 50 with adenocarcinoma formed the group. DNA sample preparation was performed at the MiSeq (Illumina, USA) device using TruSeq Amplicon Cancer Panel in accordance with the producer's user manual.

Results and Discussion. After filtering conducted by exclusion of all low-quality variants (reading depth < 50; alternative allele frequency < 15%; variants that failed to pass the PASS-filter), a total of 86 variants of somatic mutations were obtained for 101 tumor samples — 0.85 variants per tumor on average. In cases of adenocarcinoma, somatic mutations were most commonly detected in the genes *EGFR* (25.5%), *KRAS* (17.6%), *TP53* (9.8%), *ATM* (7.5%), *CTNNB1* (5.7%), *STK11* (5.7%) and in the genes *TP*53 (26.0%), *ATM* (14.0%), *FBXW7* (8.0%), *FGFR3* (6.0%), *JAK3* (6.0%), *RET* (6.0%), *APC* (4.0%), *EGFR* (4.0%), *PIK3CA* (4.0%) in cases of NSCLC.

Analysis of the association of the studied somatic mutations and the development of a certain histological type showed that mutations of *EGFR* and *KRAS* genes are associated with the development of adenocarcinoma (OR = 9.08; 95% CI 1.94–42.48; $p = 0.003$ and OR = 10.50; 95% CI 1.28–86.37; $p = 0.02$, respectively). There is an increase in the frequency of occurrence of *TP53* gene mutations in patients with NSCLC (OR = 3.23; 95% CI 1.06–9.89; $p = 0.06$). Among the carriers of *EGFR* gene mutations, women predominate: the mutation rate in women was 56.5% and in men only 3.8% (OR = 32.50; 95% CI 7.87–134.26). Somatic mutations in the *KRAS* gene were detected in 10 patients, found only in men (12.8%) and were not found in women. Somatic mutations in the *TP53* gene were found predominantly in men (21.8%) and only in one woman (4.3%).

At the next stage, studies are conducted to determine the clinical significance of the identified mutations in the prognosis of a course of the disease.

VARIABILITY OF POLYAMINE METABOLIC BALANCE DURING TUMOR DEVELOPMENT

O.A. Orlovsky, S.P. Zaletok

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine
orlovaleks1955@ukr.net

Introduction. Inhibitors of the enzymes of the polyamine (PA) biosynthesis and back-conversion are known as a kind of the effective anticancer remedies. But optimization of such "antipolyamine" therapy may be achieved only if we know real conditions of the enzymatic reaction balance variability in the PA metabolic system during the tumor development. The end expression of this enzymatic balance is a pattern of the PA spectrum in the tumor tissue and in the biological fluids of the tumor-bearing organism.

Aim. To compare the PA spectrum patterns variability during experimental and clinical tumor development.

Materials and Methods. PA spectrum patterns were studied during three kinds of experimental chemical carcinogenesis (N-nitrosodiethylamine liver carcinogenesis; 1,2-dimethyl hydrazine large and small intestine carcinogenesis) in rats as well as in the operational materials and urine of the patients with mammary, lung and rectal cancer. PA content in the biological materials was measured by the methods of thin-layer chromatography and HPLC, ornithine decarboxylase (the key enzyme of the PA biosynthesis) activity — by radiometric determination of $^{14}CO_2$ production and/or chromatographic determination of putrescine production. Student's *t*-test and, in need, Fisher's exact method were used for statistical data treatment.

Results and Discussion. Statistically significant modulations of PA spectrum were discovered during all kinds of tumor development having been studied. The modulation patterns were found to be significantly different dependently on the kind of tumor. Because of ambiguity of the PA spectrum mapping into the set of the enzyme balance stages (each pattern of PA spectrum may be obtained in two or more combinations of the enzyme activities), real optimization of the "antipolyamine" therapy requires detailed enzymological investigations.

Conclusion. Our results demonstrate significant and tumor-kind-dependent modulations of the PA balance during tumor development. This fact has to promote special enzymological investigations having a goal to optimize anticancer therapeutic schemes including the PA metabolism modulators.