Exp Oncol 2017
39, 3, 234–256

CONFERENCE REPORT

Experimental ONCOLOGY

NORMAL AND CANCER STEM CELLS: DISCOVERY, DIAGNOSIS AND THERAPY INTERNATIONAL SCIENTIFIC CONFERENCE

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October 5–6, 2017

ABSTRACTS

UNDERSTANDING THE ORIGIN OF INDUCTIVE MESENCHYME WITHIN THE WOUND-INDUCED *DE NOVO* HAIR FOLLICLES

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We recently demonstrated the existence of an adult dermal stem cell (DSC) that functions to populate both mesenchymal compartments within the hair follicle (HF): the inductive cells within the dermal papilla (DP) and the connective tissue sheath (CTS) which wraps around the distal follicle (Rahmani *et al.*, 2014). Depletion of DSCs disrupts normal HF regeneration highlighting their potential importance for restoration or prevention of hair loss (alopecia). The loss of adult HF is considered permanent but in 2007, it was shown that when the skin excisions on the back skin of mice are larger than 1 cm^2, *de novo* HFs form in the middle of the wound bed after re-epithelialization. This wound-induced HF neogenesis (WIHN) parallels the development of HFs during embryogenesis. It has been demonstrated that the nascent HFs arise from epidermal cells outside the HFs and not from the bulge stem cells within the pre-existing HFs. However, the origin of the mesenchymal compartments of these neogenic HFs is still unclear. We hypothesize that the dermal cells in the neogenic HFs originate from $aSMA^{ve+}$ cell populations within the dermis. To test that, we made full-thickness excisions (> 1 cm^2) in the dorsal skin of $\alpha SMACreER^{T2}ROSA^{YFP}$ mice. The wounds were harvested from 18 days post-wounding (dpw) up to 140 dpw to assess the dermal cells in the neogenic HFs. Our data reveal that YFP^{+ve} DSCs integrate into ~90% of the mesenchymal compartments of the nascent HFs. The neogenic HFs successfully complete their hair cycle and YFP^{+ve} DSCs appear to have re-assumed their role as DSCs within newly formed HFs.

ROLE OF USP1, CORTACTIN AND Hsp27 PROTEINS IN MOLECULAR MECHANISMS THAT AFFECT CML DEVELOPMENT

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Introduction. Development of chronic myeloid leukemia (CML) is the result of reciprocal translocation between chromosomes 9 and 22 that leads to emergence of Philadelphia chromosome. Role of BCR domains in differentiation of leukemia stem cell remains unclear. Investigation of protein-protein interactions can be one of the effective ways to reveal those molecular events that alter normal cellular processes and cause malignant transformation. Previous research showed that USP1, cortactin and Hsp27 may interact with PH domain. The main function of USP1 protein is to deubiquitinate proteins in the cell. Intracellular Hsp27 plays an anti-apoptotic role and acts as a chaperone protecting incorrectly formed proteins from aggregation. The main function of cortactin is remodeling of actin cytoskeleton during fission of clathrin-coated vesicles.

Aim. To design genetic constructs for mammalian expression and determination of protein-protein interactions between PH domain of BCR and target proteins suitable for the analysis of important signaling pathways involved in CML pathogenesis.

Materials and Methods. The standard molecular cloning techniques and expression in *E. coli* strain Rosetta.

Results and Discussion. Following PCR, we amplified target gene sequences USP1, CTTN, Hsp27. Their sizes of 2,300 bp, 1,600 bp and 650 bp are in accordance with expected. Using the ligation reaction, we produced pBluescriptSKII (+)-USP1, pBluescriptSKII (+)-CTTN and pET42a-hsp27 genetic constructs. By subcloning the CTTN gene from pBluescriptSKII (+)-CTTN plasmid, we derived pFastFT-N1-CTTN, pMediumFT-N1-CTTN, pSlowFT-N1-CTTN. Recombinant genetic constructs were successfully identified by restriction analysis. The absence of mutations and correct reading frame was confirmed by sequencing. We also produced a genetic construct pET42a-hsp27 for expression of HSP27 in bacterial host system and obtained effective bacterial expression of Hsp27. Analysis of protein expression by polyacrylamide gel electrophoresis confirmed that the expressed protein corresponds to the expected size of about 55 kDa. The purified recombinant HSP27 may be used to determine presence or absence of interaction with PH domain of BCR by far-Western.

Conclusions. All DNA constructs obtained can be effectively used to study biological role of interactions of PH domain of BCR with USP1, cortactin and Hsp27 for better understanding of molecular mechanisms of CML pathogenesis. The development of the genetic constructs with inserts corresponding to the coding sequences of CTTN, USP1 and PH domain of BCR that may be useful for determining spatio-temporal resolution of target protein inside the cell on subdiffraction level is under way.

THE CONTENT OF TUMOR STEM CELLS IN BRAIN GLIOMAS OF DIFFERENT DEGREE OF MALIGNANCY

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Introduction. At the present stage of research the possible relationship of cancer stem cells (CSCs) and the development of various tumors including brain tumors are actively studied. CSCs are responsible for metastasis, infiltrative growth, recurrence of gliomas as well as resistance to chemotherapy and radiotherapy. While the question of relationship between content of CSCs and malignancy of gliomas is controversial, it is known that a fundamental feature of CSCs is their ability to form neurospheres *in vitro*.

Aim. The purpose of the study was to investigate the neurosphere formation of CSCs from brain gliomas of varying degrees of malignancy and analyze $CD133^+$ content in such cells.

Materials and Methods. 51 biopsies of gliomas with varying degrees of malignancy were obtained during neurosurgery. Histological diagnosis of brain tumors was conducted in accordance with International Histological Classification of Tumors of the Central Nervous System (2007). The cell phenotype cells was analyzed by immunofluorescent method using monoclonal antibody to CD133 (a marker of CSCs) by cytometry protocol on flow cytometry (Beckman Coulter, USA). The CSC ability to form neurospheres was studied *in vitro* in suspension and dissociated cultures.

Results and Discussion. It is established that the number of $CD133^+$ cells in glioblastomas was $8.92 \pm 6.81\%$ and in anaplastic astrocytomas $7.98 \pm 4.62\%$. In benign gliomas of second degree of anaplasia, the content of $CD133^+$ cells was less by half than in malignant gliomas. Upon culturing of glioma cells in DMEM in the presence of EGF within 10–12 days, fluctuating neurospheres characteristic of CSCs were formed in 86.3% of glioblastoma samples, 78.5% anaplastic astrocytomas, 88.2% anaplastic olihodendrogliomas and 66% benign astrocytomas.

Conclusions. The increased content of $CD133^+$ cells was detected in malignant gliomas of III and IV degree of malignancy with accompanying increased percentage of neurosphere formation upon *in vitro* culturing. Further study of these populations of cells may provide a new understanding of the nature of tumors and the causes of treatment failure in brain gliomas.

MESENCHYMAL STEM CELL AND ONCOGENESIS

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Mesenchymal stem cell (MSC), a non-hemopoietic stem cell defined also as "mesenchymal stem/stromal cell" or "multipotent stem cell" is one of the major components of the connective tissue system. As early as in the beginning of 1970s, A. Fridenstein demonstrated that MSC may differentiate into myofibroblasts. Nowadays, the role of myofibroblasts in the formation of microenvironment of tumors has been well proven. MSCs reside in the bone marrow, dental pulp, adipose tissue and several other tissues. These cells possess the adhesive properties and do not express the markers of the hematopoietic stem cell. MSCs have the capacity to differentiate into the osteoblasts, chondrocytes, adipocytes, myocytes and neuron-like cells. The wide range of the biological features of MSC explains their implication in tumor growth. To date, several mechanisms of MSC involvement in the stimulation of tumor growth have been elucidated: a) MSCs interact with tumor cells increasing their metastatic potential and inducing EMT in tumor cells; b) MSCs secrete various soluble factors and cytokines including those stimulating tumor growth such as IL-6, IL-8, IL-10, TGFβ, HGP; c) MSCs are involved in the formation of chemoresistance that has been proved for many types of tumors; sometimes chemoresistance is provided by increasing expression of MDR, MRP, LRP; and MSC exosomes are no less important for chemoresistance. The role of MSCs in suppression of the immunological response cannot be overemphasized. Such immunosuppressive effects are displayed as follows: a) MSCs affect antigen recognition, primarily by TGFβ production; b) MSCs interfere with interplay between lymphocytes and tumor cells; c) MSCs promote the escape of tumor cells from immunological surveillance; d) MSCs inhibit cytotoxic potential of cells possessing killer activity, in particular via ROS generation; e) MSCs are actively involved in the formation of perivascular niches in all types of tissues being responsive to various extra- and intracellular signals. On the other hand, according to the general principles considering the effects of microenvironment on tumor growth one should be aware also of the MSC protective effects involving several mechanisms: a) MSCs may enhance INF gamma production followed by activation of lymphocytes and stimulation of tumor growth; b) sometimes MSC vesicles may counter-balance the negative effects of MSCs themselves; c) MSCs may protect tumors from cytostatics by inhibition of caspase activity that holds true in several tumors.

To sum up, MSCs represent the heterogeneous population capable of various activities depending on the stage of tumor growth, the source of MSCs and several other factors. The diversity of the biological effects of these cells explains why MSCs may be involved both in stimulation and inhibition of tumor growth.

IMPACT OF BONE MARROW STEM CELLS AND SOLUBLE FACTORS ON BIOLOGICAL PROPERTIES AND EPITHELIAL-MESENCHYMAL TRANSITION IN BREAST CANCER CELLS

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Background. Bone marrow is a tissue containing stem cells and cells of various degrees of differentiation.

Aim. To study the crosstalk between breast tumor cells with epithelial or mesenchymal phenotype and bone marrow stem cells of breast cancer (BC) patients using noncontact co-cultivation *in vitro* system in the presence of biological or chemical antitumor agents (IFN-α, mitomycin C, zoledronic acid), which are epithelial-mesenchymal transition (EMT) modifiers.

Materials and Methods. Human breast carcinoma cells with epithelial phenotype (MCF-7 cell line), mesenchymal phenotype (MDA-MB-231 cell line) and aspirates of bone marrow (BM) of BC patients with a different clinical course of the disease (groups "Stabilization" (BM-S) and "Progression" (BM-P)) were used in noncontact co-cultivation system *in vitro*. BM stem cells were used as a source of soluble

factors of microenvironment for tumor cells *ex vivo*. Cell culture methods, immunoenzyme and immunocytochemical analysis have been used.

Results. It was shown that co-cultivation of MCF-7 cells with BM-P did not affect the tumor cells growth and migration activity and co-cultivation with BM-S stem cells was accompanied by significant inhibition of such biological properties of tumor cells. At the same time, significant inhibition of the same parameters of MDA-MB-231 cells was observed only after their cultivation in the presence of BM-P cells.

Co-cultivation of MCF-7 cells with BM-P resulted in 30% increase of the E-cadherin expression in BC cells. The incorporation of IFN-α in such system leads to changes in E-cadherin expression (increase in MCF-7 + BM-S and decrease in MCF-7 + BM-P). The cultivation of MCF-7 cells in the presence of BM-S resulted in a 2-fold decrease in the number of Vimentin$^+$ cells, and the addition of IFN-α or mitomycin C enhanced the effect by inhibiting Vimentin expression in MCF-7 cells by 50% or 100%, respectively.

It was shown that soluble factors produced by BM stem cells practically did not affect the number of E-cadherin$^+$ MDA-MB-231cells, whereas the addition of IFN-α to this co-culture system or the complex action of BM-S and mitomycin C were accompanied by a statistically significant increase in the level of expression of this protein in these tumor cells. It was interesting to note, that a significant decrease in the number of Vimentin$^+$ MDA-MB-231 cells by 50% was observed after their co-cultivation with BM stem cells in the presence of zoledronic acid.

The analysis of the soluble factors produced by cells in the experiment showed that co-cultivation of MDA-MB-231 cells with BM cells did not change significantly the levels of VEGF and IL-6 in the culture medium. Only IFN-α in such system suppressed VEGF level by 50% compared to control. It was shown that co-cultivation of MCF-7 cells with BM cells did not affect significantly the VEGF level in the culture medium, but the addition of mitomycin C or IFN-α resulted in a decrease of VEGF level. The cultivation of MCF-7 cells with BM-S leads to two-fold increase in the level of IL-6 in the culture medium, and IFN-α in this system intensified the effect and the level of IL-6 increased 4-fold compared to control.

It should be noted, that in such co-cultivation system, regardless of the tumor cells type, the level of TGF-β1 did not change significantly in any setting of the treatment and TNF-α was not detected in the culture medium.

Conclusions. The significant changes in biological properties of BC cells dependently on their EMT status were detected after their interaction with BM cells of BC patients with a different clinical course of the disease in noncontact co-cultivation system. BM cells of BC patients had different modifying effect on proliferative and migration activity of tumor cells depending on the clinical course. It has been shown that cytokines are important elements of tumor microenvironment that influence EMT status of tumor cells.

CONTENT OF STEM-LIKE CANCER CELLS OF VARIOUS DIFFERENTIATION DEGREE AT DIFFERENT STAGES OF BREAST CANCER (EXPERIMENTAL STUDY)

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Introduction. It is known that the manifestation of malignant tumors including breast cancer (BC) is stipulated by the presence of cancer stem cells (CSCs). In this regard, the vital is the question of studying the content and functional activity of CSCs in BC development. The appropriate experimental model for the evaluation of these indices is the C3H mice with high incidence of mammary cancer.

Aim of research was to assess the proliferative potential of CSCs varying in differentiation degree in BC development in the mammary tissue itself, mammary cancer and metastases in lung.

Materials and Methods. Experiments were performed in 16-month-old female CBA and C3H mice, weighing 18–20 g, kept under standard vivarium conditions of the IPC&C of the NAS of Ukraine. The mice were divided into 5 groups of 10 animals each: Group 1 — intact CBA mice (control); Group 2 — C3H mice without tumors; Group 3 — C3H mice with tumors (0.5–1.0 cm in diameter); 4 — C3H mice with tumors (1.5–2.0 cm in diameter); 5 — those with tumors (2.0 cm in diameter) and lung metastases (0.5 cm in diameter). Content of CSCs in tissue suspensions (mammary tissue, tumors and metastases in lungs) was determined by flow cytometry (FACS Calibur) using monoclonal antibodies (BD, USA) to CD24, CD44 molecules.

Results and Discussion. In tissue of murine mammary gland of group 2, CD44^{hi} cells were detected, which were absent in the mammary gland of CBA mice. The average content of CD44$^+$/24$^-$ cells in mammary gland in mice of group 2 exceeded the same index in intact animals. The concentration of CD44$^+$/24$^+$, CD44$^-$/24$^+$ cells was lower ($p < 0.05$) than in the control group. In Group 3 of animals with minimal tumor size, the content of CD44^{hi} cells in mammary gland was unchanged if compared with group 2, while their number in tumor was twice decreased.

Thus, the content of CSCs (CD44^{hi}) in tumors of the group 3 mice was lower than in mammary gland, while a number of more differentiated CD44$^-$/24$^+$ cells on the contrary was higher than in the control group. The difference between the content of CD44^{hi}, CD44$^+$/24$^-$, CD44$^+$/24$^+$ was statistically significant in mammary gland and tumors in mice of group 4. On the background of increasing content of CD44^{hi}, a significant difference between the number of CD44$^-$/24$^+$ in mammary gland and tumor was kept. At the same time, in mammary gland the number of CD44$^+$/24$^-$ cells increased and the number of CD44$^+$/24$^+$ and CD44$^-$/24$^+$ cells decreased, in tumors, in contrast, a decrease in CD44$^+$/24$^-$ subpopulations and an increase in CD44$^+$/24$^+$ and CD44$^-$/24$^+$ cells were observed.

It should be noted that in mice of group 5 in mammary gland the concentration of CD44^{hi}, CD44$^+$/24$^-$ cells decreased and CD44$^+$/24$^+$ cells increased, while the number of CD44$^-$/24$^+$ was unchanged. In mammary tumors of this group the number of CD44$^+$/24$^-$, CD44$^+$/24$^+$ and CD44$^-$/24$^+$ cells increased significantly and exceeded the corresponding values for group 4. However, the number of CD44$^+$/24$^-$ cells in metastases did not change, and the content of CD44$^-$/24$^+$ and CD44$^+$/24$^+$ cells decreased. At the same time, the number of CD44^{hi} cells significantly increased relative to the tumor values in this group.

Conclusions. It has been found that in C3H mice, the redistribution of subpopulation composition was observed in mammary gland as far as the tumor was manifested. The results emphasize the importance of CSCs in BC pathogenesis.

STEM CELL CXCR4 RECEPTOR IN GASTRIC CANCER

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Introduction. Metastatic spread of tumor cells is the main cause of cancer-related deaths. Chemokines are the most important factors controlling cellular migration. Stem cell receptor CXCR4 as a transmembrane chemokine receptor and its specific ligand CXCL12 (stromal cell-derived factor 1, SDF-1α) play a vital role in dissemination of tumor cells from primary sites, transendothelial migration as well as homing of cancer stem cells. In the tumor microenvironment under hypoxic condition there is possible mechanism by which cells of a growing tumor are reprogrammed to express the CXCR4 receptor thereby enhancing the metastatic potential of the tumor cells. Therefore in the current work it was investigated how hypoxia in primary tumor of patients with gastric cancer (GC) affects the CXCR4 expression.

Aim of research. The aim was to find the association between CXCR4 expression in GC and bone marrow (BM) cells with tumor hypoxia, disseminated tumor cells (DTC) and clinical outcome.

Patients. All patients with primary GC were diagnosed and treated at the City Clinical Oncological Center (Kyiv). No patient received chemotherapy or radiation prior to surgery. Histological types of tumor were evaluated by WHO histological classification (2000). Tumors were classified and staged according to the 2002 version of the UICC staging system. All patients were thoroughly informed about the study that was approved by the local ethics committee.

Methods. DTCs and CXCR4 expression were detected using immunocytochemistry in BM cytospin preparations, CXCR4 expression in tumor — using immunohistochemistry, tumor hypoxia level was evaluated by ^{31}NMR spectroscopy. The statistical analyses were conducted.

Results. Overall, 78.5% of patients had tumors with $CXCR4^+$ cells. The direct correlation between $CXCR4^+$ cells and the level of hypoxia in primary tumor was shown ($r = 0.492$; $p < 0.05$); the probability of the appearance of high number of $CXCR4^+$ cells in tumor increased by a factor of 5 (OR = 4.926, 95% CI 7.027–23.628, $p = 0.046$), when tumors were characterized by severe and moderate hypoxia. Moreover, under such hypoxic condition appearance of tumor cells in BM increased by a factor of 11.4 (OR 11.4, 95% CI 2.71–47.89, $\chi^2 = 12.3$, $p < 0.001$). $CXCR4^+$ cells in tumor tissue were detected in 80% of the patients with the DTC in BM. When tumors were characterized by positivity for CXCR4 cells, the probability of appearance of DTCs in BM of patients increased by a factor of 4.0 (OR = 4.024, 95% CI 1.0597–15.2782, $\chi^2 = 4.367$, $p = 0.0408$) and the relative risk of DTCs appearance was approximately 2.0 (RR = 1.955, 95% CI 0.999–3.824, $p = 0.05$). The $CXCR^+$ cells in BM were observed in 62.1% of patients with $CXCR4^+$ tumors. Risk of unfavorable outcome in all patients with $CXCR4^+$ tumors increased almost by a factor of 3.0 (HR = 2.82; 95% CI 1.162–6.832; $p < 0.05$). It was evaluated that in patients both with M_0 category and $CXCR4^+$ BM, the risk of unfavorable outcome increased by a factor of 3.4 (HR = 3.4; 95% CI 1.156–12.054; $p < 0.03$). Overall survival (OS) was significantly longer in all patients with tumors characterized by CXCR4-negative status as compared to the patients with $CXCR4^+$ tumors (log-rank test, $p = 0.0375$), and in the patients with M_0 (log-rank test $p = 0.0137$). OS was longer in all patients with BM characterized by CXCR4-negative status as compared to the patients with $CXCR4^+$ BM (log-rank test, $p = 0.0441$).

Conclusion. Obtained results have clearly shown that overexpression of homing-protein $CXCR4^+$ in tumor, especially in the patients with M_0 category, mediated by the level of hypoxia in primary tumor, is associated with presence of $CXCR4^+$ cells as well appearance of DTC in BM and correlated with poor survival and unfavorable clinical outcome. $CXCR4^+$ BM is a risk factor of unfavorable prognosis for the patients with GC especially in the patients with M_0 category.

THE EFFECTS OF SUPEROXIDE RADICALS AND NITRIC OXIDE IN STEM CELLS

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The balance between self-renewal and differentiation is a critical function of stem cells (SC) during the development of the organism and maintaining tissue homeostasis throughout the life. Recent studies of SC in the resting phase show that their redox state is regulated by superoxide radicals (SORs) and nitric oxide (NO).

The main sources of SORs in the SC are the mitochondrial respiratory chain. It should be pointed out that SORs and NO are integral components of the redox-state of the cell and because of related functions, they affect each other. Low levels of these molecules in the SC maintain resting state and self-renewal. Increased levels of SC generation cause proliferation/differentiation, senescence, apoptosis, or depletion. Thus, generation of SORs and NO in the SC must be strictly regulated for carrying out function of redox homeostasis support and repairing damaged tissues. Generation of SORs and NO in the SC is regulated by numerous internal and external factors, and their deregulation leads to the development of pathological conditions. The consequence of aberrant generation of SORs in SC tumor is the initiation and progression of malignancy. So, today the urgent problem is the development of redox homeostasis regulators in the SC, although the mechanisms and signal pathways regulated by the SORs and NO have been not yet studied clearly. NO can cause nitrosylation of SH-groups in FeS-proteins and release of Fe ions from mitochondria Fe-S centers and other Fe-containing proteins, which lead to the impairment of their functions. In physiological concentrations, NO modulates the electron transport in the mitochondrial respiratory chain depending on the concentration of intracellular oxygen, redox-state of the electron transport chain (ETL) and the level of cellular hypoxia. SORs and NO have different chemical properties, different reactivity, time of half-life, which determines the rate of their interaction with DNA and other structural molecules in the cell. It is believed that stem cell are located in niches, which are characterized by hypoxia and low levels of SORs and NO, and it is crucial for maintaining the self-renewal potential in the SC. But, while SC are being isolated for therapeutic or research purposes, generation of SORs is enhanced and extracellular matrix undergoes degradation. SORs and NO can alter gene expression and activity of key metabolic regulators, thereby to determine the specific metabolic patterns of the cells.

Rising levels of SORs and NO cause oxidative damage to DNA, proteins, lipids, carbohydrates, premature aging and apoptosis. The accumulation of oxidation-induced DNA damage in human cord blood SC leads to the loss of its ability to restore hematopoiesis. NO inhibits SC proliferation through blocking the cell cycle and induces CD34 apoptosis. Decreased activity of NOS increases the number of SC in the bone marrow and improves hematopoiesis. Selective reactivity of SORs and NO to biotargets is the basis of the impact on specific signaling pathways that are initiated or damaged by them. These reactions cause allosteric or configurational changes in the target protein, which alter their function and interaction with other proteins, causing a variety of signaling events.

Thus, the safe use of SC in the clinic requires improvement of the production technology with strict control of SORs, NO, matrix metalloproteinases levels that will promote their ability to self-renewal, proliferation and differentiation.

CHARACTERIZATION OF CELLS ISOLATED FROM A BREAST CANCER BEFORE AND AFTER NEOADJUVANT CHEMOTHERAPY

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Introduction. Breast tumors contain heterogeneous cell populations. There can be dormant cancer cells among them that can survive therapy and give rise to secondary tumors. Therefore, in-depth studies are necessary for better understanding of the tumor pathogenesis. In this study, a standardized cell isolation and characterization methods were used to look at cell growth and differentiation state at different time points (before and after neoadjuvant therapy).

Aim. To isolate, characterize and compare cells isolated from the same breast cancer tumor biopsies before and after neoadjuvant chemotherapy. The main tasks were: (i) to isolate and culture cells from breast cancer biopsies before and after neoadjuvant chemotherapy; (ii) to perform cell growth and morphological analysis; (iii) to analyze expression of breast cancer type related genes; (iv) to compare gene expression profiles among various isolated cell fractions before and after neoadjuvant chemotherapy.

Materials and Methods. Cells were isolated from core biopsies by sequential enzymatic disaggregation collecting cell fractions obtained after each disintegration step and culturing them in four diverse cell growth media until the fourth or fifth passage (the length of cultivation depended on the numbers of cells) for further analysis and biobanking. Cells were monitored in phase contrast microscope and fed every two to three days. Several gene expression panels were developed to characterize isolated cell cultures: proliferation panel, tumor inducing cell marker panel, epithelial-mesenchymal transition marker panel, epithelial differentiation gene panel with subpanels for common differentiation, basal and Her2++, basal cell regulator, luminal cell, luminal progenitor and mature luminal cell regulators.

Results and Discussion. All isolated cultures had lower proliferation rate in comparison to reference fibroblasts (shown by growth data analysis and gene expression profile). Also, gene expression profile is different in cells cultured in media supplemented with 10% FBS from other three media that had 5% FBS and supplemental growth factors/hormones.

Patient in this study was diagnosed as luminal type breast cancer. The results from analysis of differentiation gene expression may suggest that obtained cell populations contain both luminal and basal cells. Elevated mature luminal cell regulatory gene expression may suggest that isolated populations contain cells with active epithelial differentiation program allowing them to differentiate towards more luminal cell type.

The analysis of the cultures isolated after chemotherapy is still ongoing and preliminary results will be presented in the conference.

Conclusions. In this study, we isolated different cell populations from the breast cancer core biopsies before and after neoadjuvant chemotherapy. Isolated cells show some degree of heterogeneity and gradual decrease in the proliferation potential by each passage. Cell pools probably contain both basal and luminal mammary cells capable of differentiating towards more mature luminal state.

THE USE OF BONE MARROW STEM CELLS IN RADIATION ONCOLOGY

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Despite the intensive implementation of the conformal strategy in the practice of radiation oncology, cells of normal tissues inevitably fall in the tumor irradiation zone. These are microscopic tumor infiltrates in normal tissues; tissue structures located at the inlet and outlet of the therapeutic ionizing radiation (IR) beam; blood vessels and, correspondingly, cells of the circulating blood pool, exposed to radiation at the same dose as the tumor. In the process of therapeutic irradiation of cancer patients, the early negative effects are most common in the hematopoietic system. Their severity depends both on the absorbed dose of IR and on the volume of irradiated red bone marrow. The mechanisms of these effects are due not only to the high radiosensitivity of the hematopoietic stem cells, but also to the individual radiosensitivity of the organism as a whole.

Earlier, in Ukraine, experience in bone marrow transplantation has been already accumulated (L.P. Kindzelsky *et al*., 1987, 1992, 1996, 2001) in the patients with acute radiation sickness of II–III degree who took part in the emergency operations after Chernobyl accident (April–May 1986). The purpose of this medical intervention consisted in a temporary replacement of the "failure" in the hematopoiesis using the donor bone marrow so that during this period the own pluripotent hematopoietic elements could start the production of blood elements. The period of passage through the stages of differentiation is 12–15 days for the elements of the myeloid and erythroid series. Allogeneic bone marrow was implanted 3–6 weeks after irradiation, that is, after detoxification procedures and the decay of short-lived radionuclides. First, during this time, the remaining elements in the bone marrow continued the production of blood cells; secondly, incorporated radionuclides could destroy not only own, but also transplanted myelocaryocytes. We have observed no early and late complications in any of the victims after the bone marrow transplantation.

At the present time, in order to minimize the radiation damage to healthy tissues, stem cells are used after intensive antitumor therapy. This method includes grafting bone marrow (a mixture of hematopoietic and mesenchymal stem cells), mesenchymal stem cells, the mobilization of autologous stem cells by growth factors (A.G. Konoplyanikow *et al.*, 2007; J.M. Lombaert *et al.*, 2008). Stem cell transplantation is carried out in an oncological clinic with the aim of replacing damaged and dead cells in the patients with radiation-induced damage to the tissues of vital organs. It should be noted that most of the experimental and preclinical studies in this direction were carried out with a single irradiation. Therefore, their results must be extrapolated with great care to the conditions of fractionated irradiation, when the factors of the formation of radioresistance, the processes of repopulation, etc. are involved.

EPIGENETIC AND METABOLIC REPROGRAMMING AS A TARGET FOR PROSTATE TUMOR RADIOSENSITIZATION

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Radiotherapy is a standard curative treatment for solid cancers including head and neck squamous cell carcinoma (HNSCC) and prostate cancer (PrCa). Although HNSCC and PrCa might be curable at early stages, advanced diseases have a poor prognosis. Tumor relapse after therapy is attributed to the population of tumor initiating, or cancer stem cells (CSCs) which escaped or survived the treatment. It has been proposed that CSCs in some types of tumors can be protected from the different treatment modalities by multiple intrinsic and extrinsic mechanisms that, along with CSC plasticity and heterogeneity, can be a driving force for tumor recurrence. Analysis of the phenotypes and density of CSCs in tumor biopsies prior the treatment and development of CSC-targeted therapies might be an essential approach for optimization and personalization of the treatment strategies. This lecture will review the results of studies of the Group of Biomarkers for Individualized Radiotherapy (OncoRay, Dresden) on the development of CSC-based predictive biomarkers and radiosensitizers for HNSCC and PrCa.

THERAPEUTIC RESPONSE IN BREAST CANCER BY DNA CONTENT PROFILING

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Introduction and Aims. Although the histological assessment of therapeutic response significantly helps to determine prognosis in breast cancer treated with neoadjuvant (preoperative) therapy, the problem caused by discrepancies in interpretation of results obtained from different pathologists remains actual mainly because of lack of standardized assessment criteria and methods. We propose that DNA content profiling of the affected (cancerous) tissue in combination with immunohistochemical judgment of its aggressiveness before and after therapy may facilitate interpretation of therapeutic response.

Patients and Methods. The study population consisted of 12 breast cancer patients, 11 of those 12 patients had locally advanced breast cancer. The patients underwent diagnostic procedures at the Latvian Oncology Center of the Riga East University Hospital in 2014 and 2015. The tissue specimens were collected after the patients' informed consent was obtained in accordance with the Ethics regulations. Both diagnostic biopsy and operation material, such as primary tumors surgically removed after 3–9 courses of neoadjuvant chemotherapy (NAC) using standard doses of paclitaxel and doxorubicin, were subjected to DNA content analysis with image cytometry. DNA histograms were classified according to Auer *et al.* (1980). Positions of DNA peaks were determined by calculating DNA index (DI) values. Based on the presence or absence of aneuploid DNA peaks and cells that polyploidize (> 4.5C), it seems reasonable to expect that NAC effects may vary from positive to negative (i.e., opposite). Immunohistochemical staining was applied to evaluate proliferation (Ki67), invasiveness (CD44), and self-renewal factors characteristic for stem cells (SOX2 and NANOG).

Results and Discussion. The analysis of DNA content profiles in operation material from 12 patients revealed the features of tumor non-responsiveness to NAC in 7 cases, among which 4 cases showed enhanced polyploidization, suggesting the negative NAC effect. Polyploidization is likely to be attributed to the cycling of near-triploid cells (DI = 1.26–1.74). Polyploid cells were positive for Ki67, SOX2, NANOG, and CD44. Notably, stress-induced polyploidization of tumor cells (including breast cancer cells) expressing self-renewal stem cell factors was also demonstrated *in vitro* by several groups of researchers [Salmina *et al.*, 2010; Ghisolfi L. *et al.*, 2012; Lagadec C. *et al.*, 2012] prompting us to suppose that the polyploid cells and their descendants released by de-polyploidization can possess stem cell characteristics.

Conclusions. DNA content profiling data that may not necessarily correlate with histopathologic scoring data provide the additional helpful information for interpreting therapeutic responses in NAC-treated breast cancers. Since in some cases NAC effects may be unfavorable, the use of further treatment strategy should be carefully considered. Therapy-resistant polyploid tumor cells possessing stem cell features can be induced in vivo as well, assuming that this process is not autonomous, but rather stipulated by the tumor microenvironment and intra-tumor heterogeneity.

This work was supported by European Social Fund 2013/0023/1DP/1.1.1.2.0/13/APIA/VIAA/037.

LEUKEMIC BLAST CELLS AND REVISED SCHEME OF HEMATOPOIETIC CELLS DIFFERENTIATION

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Introduction. Both classical and contemporary models of hematopoiesis are based on study of normal

stem cells and early progenitor cells (G. Mathe *et al.*, I.L. Chertkov, A.I. Vorobiov; J.E. Dick *et al.*; I.L. Weissman *et al.*). The new approach based on studying cytochemical, immunophenotypic and molecular-genetic features of leukemic blast cells seems to be advantageous for better understanding of the hematopoiesis under physiological conditions specifying and clarifying the pathways for the development of terminally differentiated blood cells of various lineages originating from hematopoietic stem cell (HSC) (A.C. Schmidt, G.K. Przybylski).

The clones of blast cells in acute myeloid leukemia represent the progenies of leukemic stem cells (LSCs). The populations of LSCs in most cases mirror normal granulocyte-macrophage progenitors (GMPs) and previously uncharacterized lymphoid-primed multipotential progenitors (LMPPs) (N. Goardon et al.).

Aim. The analysis of immunocytochemical features of leukemic cells in patients with different types of leukemia studied at the Department of Oncohematology proved to be useful to put forward several suggestions as to the possible links between progenitor cells in the scheme of hematopoietic cell differentiation.

Methods. For immunocytochemical study of leukemic blast cells (APAAP and LSAB-AP methods) we used a broad panel of monoclonal antibodies (MoAbs) proposed by WHO experts for classification of acute leukemias that include markers of hematopoietic progenitor cells (CD34, HLA-DR, CD45), B-lineage markers (CD19, CD20, CD22, CD79a), T-lineage markers (CD2, CD3, CD5, CD7), markers of myeloid lineage (CD13, CD33, CD15, MPO, CD117) and megakaryoblasts (CD41, CD61).

Results and Discussion. Our findings suggest that leukemic cells in pro-B-ALL and monoblastic leukemia (AML M5a) may derive from the common LSC analogous to the presumed progenitor for B cells and monocyte/macrophages in normal human hematopoiesis. We also demonstrated the similarities of immunophenotype between blast cells in acute erythroid leukemia (AML M6b) and megakaryoblastic leukemia (AML M7). Such similarity hints to the existence of the common bipotent progenitor cells in AML M6b and AML M7, which is analogous to precursor cell common for megakaryocytopoiesis and erythropoiesis.

The important data for understanding the hierarchy in normal human hematopoiesis were obtained in studying some types of acute leukemias of ambiguous lineage (acute undifferentiated leukemia, mixed phenotypic acute leukemias — B/myeloid and T/lymphoid). In some cases of acute lymphoblastic leukemia (ALL) from earlier B cell progenitors, co-expression of myeloid markers (CD13, CD33, CD15) is observed. However, we have never seen T cell lineage associated antigens in B-ALL leukemic blasts. Also, in ALL of T cell origin we do not find out B cell differentiation antigens. Our findings call into question the real existence of common lymphoid progenitors (CLP) in human hematopoiesis postulated elsewhere.

Conclusions. The data on the immunophenotype of leukemic blast cells in various forms of leukemia may be useful for the analysis of hematopoiesis under physiological conditions. Further studies may challenge several conventionally accepted differentiation pathways in existing schemes of human hematopoiesis.

CONTENT OF CD133$^+$ AND CD15$^+$ STEM CELLS IN GLIOMAS AND RATIO OF PERIPHERAL BLOOD CELLS OF DIFFERENT HEMATOPOIETIC LINEAGES IN PATIENTS WITH BRAIN TUMORS

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Introduction. In patients with brain tumors, changes in the content of stem cells in gliomas and cells of peripheral blood are noted (neutrophilia, lymphopenia, thrombocytosis).

Aim. To compare the ratios of peripheral blood cells: platelet to lymphocyte ratio (PLR), platelet to neutrophil ratio (PNR), neutrophil to lymphocyte ratio (NLR) with the content of stem cells in gliomas of varying degrees of anaplasia.

Methods. The hemogram indices of 105 patients with brain tumors and 28 healthy persons were analyzed. The blood indices were measured on the automatic hematological analyzer Mindray 3000 plus, the ratio of PLR, PNR and NLR was determined in the peripheral blood. The expression of receptors for CD133 and CD15 in tumor cell suspension was studied by flow cytometry on CYTOMICS Fc 500 (Beckman Coulter USA) using anti-CD133 and CD15-FITC antibodies (Millipore).

Results and Discussion. In gliomas of the I–II degree of anaplasia, the content of CD133$^+$ cells was 3.3 ± 1.1%, in anaplastic gliomas — 5.1 ± 3.2%, in glioblastomas — 8.1 ± 2.2%. CD15$^+$ cells (embryonic cell receptor) were detected in gliomas twice as often as CD133$^+$ for each degree of anaplasia. The content of stem cells CD133$^+$ CD15$^+$ in gliomas increased with an increase of the degree of anaplasia. In this case, the percentage and absolute content of lymphocytes in the blood decreased. In malignant gliomas, an increase in PLR, NLR was observed in comparison with astrocytomas. It has been established that low values of PLR and NLR are observed with a relatively higher remission in patients with glioblastomas.

Conclusions. In malignant gliomas, the content of stem cells in the tumor tissue increases, and in the peripheral blood the values of cells PLR and NLR reliably increases and PNR decreases. Hematological indices can be used as prognostic markers of clinical course of gliomas and the effectiveness of treatment.

CONTRIBUTION OF EHRLICH CARCINOMA SUBPOPULATION COMPOSITION IN MAINTAINING TUMOR GROWTH

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Introduction. Development of malignant tumors results from expansion of cancer stem cells (CSCs) possessing unlimited potential of self-maintenance. There is a need in further study of functional and structural characteristics of these cells in various types of model systems.

Aim of the work was to identify the subpopulation composition of Ehrlich carcinoma cells including those with the signs of CSCs as well as their tumorigenicity.

Materials and Methods. Ehrlich carcinoma cells were transplanted intraperitoneally into BALB/c mice. Total population of Ehrlich carcinoma cells was divided by magnetic separation method with BD IMagnet cell separation system (USA) into $CD44^+$- and $CD44^-$-fractions. The growth potential of total population of Ehrlich carcinoma cells, $CD44^+$- and $CD44^-$-cell fractions was assessed after 7 days following transplantation. The volume of ascitic fluid in the peritoneal cavity was measured and cell concentration was counted. Immune phenotypic analysis of the subpopulation composition of tumors was performed using monoclonal antibodies to CD4, CD24, CD117, Sca-1 molecules with flow cytometer "FACS Calibur" ("BD", USA).

Results. Phenotypic analysis demonstrated that Ehrlich carcinoma is a heterogeneous population of tumor cells of various differentiation degree. Identification of the Sca-1 structure in virtually all the cells of Ehrlich carcinoma allows us to consider it as a versatile marker of this type of tumor. The particular significance of the subpopulation of $CD117^+$ cells in maintaining microenvironment for tumor growth was demonstrated. Cells with $CD44^{high}$ phenotype, being the part of the $CD44^+$ $CD24^-$ population may be considered as the candidates for CSCs in this model system. This assumption was confirmed by a comparative study of tumorigenic potential of the cells from the fractions $CD44^+$ and $CD44^-$. It was shown that the $CD44^+$-fraction cells had a much higher ability to form a tumor with the predominant content of highly potent tumor-inducing $CD44^{high}$ and $CD44^+CD24^-$ cells. It was demonstrated that the cells of $CD44^+$ fractions were able to grow even at a 100-fold lower concentration (10^4 cells/mouse) used for transplantation as compared to unfractionated Ehrlich carcinoma population (10^6 cells/mouse). On the contrary, tumorigenic potential of $CD44^-$ fraction was much lower. Upon administration of 10^6 $CD44^-$ cells/mouse, ascite developed only in 50% of animals with total cell count in the peritoneal cavity being 5 times less compared to transplantation of unfractionated Ehrlich carcinoma cells.

Conclusion. The subpopulation composition of inoculated tumor cell line of Ehrlich carcinoma was studied and the significance of $CD44^+$ subpopulation in maintaining the growth of this type of tumor was established. Cells with the $CD44^{high}$ phenotype being the part of the population of $CD44^+CD24^-$ could be considered as CSCs in this model system.

THE EFFECT OF DEUTERIUM DEPLETED WATER ON THE FUNCTIONAL CHARACTERISTICS OF NORMAL AND CANCER CELLS IN CULTURE *IN VITRO*

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Introduction. Deuterium depleted water (ddw), in its physico-chemical characteristics, differs from the water of natural isotope composition (V. Goncharuk, 2011). However, the biological activity of ddw is poorly understood. The ddw protective properties are confirmed by toxicological studies, from which it follows that ddw, due to its transport properties, effectively removes toxins and metabolic products from the body (R. Robins, 2012). Different concentrations of deuterium in water can differently affect the proliferative activity of the cell culture of prokaryotes and eukaryotes *in vitro* (G. Somlyai, 2010; O. Mosin, 2014). Positive effect of ddw in studies with transplantable tumors in laboratory animals and in other *in vivo* and *in vitro* experiments (H. Wang, 2013) was noted.

Aim. To study the effect of ddw on the functional characteristics of cells *in vitro*.

Materials and Methods. After preparing growth media based on ddw and deionized water (miliQ), the final concentration of deuterium in water was D/H = 30 ± 2 ppm and 150 ± 2 ppm, respectively. Skin biopsies were dissociated by enzymatic treatment for 1 h in 0.05% collagenase IA and 0.05% pronase. The cells were cultured in DMEM:F12 supplemented with 10% FBS, 2 mM L-glutamine and 1 ng/ml FGF-2 in a multigas incubator at 5% CO_2 and 5% O_2. The following assays were done: clonogenic potential (CFU test) at 2^{nd} and 4^{th} passages and counting cell doubling time (CDT) at 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} passages. Human cancer cell lines A549 (lung carcinoma) and HT29 (colon adenocarcinoma) were used. The method of scratching was used to study the "amoeboid" movement of cancer cells within 12–24 h.

Results and Discussion. It was established that growth media based on ddw contributes to a significant push to the colony formation of human dermal fibroblasts in the early passages by 15–20% more than in water of the natural D/H ratio. The population doubling times in colonies of human dermal fibroblasts, in late passages, are shorter by 4–6 h in ddw than in the water of the natural isotope ratio D/H. This may indicate a larger reserve of culture dividing cycles in a growth medium based on ddw than in the water of the natural isotope ratio D/H. A549 and HT29 cancer cells filled the monolayer damage zone 50–70% slower with the addition of ddw than the water-based control of the natural D/H ratio. The data obtained require additional studies to determine the molecular mechanisms of deuterium action on intracellular structures.

Conclusions. The effect of the isotope ratio of D/H in water on the proliferative potential and the rate of colony-forming units of human dermal fibroblasts *in vitro* was established. It was shown that ddw contributes to a significant slowing of the "amoeboid" movement of A549 and HT29 human cancer cells. However, according to the results of our work, the role of deuterium in biological systems *in vitro* is not completely determined, which requires additional studies in the field of chemistry, physics and biology of water.

POSTNATAL EXTRA EMBRYONIC TISSUES AS A SOURCE OF MULTIPLE CELL TYPES FOR REGENERATIVE MEDICINE APPLICATIONS

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Introduction. Placenta, amnion and umbilical cord are usually discarded after childbirth whereas these

tissues may serve as a valuable source of different cell types. These postnatal extra-embryonic tissues are readily available and their potential use for cell therapy poses no ethical issues for either autologous or allogeneic application.

Aim. We aimed to isolate and characterize the cell types which could be obtained from postnatal extra-embryonic tissues.

Materials and Methods. Fresh tissues (no more than 12 h after delivery) were used for enzymatic or explants methods of cell isolation. Obtained cultures were further maintained at 5% oxygen. At P3 cell phenotype was assessed by FACS and the multilineage differentiation assay was performed.

Results and Discussion. We have isolated multiple cell types from postnatal tissues. Namely, placental mesenchymal stromal cells (Pl-MSCs) from placenta chorionic disc, chorionic membrane MSCs (ChM-MSCs) from free chorionic membrane, umbilical cord MSCs (UC-MSCs) from whole umbilical cord, human umbilical vein endothelial cells (HUVECs) from umbilical vein, amniotic epithelial cells (AECs) and amniotic MSCs (AMSCs) from amniotic membrane. All isolated cell types displayed high proliferation rate together with the typical MSCs phenotype: $CD73^+CD90^+CD105^+CD146^+CD166^+CD34^-CD45^-HLA\text{-}DR^-$. HUVECs constitutively expressed key markers CD31 and CD309. All MSCs and AECs were capable of osteogenic and adipogenic differentiation. The only exceptions were the Pl-MSCs and isolated by enzymes UC-MSCs that failed typical osteogenic differentiation. Surprisingly, UC-MSCs isolated via explants qualitatively demonstrated osteogenic differentiation. Thus obtained MSCs cultures fulfill mostly all minimal MSCs criteria. Of note, AECs, initially possessing typical epithelial morphology, progressively acquired fibroblastoid appearance at P2-P3 probably due to the EMT.

Conclusions. We have shown that a wide variety of cell types can be easily isolated from extra-embryonic tissues, banked and expanded *ex vivo* for regenerative medicine applications. These cells possess typical MSCs properties and can be considered an alternative to adult MSCs, obtained from bone marrow or fat, especially for allogeneic use.

THE EXPRESSION OF THE EPITHELIAL-MESENCHYMAL TRANSITION MARKERS IN 2D AND 3D CELL CULTURES OF MCF-7 UNDER THE INFLUENCE OF C-MEDIUM FROM MESENCHYMAL STEM CELLS

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Introduction. It is known that breast cancer (BC) consists not only of the neoplastic cells but also of the changed stroma. The stroma, which is associated with metastases, includes the proteins of extracellular matrix and the cellular components, in particular mesenchymal stem cells (MSCs), the blood vessels and the immune cells. The various studies have shown conflicting data on the MSC role in the development of BC. Such contradictions may depend on a wide range of the factors, including differences in the setting of experiments, the heterogeneity of MSC populations, the dose and the time of injection of these cells, and also on the model. The study of the influence of the humoral factors from MSCs on the development of BC is very relevant in recent years. In addition, it is important to study the change in the epithelial phenotype to the mesenchymal phenotype in the tumor cells. EMT cells actively proliferate and self-renew, which leads to an increase in the number of the heterogeneous populations. It has been shown that the most adequate model of the study of tumor population *in vitro* is a multilayer (3D) cell culture. The target effect on the expression of the molecules that regulate EMT is promising for cancer therapy, including BC.

Aim of the study was to compare the expression of the major EMT markers in the tumor cells of breast adenocarcinoma (MCF-7 cell line) under the influence of c-medium from MSCs in 2D and 3D cell cultures.

Materials and Methods. MCF-7 cell line (breast adenocarcinoma) was chosen as an experimental model *in vitro*. The monolayer cell culture was cultured in standard conditions (37 °C, 5% CO_2, humidity 95%). The initial density of culture was 2×10^4 cells/cm^2. To initiate the generation of the spheroids, the monolayer cell culture was removed off the substrate after four days of the incubation, using 0.25% Trypsin-EDTA, and placed in the nutrient medium with 5% carboxymethyl cellulose, 5×10^5 cells/ml. Then the plates were incubated on an orbital shaker at 50 rpm for 3–5 h. Human BM-MSCs were cultured with MCF-7 cells and multicellular tumor spheroids by non-contact method. Detection of the markers (EpCAM, vim, CKs) in 2D and 3D cell culture was performed using IHC method with the primary monoclonal antibodies.

Results and Discussion. Our results demonstrate the differences in the expression of the epithelial-mesenchymal transition markers in 2D and 3D breast cancer cell cultures. Thus, the percentage of epithelial markers (cytokeratines and epithelial cell adhesion molecule) in the tumor spheroids is less than in the cells of monolayer, however, the spheroids cells begin expressing a mesenchymal marker — vimentin. C-medium from hBM-MSCs reduces the volume of the spheroids by 61% compared with the control and promotes an increase in the expression of the tumor-associated markers — cytokeratins and EpCAM in 2D and 3D cell cultures, but only in 3D culture the vimentin expression increased.

ADAPTOR PROTEIN RUK/CIN85 ENHANCES THE FEATURES OF CSCS IN BREAST CANCER CELLS

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Introduction. Cancer stem cells (CSCs) are known as a population of tumor cells with high tumorigenic potential and self-renewal ability. These cells are characterized by a number of properties, such as expression of specific molecular markers, higher invasiveness and metastatic potential, drug resistance, expression of "embryonic" genes. Adaptor proteins play significant role in assembly of signaling complexes mediating various cellular processes including acquiring for the development of stem-like phenotype. In this study we investigated the effect of adaptor protein Ruk/CIN85 on the CSSs properties of breast cancer cells.

***Materials and Methods*.** As a model we used two breast cancer cell lines: weakly-invasive human MCF-7 cell line and highly-invasive mouse 4T1 cell line. In order to analyze the role of Ruk/CIN85 in the manifestation of CSCs features, we generated stable sublines with overexpression of Ruk/CIN85 and with its downregulation. Proliferation rate was estimated by MTT-assay and growth curves. Invasive ability was analyzed using Boyden chamber assay. Adhesion-independent growth ability was estimated by mammospheres formation assay. Gene expression was evaluated by RT-qPCR.

***Results*.** In 2D cell culture, the transition of both human and mouse Ruk/CIN85 overexpressing breast cancer cells from epithelial to rounded and mesenchymal phenotype was observed. It was also demonstrated that Ruk/CIN85 negatively regulates MCF-7 and 4T1 cell proliferation and adhesion while substantially enhances invasiveness of Ruk/CIN85-overexpressing cells. It turned out that in both cases cells with overexpression of Ruk/CIN85 produce more mammospheres than control ones. Analysis of stemness related genes expression demonstrated that in MCF-7 cells with Ruk/CIN85 overexpression *NANOG, ID-1, Oct4, KLF-4*, and *CCL-2* genes were up-regulated, while in 4T1 cells Ruk/CIN85 overexpression resulted in up-regulation of *NANOG, KLF-4, SNAIL1, Lcn2* and down-regulation of *Myb* genes.

***Conclusion*.** The obtained data suggest that adaptor protein Ruk/CIN85 is a key regulatory factor involved in the development of stemness features in breast cancer cells.

This study was partially supported by SC STCU and SCOPES grants.

COMPARISON OF EXTRACELLULAR MATRIX AND ADHESION MOLECULES EXPRESSION PROFILES OF DIFFERENT ORAL MESENCHYMAL CELL CULTURES

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***Introduction*.** A set of mesenchymal stem cells from oral tissues were described in the last decade. Detailed characterization and comparison of the ECM and adhesion profile of different mesenchymal stem cell cultures has not yet been performed.

Aim of this research is to isolate primary mesenchymal cell cultures from adult (DPSC) and deciduous tooth pulp (SHED), periodontal ligament (PDL), dental apical papilla (SCAP), bone marrow (BMC) and gingiva (GMSC) and to characterize them for expression of specific extracellular matrix and adhesion molecules using RT Real time PCR.

***Materials and Methods*.** DPSC, SHED, PDL, SCAP, BMC, GMSC were isolated from unadulterated dental, alveolar bone and gingival explants from patients undergoing routine surgical operations in Oral and Maxillofacial Surgery Clinic of Dental Faculty, Medical University — Sofia, Bulgaria, after acquiring informed consent. Explants were digested in 3 mg/ml Collagenase I/ Dispase and seeded in culture flasks with DMEM complemented with 20% FBS and antibiotics. Cells were passaged after reaching 70% confluence. Cells up to the 3rd passage were used for the experiments. Total RNA was extracted with RNeasy Plus Mini kit, cDNA was synthesized with RT2 First Strand Kit and analyzed with RT2 Profiler PRC Array, Human Extracellular Matrix & Adhesion Molecules Array (all QIAGEN, Germantown, MD, USA) following manufacturer's instructions.

***Results and Discussion*.** Analysis of adhesion and ECM markers showed significant differences between different oral mesenchymal stem cell cultures. Comparison of collagen isotypes expression showed also significant differences in fibrillar collagen expression and particularly Collagen I, with highest expression in BMC and lowest in SCAP.

Analysis of integrins showed similarity for the most abandoned β subunit is ITGB1 followed by ITGB5. ITGB3 on other hand were mostly expressed by dental mesenchymal stem cells.

Significant differences in expression of integrin α subunits were discovered. ITGA8 was the most abundant except in PDL and BMC where ITGAV and ITGA5 prevailed respectively. Stem cell related ITGA1 and ITGA6 were expressed in DPSC, RDL and SCAP and ITGA6 expression was much lower than ITGA1 in other three sell types.

Comparison of matrix-metalloproteinases (MMP) showed the highest amounts of MMP2 and MMP14 in all cells with significant difference in other minor expressed types.

Cluster analysis of total examined gene expression revealed similar expression profiles of SCAP and PDL cells, differing significantly from BMC and GMSC, whereas DPSC and SHED showed bigger similarities with BMC and GMSC respectfully.

All these results suppose the differences in potential for wound healing, cell migration, modulation of the immune response and participation in carcinogenesis of the mesenchymal stem cell subpopulations.

***Conclusions*.** Oral tissues contain a set of different mesenchymal stem cells, having different adhesion and ECM profile, thus possessing different stem cell properties. Further analysis and comparison between mesenchymal stem cell population gene expression is needed for better understanding of stem cell potential of these adult stem cells. This understanding would refine the knowledge about adult stem cells and their role in healthy and pathologic conditions and may lead to development of regenerative and cell therapy methods.

CYTOCHEMICAL AND IMMUNOPHENOTYPICAL FEATURES OF CELLS IN BLAST CRISIS OF CHRONIC MYELOID LEUKEMIA

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***Introduction*.** Multistage process of hematopoiesis is sustained by hematopoietic stem cells (HSCs) and progenitor cells that are generated in embryonic development. Being at the base of the whole hematopoietic hierarchy, HSCs finally give rise to all blood lineages in the adult organism. The study of the upper hierarchy of hematopoietic system is in fact the topical problem. The molecular biology techniques, genetic engineering, and flow cytometry are widely used nowadays with the aim of elucidating the origin of specified hematopoietic lineages. The study of morphocytochemical and immunocytochemical features of leukemic blast cells of various origin and differentiation levels originating by transformation of the correspond-

ing progenitor cells may be also useful as one of the approaches to clarify some challenging aspects relating to normal hematopoiesis.

Aim. To define linear origin and differentiation level of leukemic cells in various forms of blast crisis of chronic myeloid leukemia (CML BC).

Methods. Cytomorphological, immunohistochemical, cytochemical, statistical.

Results and Discussion. When CML BC of monocytic type was compared to acute monoblastic leukemia originating *de novo*, no differences could be observed in cytomorpholgy and cytochemical features of leukemic cells. Nevertheless, immunophenotyping revealed expression of CD19, CD36, and CD61 in CML BC contrary to *de novo* acute monoblastic leukemia (AML M5). Furthermore, the expression of CD7 and CD56 in CML BC was much more intensive as compared to *de novo* AML M5.

Despite the common morphological features of blasts in megakaryocytic CML BC and *de novo* acute megakarioblastic leukemia (AML M7), some differences were evident upon their cytochemical study. Namely, in CML BC activity of acid phosphatase was manifested as the granules against the background of the diffusely stained cytoplasm whereas in *de novo* AML M7 cytochemical features of blast cells varied. In cases of less differentiated cells in AML M7, the cytochemical reactivity of blast cells was rather inert (except for acid phosphatase) similar to that in blasts of CML BC. In more mature blasts in AML M7, some other enzymes were detected by the cytochemical techniques (except for myeloperoxidase). Analysis of immunophenotype revealed several stages of blast differentiation in *de novo* AML M7. On the contrary, phenotype of blast cells in CML BC was monomorphic in all patients. Moreover, contrary to CML BC, blast cells in AML M7 expressed CD7 and CD36. When cytomorphological, cytochemical and immunophenotypical features were compared in cells of *de novo* acute myeloid leukemia with minimal signs of differentiation and CML BC with minimal signs of differentiation, no distinctions were evident.

Conclusion. The origin of different types of BC CML indicates to the secondary leukemic transformation that occurs at the level of multipotent progenitor cells, whereas in *de novo* acute myeloid leukemia transformed leukemic cells seem to originate from oligolineage hematopoietic progenitor cells.

ETHICAL ISSUES OF RESEARCH, BANKING AND CLINICAL APPLICATIONS OF HUMAN CORD BLOOD HEMATOPOIETIC STEM CELLS

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Introduction. Umbilical cord blood hematopoietic stem cells (UCB-HSCs) are in the category of the most studied adult multipotent stem cells (M.S. Santos, P. Ventura-Junca, 2012). As a component of the already formed human body, which is in the neonatal period of its own development, UCB-HSCs have the property of replicating themselves, maintain their differentiation potential and, consequently, are capable of hematopoietic tissue regeneration (D.L. DiGiusto, 1996; Q.L. Hao *et al.*, 2001). In general, the study and use of adult stem cells does not raise such serious ethical issues related to the source of extraction, which are painful for human embryonic stem cells. At the same time, a number of common problems associated with all stem cells are actual for the UCB-HSCs, in particular, issues related to clinical research in this field. There are also the specific nature issues that are inherent in this type of human stem cells.

Aim. To discuss specific and common ethical aspects of research, banking and clinical applications of UCB-HSCs.

Methods of research. Systemic causal-dialectical analysis of practical research experience, world-wide scientific literature data, international legal standards and recommendations.

Results and Discussion. Cord blood as an effective alternative source of hematopoietic tissue is recognized due to a number of important advantages over other unrelated allogeneic hematopoietic stem cell transplants (E. Gluckman, 2009; A.R. Smith, J.E. Wagner, 2009). These benefits include: lack of donor risks, availability of frozen samples, reduced incidence of graft *versus* host and relapse with similar overall survival (J. Munoz *et al.*, 2014). Current research on the development of methods for improving engraftment, restoring the recipient immune system is accompanied by ethical issues that are common to transplantology and regenerative medicine: the inadmissibility of commercialization of the human body, the balance between the desire for clinical progress and scientific caution (C. Petrini, 2012; N. King, J. Perrin, 2014). The functioning of the two main options of umbilical cord blood banks (public and private) raises specific ethical and legal issues of control and equitable access to medical care (N.M. Fisk *et al.*, 2005; N.M. Fisk, R. Atun, 2008; C. Petrini, 2010; E. Gluckman, 2012; C. Parco *et al.*, 2013; G.M. Guilcher *et al.*, 2015). Provided the absolute benefits of altruistic cord blood donation in public banks for the sake of public interest over autologous private storage, there are problems for priority resolution, such as the need for clear regulation of property rights, as well as the wording of the rules for granting consent to the definition of the concept of a donor, consideration of interests of the newborn and his family, confidential safekeeping of medical and social information data, development of information forms for the donor family, which are connected with the prospects of material use (E.A. Meyer *et al.*, 2005; A. Mohr *et al.*, 2012; C. Patrini, 2013; R. Isasi *et al.*, 2013; C.L. Stewart *et al.*, 2013).

Conclusion. Considering the prospect of the UCB-HSC scope expanding, the designated complex of ethical issues requires regular monitoring and attention.

EFFECT OF CRYOPRESERVATION FACTORS ON BIOLOGICAL PROPERTIES OF UMBILICAL CORD BLOOD STEM CELL TRANSPLANT

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Introduction. Umbilical cord blood (UCB) is widely used as a source of stem cells for hematopoietic reconstitution (J.E. Wagner, E. Gluckman, 2010; J.N. Barcer, J. Kurtzberg, K. Ballen *et al*., 2017). The limit of cell content and the forced use of cryopreservation technologies cause profound study of opportunities for saving and expanding the tissue transplantation potential (P.R. Motta *et al.*, 2014; G. Chen *et al.*, 2015; J. Kieman, P. Damien *et al.*, 2016). It is known that the cryoprotection effectiveness consists in controlling the ice

formation, the dehydration development and the solution effect with the help of cryoprotective substances, directly proportional to their concentration (H.T. Meryman, 1971; G.M. Fahy et al, 1984; M.J. Taylor *et al.*, 2004; P. Mazur, 1977, 1984, 2004; J.O. Karlsson, M. Toner, 1996; M.S. Berrada, G.S. Bischof, 2001; L.E. Ehrlich *et al.*, 2015).

The effectiveness of umbilical cord hematopoietic tissue cryopreservation under the protection of dimethyl sulfoxide (DMSO) has been proved by numerous studies (R. Syme *et al.*, 2003; D. Bauwens *et al.*, 2005; A.S. Chen-Plotkin *et al.*, 2007; A.M. Junior *et al.*, 2007; A. Abdelkefi *et al.*, 2009; A. Michael *et al.*, 2012). Therefore, maintaining a balance between preservation of biological structures and reduction of the potential threat from using of cryoprotective agents is considered one of the biggest problems in the cryopreservation of hematopoietic stem cell transplants (HSCT).

***Aim*.** To investigate the changes in the biological properties of the UCB HSCT during cryopreservation in order to determine the ways of their correction.

***Materials and Methods*.** The isolated mononuclear fraction was frozen under protection of 5 and 10% DMSO. The evaluation of the cell state was carried out by morphological, functional, biochemical (determination of the activity of lipid peroxidation processes (LPP) of neutral lipids and phospholipids) methods. The study of apoptotic stages was carried out by double annexin V (Ann V) and propidium iodide (Pi) staining.

***Results and Discussion*.** After exposure with DMSO (5% concentration) Ann V^+/Pi^+ — cell content and cells with damaged membranes increased to 2.2 ± 0.3% ($p < 0.001$) and to 1.1 ± 0.5% ($p < 0.02$), respectively. The exposition with higher concentrations of DMSO — 10% caused the increasing early stages of apoptosis to 16.1 ± 1.8% ($p < 0.05$). Provided that the cryoprotective concentration was decreased, there was a reduction in the granulocyte-macrophage progenitor cell (CFU-GM) number ($p < 0.001$) and a decrease of cell viability (from 92.9 ± 0.8% to 85.9 ± 1.0%; $p < 0.01$) in defrosted samples. The correlation of the LPP activity with the apoptosis indices and the functional activity of the CFU-GM number was identified.

***Conclusion*.** The enhanced activation of the lipid peroxidation in the UCB HSCT both indirectly through the induction of apoptosis and as a result of the toxic effects of the peroxidation products on the cell membranes leads to a reduced quality of transplant material. The need for correction of these changes should be considered at the development of long-term storage technologies.

ARSENIC-INDUCED LIVER CARCINOGENESIS: IS THERE A LINK TO IRON METABOLISM DEREGULATION IN PROGENITOR CELLS?

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***Introduction*.** Inorganic arsenic is a common environmental contaminant and known human carcinogen. Liver is the main organ for iron storage and metabolism and an important target for arsenic carcinogenesis. However, the impact of arsenic on hepatic iron homeostasis is not clear.

Aim of this study was to investigate the effect of inorganic arsenic on iron metabolism in human liver HepaRG cells.

***Materials and Methods*.** Gene expression in the human HepaRG cell line was analyzed by whole genome microarray and quantitative reverse transcription-PCR techniques. Protein level of transferrin (TF) was determined by Western blot analysis, and iron content was measured by inductively coupled plasma mass spectrometry.

***Results and Discussion*.** The results showed that treatment of human liver HepaRG cells with 1 μM of inorganic arsenic (as $NaAsO_2$) for 14 days increased the expression of iron metabolism-related genes, *TFRC*, *FTL, FTH1* and *SLC40A1*, while the level of *TF* mRNA and TF protein was decreased. Additionally, exposure of HepaRG cells to arsenic resulted in an increase of cellular iron content. These changes were accompanied by a decrease in a number of mature functional hepatocytes and expansion of liver cancer stem cells, evidenced by the accumulation of progenitor like cells (e.g., primitive biliary cells).

***Conclusion*.** Our results demonstrate that chronic exposure of human liver HepaRG cells to a low dose of inorganic arsenic resulted in substantial iron metabolism alterations, loss of functional hepatocytes, and activation of liver cancer stem-like cells. These findings indicate that arsenic-induced iron metabolism dysregulation may be one of the underlying mechanisms of arsenic-related liver carcinogenesis.

THE RESULTS OF 5-YEAR OVERALL SURVIVAL OF STANDARD AND HIGH-RISK NEUROBLASTOMA PATIENTS TREATED WITH MYELOABLATIVE THERAPY AND PERIPHERAL BLOOD STEM CELL RESCUE (ONE CENTRE EXPERIENCE)

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***Introduction*.** Intensification of consolidation therapy with autologous stem-cell rescue after myeloablative doses of chemotherapy could contribute to the improved survival of children with high-risk neuroblastoma. However, long-term cure rates remain low, presumably because of the emergence of resistant clones or persistence of minimal residual disease.

***Aim*.** To investigate the 5-year overall survival of patients with high-risk neuroblastoma treated with myeloablative therapy and peripheral-blood stem-cell rescue (PBSCR) in the National Cancer Institute of Ukraine.

***Methods*.** In 2007–2016, 143 patients with neuroblastoma (74 standard-risk (SR) patients and 69 high-risk (HR) patients) received treatment in the Scientific and Research Department of Pediatric Oncology at the National Cancer Institute. The risk group of patients was determined on the basis of the most common prognostic factors including the child's age, stage, genetic markers (*N-MYC* oncogene amplification, DNA diploidy). All enrolled patients received treatment according to NB-2004 and HR-NBL-1/ESIOP protocols. During the late stage of treatment, 54 high-risk patients were cured with high-dose chemotherapy (HDHT) with autologous stem cell support; of these, 13 patients were cured with tandem HDHT.

***Results*.** The 5-year overall survival (OS) was 67% for SR patients and 30.4% for HR patients. Depending on the child's age: the OS was 58.8% for patients under 1 year of age, while the OS was 19.2% for patients aged 1 year or over. We also analyzed the survival rate of high-risk patients based on whether the patient has *N-MYC* gene amplification. The OS was 49.8% for *N-MYC* negative subjects and 24.3% for *N-MYC* positive subjects. Currently, the OS is 69.2% for high-risk patients who were treated with tandem HDHT with autologous stem cell support.

***Conclusion*.** The obtained results show that the survival in high-risk patients aged 1 year or over positive for *N-myc* oncogene was worse greatly than in patients with normal *N-MYC* status. The results of treatment of HR patients who received tandem HDHT with autologous steam cell support are encouraging.

HUMAN MESENCHYMAL STEM CELLS FOR BONE TISSUE ENGINEERING: MODERN GENE DELIVERY TOOLS IN OPTIMIZATION OF OSTEOGENESIS

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***Introduction*.** Human skeleton is completely remodeled by mesenchymal stem cells (MSC) within roughly four-and-half years. Bone morphogenetic proteins (BMP) are main inducers of bone formation and maintenance apart of their crucial roles in morphogenesis of other tissues and organs. Optimal activity of BMPs in bone usually requires coordinate signals with the Wnt pathway. Consequently, several bone diseases occur when BMP or Wnt pathways are misregulated. Therefore, proper optimization of the osteogenesis requires efficient gene delivery tools on the way of modulation of osteoblast differentiation of MSC. Unfortunately, classical transfection with multiple transfection reagents triggers massive cell death in transduced MSC. On the other hand, viral tools for gene delivery allow to get 100% of efficiently transduced stem cells without any detection of toxicity.

***Materials and Methods*.** Human MSC cell were isolated from bone marrow aspirates obtained under patients' consent from surgical material upon hip joint arthroplasty or from fat using a liposuction. Adv5-based vectors were used for efficient overexpression of recombinant genes into the mesenchymal precursor cells and lentiviral vectors were used for small hairpin RNA (shRNA)-mediated knockdown of gene expression.

***Results and Discussion*.** The set of adenoviral and lentiviral vectors generated at our lab allows to overexpress or correspondingly downregulate expression of different genes important in regulation of osteogenesis. Usage of the gene delivery tools generated at our lab allowed us to describe a molecular mechanism that mediates osteogenesis regulation by secreted phosphoprotein 2.

***Conclusions*.** Modern viral gene delivery tools open a wide window for biomedical studies aiming to investigate the molecular mechanisms of human MSC differentiation and to optimize the efficacy of regenerative skeletal tissues engineering based on MSC incorporation into the grafts.

EPITHELIAL-MESENCHYMAL TRANSITION IN HUMAN STEM CELL LINE 4BL

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Introduction. We obtained a new human stem cell line from peripheral blood cells of adult healthy donor. It successfully overcame the Hayflick limit and is stable (immortalized) cell line. During morphological analyses of stained cells at different passages we observed two types of cells: epithelioid and fibroblast-like.

***Aim*.** Presence of two morphological cell classes is an interesting phenomenon, thus the aim of research was to study epithelial-mesenchymal transition (EMT) in 4BL cell line.

***Materials and Methods*.** Cells were cultivated in DMEM with 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin. For morphological studies cells were stained with 1% neutral red. The immunophenotype was analyzed by flow cytometry on BD FACS Aria (BD, USA). Soft agar test was performed in 0.3% agar on Petri dish. For array CGH analyses DNA from the cells was extracted with QIAamp DNA Blood Mini Kit and purified with QIAquick PCR Purification Kit (Qiagen, UK). 1 µg of cell DNA was hybridized on CytoSure Aneuploidy array slide followed by the analysis by the Innopsys Innoscan 710 device with OGT CytoSure Interpret Software 3.3.2 due to the recommendations of the Oxford Gene Technology.

***Results and Discussion*.** Maintenance of two morphological cell types: epithelioid and fibroblast-like, first of all is concerned with stem cell plasticity and EMT. Stem potential of 4BL cell line was revealed in the previous studies by demonstrating adipogenic, osteogenic and myogenic differentiation using special induction mediums.

Presence of different cell types in cell population can provide advantages of survival in changing culture conditions. Thus, in soft agar test 4BL cells grew well, proliferated without attachment to the substrate and formed colonies. It is worth mentioning that ability to grow in semi-liquid medium is characteristic both of mesenchymal stem cells and cancer cells. We detected the expression of MSC markers: $CD73^+$ and $CD105^+$ with flow cytometry. Usually EMT is associated with acquisition of malignant state, but we have not revealed the expression of Oct4, the marker of embryonic stem cell, which is re-expressed in cancer stem cells. By array CGH data we have detected partial deletion of 12p11.1pter, where *Nanog* gene is located, which is also secondarily activated in cancer cells.

Due to array CGH we have revealed the duplications of chromosomes with genes, which take part in differentiation in bone tissue — *FRZB* (2 q31.1–q33.1) and *FGFR3* (4p16.1-pter); in myogenic tissue — *M-cadherin* (16q22.1qter) and *NODAL, MYOZ1 myozenin 1* (10q22.1q22.2); stem cell potential maintenance — *MATK, KLF1, GDF15, BST2* bone marrow stromal cell antigen 2 (19 p13.3–p12), and also *PARD6A*, which plays role in asymmetric cell division and EMT (16q22.1qter).

***Conclusions*.** Population of the stem cell line 4BL maintains two morphological cell types: epithelioid and fibroblast-like, which is concerned with stem cell plasticity. It is verified by cell differentiation into three types of lineages and by easy adaptation of cells to microenvironment change such as growth in soft agar.

AGE-RELATED CHANGES OF BIOLOGICAL PROPERTIES IN THE MULTIPOTENT MESENCHYMAL STROMAL BONE MARROW CELLS, FUNCTIONING OF THYMUS AND PINEAL GLAND IN MICE OF DIFFERENT STRAINS

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***Introduction*.** The bone marrow multipotent mesenchymal stromal cells (BM-MSCs) can differentiate into various

directions, produce trophic and immunomodulatory effects, influence the differentiation of hematopoietic stem cells. With age, the BM-MSCs properties undergo changes, while the occurrence of malignancies rises (Gross, 2007; Anisimov *et al.*, 2015). Thymic hormone thymuline influences the proliferative capacity of BM-MSCs (Labunets *et al.*, 2012). The thymus function is under influence of pineal gland and its main hormone, melatonin, possesses geroprotective and antitumor properties. In aging, bone marrow-immune-endocrine interrelations may be linked with genotypes.

***Aim*.** Comparative analysis of the biological properties of BM-MSCs, functions of the thymus and pineal gland in FVB/N and CBA/Ca mice, varying significantly in their life duration, frequency of malignancies (leukemia/lymphomas) and general adaptability.

***Materials and Methods*.** The studies were conducted on adult (4–6 months) and old (20–22 months) mice. FVB/N versus CBA/Ca mice are characterized by both, high frequency of spontaneous bone marrow chromosomal aberrations and leukemia/lymphomas in aging. These mice are sensitive to the B strain of Friend leukemia virus and reveal low immune response. In our study, the number of bone marrow colony-forming unit fibroblasts (CFU-Fs) was measured by the bone marrow monolayer culture technique. The number of granulocyte/macrophage colony-forming cells (GM-CFCs) in the semi-agar cultures was assessed. The phenotyping of BM-MSCs was performed using the monoclonal antibodies to mouse membrane antigens labeled with fluorochromes. To determine the immunomodulatory properties of BM-MSCs, we performed a joint cultivation of MSCs from mice of different age with syngeneic splenocytes of young mice stimulated by T-cell mitogen phytohemagglutinin. The melatonin and thymuline levels were determined in the serum.

***Results and Discussion*.** The stromal progenitor cells significantly increase in their number only in bone marrow of old FVB/N mice, indicating age-associated elevated ability of the BM-MSCs to proliferation. The number of GM-CFCs also increased in old FVB mice. On the contrary, changes of indices in the old CBA/Ca mice are slight. The immunosuppressive effect of BM-MSCs from adult mice on lymphocyte proliferation was higher than in old mice. The blood thymuline level falls significantly in old CBA/Ca mice and remains unchanged in FVB mice. The melatonin level decreases more intensely in old FVB/N versus CBA/Ca mice.

***Conclusions*.** The genetic differences in biological parameters of BM-MSCs may be linked with particularities of interrelations between pineal gland and thymus functions in aging mice (a), may contribute to the development of certain type spontaneous tumors in old organism (b).

STEM CANCER CELLS. A NEW CHALLENGE IN STUDYING ONCOGENESIS

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The last 20 years due to the great success in the study of the biology of human stem cells, a new direction in oncology has been defined — the study of the nature of stem tumor cells. The theory of stem cells is not recognized by all, although there are indisputable facts proving their existence, in particular, with glioblastomas.

Thanks to the discovery of cancer stem cells (CSC), not only the theory of carcinogenesis has changed, but also the concepts of infiltrated growth, the resistance to adjuvant methods of treatment acquired new outlines. Such properties of CSC as angiogenic potential, heterogeneity, differentiated mimicry, migratory and tumor-inducing abilities have been proved experimentally. CSC are ten times more potent in experimental induction of tumors in mice than non-CSC tumor cells. It has been established that CSC of different types of human tumors have certain phenotypic markers, which allows using them for diagnosis and prognosis.

At the same time, there are many challenging questions related to CSC. There are no clear phenotypic markers for tumor and non-tumor stem cells. Theoretical assumptions about the possibility of anti-CSC therapy of carcinogenesis have not yet been confirmed. Antibodies against marker CSC molecules (anti $CD133^+$) can both stimulate and suppress tumor growth in the experiment. Induction of tumor growth in mouse experiments with tumor cells without phenotypic signs of CSC ($CD133^-$ cells) casts doubt on the assumption that CSCs are the only or basic cells capable of inducing tumor growth.

Therefore, today the questions about the role of cancer-inducing cells in oncogenesis as well as the possibility of controlling carcinogenesis with the help of CSC remain unresolved.

CONTENT OF CANCER STEM CELLS IN GLIAL BRAIN TUMORS

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***Introduction*.** In recent years special attention has been paid to the study of the role of cancer stem cells (CSCs) in the pathogenesis of malignant growth. CSCs are responsible for migratory ability, radio- and chemoresistance, for induction of new tumors in the experiment, which is important for determining the characteristics of tumors and predicting the outcome of the disease. The available data on the CSC content in brain tumors are contradictory.

***Aim*.** To study the content of CSCs in brain tumors of various histology.

***Materials and Methods*.** 134 samples of biopsy material of brain tumors were examined with the aid of immunohistochemical, immunofluorescence and flow cytofluorometry methods. The monoclonal antibodies to CD133 (Millipore, USA) CD15 and CD34 (Becton Diskinson, USA) were used.

***Results and Discussion*.** $CD133^+$ CSCs have been detected in various brain tumors. Their highest content was found in glioblastomas and medulloblastomas. After repeated surgery, the CSC content in the tumor tissue increased. In addition to $CD133^+$ cells, a small number of $CD34^+$ and $CD15^+$ cells was also detected. It was found that an increase in the content of $CD133^+$ cells in tumor tissue is accompanied by a decrease in lymphocyte count in the peripheral blood. Even the CSC content in tumors of the same histological type (glioblastoma or medulloblastoma) varied significantly.

***Conclusion*.** Determination of CSCs in tumor tissue can serve as an additional indicator for choosing methods of combined treatment and predicting the duration of remission.

EXPRESSION OF CD133 BY GLIOMA C6 CELLS UNDER THE INFLUENCE OF RAT FETAL BRAIN NEUROGENIC CELLS CONDITIONED MEDIUM

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Introduction. Current state of development of neurooncology is focused on searching the criteria of treatment personalization on the basis of identifying a unique set of molecular changes in tumor cells. The neuroepithelial stem cell markers are considered among the targets for brain glial tumors. CD133 (prominin-1) molecule may be considered as one of the most characteristic biomarkers of brain tumor stem cells (BTSC). Cellular and molecular similarities between BTSC and normal neurogenic stem/progenitor cells (NSC/NPC) substantiates the search for new methods of treatment of malignant glioma using NSC/NPC (K.S. Aboody *et al.*, 2013; R.A. Morshed *et al.*, 2015; J. Portnow *et al.*, 2016). In particular, immunobiological properties of NSC/NPC may be realized via the expression and production of immunoregulatory cytokines and growth factors.

Aim. To study the influence of rat fetal brain neurogenic cells conditioned medium (NCCM) on the content of $CD133^+$ cells in rat glioma C6 and normal rat fetal brain cell cultures.

Materials and Methods. NCCM was obtained from suspensions of neurogenic rat brain cells on the 14^{th} (E14) day of gestation. The cells of rat brain glioma (cell line C6, n = 6) and cells of rat fetal brain (E14, n = 6) served as the material for culturing. The study was performed in control cultures (standard cultivation conditions without NCCM adding) and research cultures (with adding NCCM (0.10 mg/ml) for 48 h). Immunocytochemical staining for CD133 molecule was performed using murine monoclonal antibodies to CD133 (Millipore, USA). In cytological specimens the changes in cellular composition and the number of immunopositive cells were determined and morphometric studies were performed.

Results and Discussion. According to the immunocytochemical staining, BTSC or $CD133^+$ cells accounted for 7.2–16.8% of the total number of cells in C6 glioma cultures and 25.0–49.7% in fetal rat brain (E14) cultures. $CD133^+$ cells were smaller in size than $CD13^-$ cells (average values of cell sectional area, nucleus cell-sectional area) and had greater nuclear-cytoplasmic ratio. $CD133^+$ cells and their nuclei in cell cultures of fetal rat brain were twice larger in size than such cells in cultures of C6 glioma.

NCCM (0.10 mg/ml for 48 h) reduced 4-fold the number of $CD133^+$ cells in C6 rat glioma cell cultures ($p = 0.02$ compared to control, U-Mann — Whitney test). Cell cultures of fetal rat brain did not reveal such effect.

The functional role of CD133 in NSC/NPC remains poorly understood. According to J.M. Angelastro and M.W. Lame (2010), exogenous expression of CD133 caused a 2–4-fold decrease in apoptosis of tumor cells in response to therapeutic agents (doxorubicin, kaptotecin) and $CD133^+$ glioma C6 cells had increased by 62% expression of one of ABC-transporters (P-glycoprotein), leading to drug resistance, which support the hypothesis of antiapoptotic CD133 functional role in protecting tumor cells, particularly from chemotherapeutic agents. Our results demonstrate that NCCM reduces the number of CD133-expressing BTSC cells in cultured glioma C6 cells but not in cultures of fetal rat brain. Moreover, the size of $CD133^+$ cells in cultures of glioma C6 and fetal brain differ in size that suggests that BTSC of glioma C6 may be qualitatively different from NSC/NPC (probably express ligands for the active molecular agents of NCCM).

Conclusions. Since CD133 is regarded as critical therapeutic target for therapy of brain tumors, the results of reducing the number of CD133-expressing cells (BTSC) in C6 glioma cell culture under influence of the NCCM can be the basis for a comprehensive study of preparations derived from neurogenic fetal cells for the purpose of theoretical justification of their application in pathogenetic treatment of patients with brain gliomas.

NEW TUMOR STROMAL CELL LINE WITH CHARACTERISTICS OF STEM CELLS TO ASSESS THE IMPACT OF MICROENVIRONMENT COMPONENTS ON TUMOR CELLS *EX VIVO*

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Introduction. The basis of the formation of metastatic phenotype of tumor cells is a phenomenon of epithelial-mesenchymal transition (EMT). The key regulator of this process in tumor cells is their microenvironment and, in particular, the cellular components of the tumor stroma. Mesenchymal stem cells as key elements of the stroma are important regulators of tumor progression and its sensitivity to antitumor factors.

Aim. To obtain and characterize new stromal cell line for studying the interaction between malignantly transformed cells and their microenvironment *ex vivo* and assessing new approaches to anti-stromal therapy.

Materials and Methods. Cell culture methods, immunocytochemical and statistical analysis.

Results. SEBR line of stromal cells from the spontaneous breast tumor adenocarcinoma of the rat was established and characterized by morphology, growth parameters, immunophenotypical profile and cytogenetic features. SEBR cells are characterized by fibroblast-like morphology, a nuclear-cytoplasmic ratio is similar to normal cells, morphology and number of chromosomes was as in normal rat cells (2n = 42). The cells are characterized by the dominance of mesenchymal cell antigens: high expression of N-cadherin (262 ± 33), Vimentin (280 ± 11), and a low number of cells with pan-cytokeratin expression. A high level of the expression of stem cell marker CD44 (> 200 points by H-Score system) was found. The SEBR cells were used in the co-cultivation system with malignant transformed rat mammary cells (MRS, MRS-T5 cell lines) with different morphological characteristics and the initial status of EMT associated proteins. The aims of investigation were to assess the impact of stromal cellular elements on the proliferative and immunophenotypical properties of tumor cells. In parallel, the effect of normal embryo fibroblasts on tumor cells and additional exogenous factors with antitumor activity (chemotherapy drugs, cytokines) were studied. The conditioned culture medium (CM) was collected and assayed at different phases of cell growth. It has been shown that CM from stromal SEBR cells significantly stimulates the proliferation of MRS-T5 cells (tumorigenic mesenchymal phenotype) and does not stimulate MRS cells (nontumorigenic epithelial phenotype). CM from normal

fibroblasts does not activate the cellular metabolism of MRS and MRS-T5 cells. Moreover, under specific conditions (depending on cell concentration and CM content), a significant inhibition of cellular metabolism and proliferation (up to 50%) is evident. CM from tumor cells was shown to inhibit SEBR cell growth and metabolism (20 to 60%) and stimulate normal fibroblasts (from 10 to 80%). In the study of sensitivity of stromal and tumor cells to drugs, it was found that cells are characterized by similar sensitivity to zoledronic acid (IC50 1–2 μg/ml), cisplatin (IC50 5–10 μg/ml) and paclitaxel (IC50 0.1–0.2 μg/ml). At the same time, the response of these cells to the TNF and mitomycin C was different, in particular, the tumor cells were more sensitive to mitomycin C and less sensitive to TNF (at doses lower than 2000 IU/ml, the stimulation of cellular proliferation was observed) as compared to SEBR.

Conclusion. A new cell line SEBR of stromal origin with characteristics of stem cells was established and characterized. A significant difference between the responses of tumor cells with various EMT status on cocultivation with SEBR cells was shown.

EFFECT OF PDGF-BB ON PROLIFERATION, MATRIX SYNTHESIS AND MINERALIZATION OF HUMAN PDL STEM CELLS

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Introduction. PDGF is one of the main active morphogenes in the platelet concentrates which have been increasingly used to stimulate tissue healing at the injury site. Although in recent years many clinicians apply autologous platelet derived products to enhance healing process and regeneration of PDL, the effect of PDGF on stem cell markers expression, enzymatic activity, ECM synthesis and mineralization in PDL has not been studied in detail.

Aims. The purpose of the present study is to determine the presence of cells in human PDL capable of expressing stem cell and differentiation markers and to determine the effect of PDGF-BB on cell proliferation, marker expression, collagen synthesis, enzymatic activity and mineralization.

Materials and Methods. PDL cells were isolated and characterized using stem cell and differentiation markers via immunofluorescence and flow cytometry and then cultured *in vitro* and treated with different concentrations of PDGF-BB. The effect of PDGF-BB on cell proliferation, stem cell and differentiation markers expression, soluble collagen production, lysyl oxidase (LOX) activity, alkaline phosphatase (ALP) activity and calcium nodules formation was assessed.

Results and Discussion. PDGF-BB stimulated the proliferation of cells with the maximum effect at 50 ng/ml. The growth factor increased the expression of stem cell markers and SPARC; Col1a2 expression was decreased, whereas the expression of Col3a1 and BSPII remained unchanged. Soluble collagen production, ALP activity and calcium nodules formation were also significantly decreased by PDGF-BB; LOX activity was significantly increased. Our results show that PDGF-BB initiates self-renewal through maintenance of cell proliferation and stem cell properties on the one hand, and inhibition of abnormal mineralization in human PDL on the other.

Conclusions. PDGF-BB is a powerful promoter of cell proliferation that increases the expression of stem cell markers; inhibits collagen production and mineralization but accelerates the maturation of collagen chains through increased LOX activity and SPARC expression.

INTERPLAY BETWEEN MITOCHONDRIAL RIBOSOMAL PROTEIN S18-2 AND RETINOBLASTOMA PROTEIN IN REGULATION OF CELL STEMNESS AND DIFFERENTIATION

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The retinoblastoma (RB) pathway is inactivated in several different human cancer forms, including RB, osteosarcoma, melanoma, and neuroblastoma. These tumors are derived from neural crest from cells that fail to undergo terminal differentiation.

We have found that mitochondrial ribosomal protein S18-2 can bind RB. Thus, we discovered a novel mechanism of cell fate regulation, which depends on overexpression of S18-2 (MRPS18-2).

Noteworthy, overexpression of the human S18-2 immortalized primary rat embryonic fibroblasts (REFs) that showed properties of embryonic stem cells. Moreover, S18-2 is expressed at the high levels in endometrial, breast and prostate cancer tissues, as we shown by immunohistochemistry and q-PCR.

Moreover, analysis of the available data bases revealed the elevated expression of S18-2 at the mRNA levels in stem cells and many cancer types. This raises the question of whether this protein co-operates with the RB protein in differentiation and carcinogenesis. We also hypothesized that simultaneous expression of RB and S18-2 proteins at the high levels might support stemness.

Here we discuss the mechanisms of how S18-2 protein, together with RB, is involved in the maintenance of cell stemness.

BONE MARROW MESENCHYMAL STEM CELLS AND MORPHOFUNCTIONAL STATUS OF THE TRANSPLANT

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Introduction. In the transplantation immunology, T- and B-lymphocytes play an important role in maintaining morphoregulatory processes and implementing an immunological surveillance to maintain cellular homeostasis. The posttransplant period is affected by the immune system imbalance, in particular, of T-cells (CD3), T-helper cells (CD4), cytotoxic T cells (CD8), NK cells, plasma cells, regulatory lymphocytes ($CD4^+CD25^+FOXP3$) and cytokines produced by these cells.

Aim. The aim of the study was to investigate the morphofunctional status of the transplant in the post-transplant period and transplantation of the bone marrow derived mesenchymal stem cells (BM-MSC).

Materials and Methods. In the study, two groups of mice were involved: a control group (n = 30) with a skin graft transplantation and main group (n = 30) with the skin graft transplantation combined with BM-MSC. Paraffin technique was used for morphological control of transplant engraftment after 7, 14, and 21 days. Briefly, the tissue was fixed in 10% neutral buffered formalin, and then the tissue samples were cut and embedded in paraffin. Tissue samples were stained with hematoxylin and eosin. Further histological slides were studied by microscopy and photographed with a digital camera Axiocam color (Germany).

Results and Discussion. According to the histological analysis and macroscopic observations at day 7, 14, and 21 significant differences were seen among the study groups. Thus, at day 7, in the control group the state of the transplant was unsatisfactory with necrotic graft changes and edema occurred. This picture was observed almost throughout the study period (at day 7, 14 and 21). After fortnight, the transplant of the mice of the control group appeared a dark pale in color. A half size of the transplant of the control group was rejected, there were eruptions of the surgical sutures. Necrosis and edema of the graft occurred as well. At the end of the third week, there was a complete rejection of the graft in the control group. In the main group, during all observation periods (at day 7, 14 and 21), the functioning of the transplant was noted. It should be noted that after a week of surgical procedure there were marginal necrosis, edema and a pale coloration of the skin. The mice of the main group had engraftment of the graft and had no signs of transplant rejection. Under the microscope, moderate edema of the graft and lymphohistiocytic infiltration was noted. Overall, the function of the graft was retained. At the end of observation period, the mice of the main group had the complete engraftment and the function of the transplant maintained.

Conclusions. The study has allowed establishing a connection between transplant engraftment and immunoregulatory properties of mesenchymal stem cells, both inducers of immunological tolerance, and their regenerative properties, which contributed to the full engraftment of the graft.

MESENCHYMAL STROMAL CELL-BASED TISSUE ENGINEERING

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Introduction. Due to ability to produce a variety of cell types multipotent mesenchymal stromal cells (MSCs) are promising cellular component for tissue engineering.

Aim. Development of tissue engineered constructs on the base of MSCs derived from adult human tissues and various 3-D scaffolds.

Materials and Methods. MSCs were isolated from adult human bone marrow, dental pulp, adipose and dermal tissues after the patient informed consent in appliance with local Ethical guidelines. MSCs were seeded into alginate microbeads (AMB) and wide-porous scaffolds prepared on the base of alginate, gelatin, collagen, demineralized chitinous skeletons of marine sponges and dental osteoplastic composite materials. MSCs viability, proliferation and capacity to differentiation toward adipogenic and osteogenic lineages were studied during 3-D culturing.

Results and Discussion. MSCs isolated from adult human bone marrow, dermal and adipose tissues after expansion in monolayer culture had similar phenotype and growth patterns as well as ability to induced multilineage differentiation. After encapsulation in AMB, MSCs had spherical shape and during 3-D culture they stopped to proliferate, but possessed viability and capability to differentiation toward adipogenic and osteogenic lineages. After seeding in 3-D wide-porous scaffolds, cells attached to porous surface, proliferated and filled pores during culturing. In the presence of definite inducers, MSCs were able to multilineage differentiation. The efficiency of osteogenic and adipogenic differentiation depended on scaffold type and composition.

Some approaches for storage, transportation and cryopreservation of MSC-based tissue-engineered constructs are presented and discussed.

Conclusions. Varying the properties of MSCs and scaffolds made it possible to generate different tissue engineered constructs that can be applied for correction of a wide range of tissue defects.

EXPERIMENTAL STUDY OF CELL AND TISSUE THERAPY PROTOCOLS IN REHABILITATION AFTER CHEMOTHERAPY-INDUCED OVARIAN FAILURE

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Introduction. Premature ovarian failure is observed in 1% of women under 40 and 0.1% under 30 years; among those 37% cases are due to iatrogenic causes (mainly after cancer treatment). Problem of rehabilitation after chemotherapy is more important for women, because chemotherapy often leads not only to hepatic and renal dysfunction, but to severe menopause, psycho-emotional, sexual, disorders, cardiovascular diseases (A.J. Fenton *et al.*, 2015). In recent years, cell and tissue therapy is considered as an alternative method of treatment of iatrogenic menopausal disorders and premature ovarian failure. The human placenta is a promising source of stem cells and biologically active substances capable of affecting the female organism. For reasons of biosafety, only cryopreserved material is possible to use for clinical application (O.S. Prokopyuk *et al.*, 2017).

Aim of this study was to compare different methods of tissue and cell therapy in the rehabilitation and recovery after chemotherapy in female mice.

Materials and Methods. The research was performed on BALB/c mice model of ovarian insufficiency induced by cyclophosphamide and busulfan (G.Y. Xiao *et al.*, 2014). The dynamics of ovarian, sexual function, morphology of liver and kidney, the general state of the animals was studied after application of cryopreserved

explants, extract, placental MSCs, adipose tissue MSCs obtained by the previously developed methods (D. Pogozhykh *et al.*, 2015).

Results and Discussion. In the chemotherapy group without rehabilitation, loss of weight, absence of regular changes in vaginal cytology, atrophy of the ovaries and uterus, cirrhotic changes in the liver, a sharp edema of the cortical and medullar layers in the kidneys was observed. The most rapid and complete recovery of the reproductive system, liver and kidney was noticed using placental explants, MSCs or extract. Application of adipose tissue MSCs led to slower recovery, but prevented critical changes (cirrhotic changes in the liver, kidney tubular necrosis). Recovery of morphology of liver and kidney, regular changes in vaginal cytology, sexual function, but not fertility was observed since follicles were lost in this model. Sensitivity to chemotherapy and treatment in some mice depended on the individual characteristics of the organism.

Conclusions. It was shown that the different methods of cell and tissue therapy are comparably efficient in the rehabilitation after chemotherapy and treatment of chemotherapy-induced ovarian failure. The application of placental preparations leads to faster and more complete recovery of the female reproductive system.

ARE $CD44^+/CD24^-$ CELLS THE ASSUMED CANCER STEM CELLS IN BREAST CANCER?

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Introduction. Identification and characterization of the population of cancer stem cells (CSC) depends on several cellular markers, which combination is specific for the phenotype of CSC in the corresponding tumor. Several markers of CSC have already been identified in breast cancer (BC), but there are no universal indicators that could specifically identify the CSC in BC.

Aim. To determine the validation of the CSC model for cell surface markers such as CD44 and CD24 and their clinical significance.

Materials and Methods. Primary tumor samples of 45 patients with invasive BC without chemotherapy prior to surgery were examined in paraffin blocks. CD44 and CD24 expression was evaluated by the percentage of positive cells using different chromogens and the MultiVision detection system (Thermo Scientific, USA) by immunohistochemical (IHC) method. In this research the evaluation was determined by the following criteria: (−), negative — expression in < 10% of tumor cells; (+), positive — expression in ⩾ 10% of cells. The same scoring system was applied for the expression of $CD44^+/CD24^-$.

Results and Discussion. 62.2% of investigated patients were older than 50 years and most of them had stage II of the disease (71%) and luminal tumor subtypes (68.9%). We analysed the expression of CD44 and CD24, $CD44^+/CD24^-$ for different patients by dividing them into two groups. The group A consists of the patients with unfavorable prognosis (relapses and metastases have occurred in the first three years after diagnosis), and the group B — with a favourable prognosis (the development of metastases after three years). Median disease-free survival in the group A is 19 months, in the group B — 46 months. The difference between the overall survival (OS) curves in the groups A and B is statistically significant ($p < 0.001$), the risk of death was higher in the group A [HR 5.9; CI 2.3–15.2].

The content of CD44 cells did not differ statistically between groups A and B ($p = 0.18$), but there was a tendency for increasing in OS with the existence of $CD44^+$ cells ($p = 0.056$). The distribution of the expression of CD24 marker did not differ between the groups ($p = 0.36$) as well as the OS curves ($p = 0.59$).

Analysis of the expression of $CD44^+/CD24^-$, which were considered as possible CSC, revealed a paradoxical increase ($p = 0.03$) in the frequency in patients of the group B (40.9%) compared to the group A (8.7%). Nevertheless, the comparison of the clinical outcomes did not reveal a statistically significant difference in the survival curves in the groups with existence and absence of $CD44^+/CD24^-$ expression ($p = 0.08$). The analysis showed the increase of the risk of worse clinical outcomes in the cases of absence of $CD44^+/CD24^-$ expression [HR 2.8 CI 1.1–6.8].

Conclusions. As a result of our research, the analysis of the percentage of assumed stem cells of the BC, which were identified by IHC as CD44 and CD24 cells, failed to detect a statistically significant relation between groups of patients with different prognosis, and the identification of their expression is not enough for the characteristics of CSC.

The obtained data demonstrating the worst clinical outcome in the cases of absence of $CD44^+/CD24^-$ expression apparently require further investigations and the validation of the IHC method with the determination of the cut-off line in defining of CD44 and CD24 status.

EFFECT OF THE OLFACTORY BULB NEUROGENIC STEM CELLS TRANSPLANTATION ON THE MOTOR FUNCTION IN AN EXPERIMENTAL SPINAL CORD INJURY

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Introduction. Transplantation of neurogenic stem cells is increasingly used in the treatment of a number of degenerative diseases of the central nervous system. Neurogenic cells of the olfactory bulb of humans and mammals possess stem properties: when cultured in a nutrient medium with mitogens they form neurospheres, and in the presence of retinoic acid they differentiate into neurocytes and gliocytes.

Aim. To study the effect of transplantation of olfactory bulb cells (TOBC) on the motor activity of rats in experimental spinal cord trauma.

Materials and Methods. Experimental groups of animals (white outbred male rats) included: 1) modeling of the left-sided half-intersection (LHI) of the spinal cord in the lower thoracic region (n = 24); 2) and 3) — TOBC in the tissue of the spinal cord below the LHI at 7 (n = 4)

and 13 (n = 4) weeks after injury. Cells from the rat olfactory bulb were cultured in the presence of growth factors hEGF and hFGF for 10 days. Estimation of motor activity of experimental animals in comparison with control animals was taken into account according to D.M. Basso, M.S. Beattie and J.C. Bresnahan (BBB) scale and parameters of electroneuromyography (gastrocnemius of the posterior ipsilateral limb) with direct stimulation of the spinal cord above the LHI.

Results. TOBC at 7 weeks after modeling of LHI in rats causes an increase in the rate of the hind limbs function growth. After TOBC the magnitude of the maximum amplitude of the M-response of the muscle being studied was significantly lower than that in group 1 compared to group 2.

Conclusions. TOBC in the long-term post-traumatic process provides a positive functional effect against the background of a decrease in electrical activity in the efferent parts of the motor system of the spinal cord, which reflects the antispastic potential of the method.

ABERRANT PLACENTAL ALKYLINE PHOSPHATASE (ALPP) IS THE BIOMARKER OF MYELOID LEUKEMIC STEM CELL NICHE MICROENVIRONMENT

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Human ALP is classified into four isoforms: intestinal (ALPI), placental (ALPP), germinal (ALPG) and tissue nonspecific (bone/kidney/liver) (TNAP). Intestinal, placental and germinal ALPs have higher homology between themselves than TNAP. The first three *ALP* genes are located on chromosome 2q34–37, in contrast the *TNAP* gene that is located on chromosome 1p36.1–34. It is referred that alkaline phosphatase is considered a non-specific tumor-related marker. However, the origin and nature of tumor-related alkaline phosphatase is not clear. We have focused on the role of ALP in chronic myeloid leukemia (CML) progression.

Hematopoietic stem cells (HSCs) possess the ability to self-renew and to differentiate to mature progeny along multiple different hematopoietic lineages. The function of HSCs depends upon the signals from surrounding cells found within the highly specialized microenvironment termed the hematopoietic stem cell niche. HSC niche was first conceptualized by Schofield in 1978 and was defined as “an entity in which the stem cell’s maturation is prevented and the properties of ‘stemness’ are preserved”. In addition, we suggest that hematopoiesis taking place in the bone marrow is orchestrated by HSC niche. Understanding and exploiting the HSC niche is a goal of basic research of the hematological malignancies. Osteocytes in bone marrow niche are essential for granulocyte colony-stimulating factor (G-CSF), which is the major cytokine regulator of G-CSF-induced hematopoietic stem/progenitor mobilization (N. Asadaet *et al.*, 2011). Myelopoiesis is regulated by HSC niche’s osteoblast/osteocytes signaling through HSCs granulocyte colony-stimulate signaling (K. Fulzele *et al.*, 2012). ALP activity was detected widely in osteoblasts of HSC niche in the bone marrow, about 20% of bone marrow cells expressed ALP activity (D. Miao, A. Scutt, 2002). Taken together, ALP is secreted by bone marrow HSC niche osteoblasts and has role in regulation of myelopoiesis through G-CSF signaling. As a result, the niche-associated ALP gene expression was absent in blood cells of healthy persons as we have shown. In addition to this, normal HSC is marked by G-CSFR (granulocyte colony-stimulate factor receptor), while leukemic hematopoietic cells lose this marker. Unexpectedly, we have observed the aberrant placental ALP gene expression in the peripheral blood cells in CML patients (n = 8). The aberrant placental ALP expression was investigated by real-time RT-PCR with consequent amplicone-DNA sequencing analysis. We first found the phenomenon of the phenotypic molecular switching from normal TNAP to ALPP in the hematopoietic niche induction during leukemogenesis. We have proposed that aberrant ALPP highlights the leukemic stem cell niche feature in CML progression. Moreover, we have firstly elucidated the epigenetic reverse regulation of aberrant ALPP gene expression in CML patients by histone deacetylase inhibitor (HDACi) sodium butyrate in culture of blood cells. In addition, the targeted increase of G-CSFR mRNA expression has been shown. In conclusion, we have suggested that aberrant placental ALP is the leukemogenic-derived alkaline phosphatase that drives HSC plasticity in leukemic stem cell survival. Based on the date obtained, we discuss targeting potentially capable of epigenetic reversion of the leukemic stem cell phenotype.

AUTOSELECTION PHENOMENON IN THE NORMAL STEM CELLS: FROM POPULATION HETEROGENEITY TO CANCER PHENOTYPE

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Introduction. The development and functioning of a multicellular organism is impossible without regulation of cell division and cell differentiation processes. It is just their mutual regulation, on the one hand, causes step-by-step narrowing of cell potentiality during embryogenesis and, on the other hand, maintains the renewal of cellular composition and spatial arrangement of tissues during the reparation processes in adult organism. Disturbances in the above regulation are then considered to be responsible for a number of pathological processes in organism including carcinogenesis.

At present there is no consensus on the mechanisms of the regulation of cell division/differentiation processes. However, none of the existing concepts are capable of giving a complete solution of the regulation problem till they do not touch the question of interdependence between the proliferation and differentiation processes in the heterogeneous populations of normal stem cells.

Aim. The purpose of the present study is to show that in the heterogeneous population of stem cells the mechanism of proliferation — differentiation processes based on the inhibition of the proliferating stem cells by differentiation factors (special messengers synthesized by the differentiated cells) can result in the selection of actively proliferating cells that are insensitive to the action of differentiation factors.

Methods. The problem of regulation of the proliferation — differentiation processes is analyzed in the framework of the mathematical model of unipotential stem cell clone dynamics, assuming two alternative scenarios of an every new-born cell development, which in the long

run define correspondingly the proliferating or differentiated cell status. The scenario choice is then supposed to be a random event influenced by both the inherent mitotic activity of stem cell and the differentiation factors.

Results and Discussion. Taking into account the heterogeneity of stem cells it was shown that such mechanism of the regulation of proliferation — differentiation processes can result in the selection of actively proliferating and cells that are insensitive to the action of differentiation factors (that is to say, the cells with cancer phenotype). We called this phenomenon an autoselection. In the framework of the model, the low level of the intraclonal heterogeneity of the mitotic activity index of stem cells is necessary but not sufficient for the emergence of the cells with cancer phenotype. Another important condition, which is necessary for the appearance of such cells in the clone, is the increase of the interclonal heterogeneity of stem cells.

Conclusions. The stem cells with cancer phenotype appears through the series of the permanent nonmutational changes by autoselection mechanism retaining for the division and generation of the progeny only that actively proliferating cells which are less sensitive with respect to the action of the differentiation factors. The progression of normal stem cells towards the appearance and increase of the number of the cells with cancer phenotype can be considered as a possible mechanism of carcinogenesis.

MODIFYING EFFECT OF EXOGENOUS LACTOFERRIN ON THE HUMAN BREAST CANCER CELL LINES WITH PHENOTYPE OF CANCER STEM CELLS

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Introduction. Cancer stem cells (CSCs) today are the subject of intensive research in experimental and clinical oncology. Their presence in the tumor area is associated with aggressive course and resistance to therapy. Currently, the search and development of new therapy approaches for the treatment of malignant tumors with CSCs is conducted worldwide. Experimental studies have shown that higher antitumor activity have natural antioxidant proteins, including lactoferrin (LF), compared to synthetic analogs. LF is characterized by a unique set of biological properties of a different nature. At the same time, the peculiarities of the LF influence on breast cancer (BC) cells with the CSC phenotype have not been finally determined.

Aim. To investigate the cytotoxic and genotoxic effects of exogenous LF on hormone receptor-positive and receptor-negative human BC lines with CSC phenotype.

Materials and Methods. The hormone receptor-positive (MCF-7) and receptor-negative (MDA-MB-231) human BC cell lines, positive for CSC markers, were cultured with an exogenous LF at a dose corresponding to IC30 (10 μg/ml for receptor-positive cells, 5 μg/ml for receptor-negative cells). Flow cytometry, immunocytochemical, and statistical methods were used.

Results. Cultivation with exogenous LF resulted in reducing the number of cells with the CSC-like phenotype both in hormone-receptor-positive and receptor-negative BC cell lines. This is evidenced by a reduction in the number of cells positive for CD44 expression, in MCF-7 by 55% and the 4.5-fold increase of CD24 expression in MDA-MB-231cells. We have found that after incubation with LF, MCF-7 and MDA-MB-231 cells demonstrated increased levels of reactive oxygen species (2.0–1.7-fold, $p < 0.05$), inducible NO generation rate of NO-synthase (1.6–1.9-fold, $p < 0.05$) and the level of "free iron" (1.7–2.3-fold, $p < 0.05$). In addition, the effects of LF were more pronounced in MDA-MB-231 receptor-negative cell line. These changes resulted in the increased expression of proapoptotic protein Bax (by 10–16%, $p < 0.05$), reduced expression of the antiapoptotic protein Bcl-2 (16–21%, $p < 0.05$) and lowering level of unoxidized mitochondrial phospholipid cardiolipin (by 1.6–2.4 times, $p < 0.05$). This, in turn, caused an increase in the number of cells killed by apoptosis (14–29.1%, $p < 0.05$). Cytotoxic effects of LF were accompanied by an increase in the percentage of DNA in "comet tail" (by 1.8–4 times, $p < 0.05$) and the blockage of cell cycle at G_2/M phase, especially in receptor-negative cell line.

Conclusions. The obtained data indicate that exogenous LF shifts pro-/antioxidant balance of hormone in receptor-positive and receptor-negative human BC cells with the CSC phenotype by increasing the level of reactive oxygen species, "free iron" and the rate of NO generation. These disruptions lead to the blockage of the cell cycle in the G_2/M phase and the death of malignant cells by apoptosis. Further study of the mechanisms of LF influence will justify the need for its use to modify the phenotype of cells positive for the expression of CSC markers.

PROTEOME RESPONSE OF DPSCS TO TREATMENT WITH EXOGENOUS FGF8

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Introduction. Human dental pulp is a rich source of postnatal mesenchymal stem cells which can self-renew and undergo differentiation into multiple lineages. FGF8 is a paracrine-acting FGF family ligand, expressed during the earliest stages of tooth site determination and indispensable for mediating epithelial-to-mesenchymal interactions initiating odontogenic differentiation during embryogenesis.

Aim. To examine differential protein analysis in adult human dental pulp in response to short- and long- term treatment with FGF8 in order to assess its value as a therapeutic agent for the purposes of pulp regeneration.

Materials and Methods. Intact human premolar teeth were obtained from healthy patients between the ages of 18 and 40 following the acquisition of written consent. Dental pulps were exposed using diamond burs and extracted using sterile barbed broaches. Tissue explants were enzymatically digested with collagenase type I (3 mg/ml) and disease (4 mg/ml) for 1 h at 37 °C and

cultured in DMEM with added 20% heat-inactivated fetal bovine serum (FBS). Heterogenous cell culture was maintained at standard culture conditions with supplemented 10% FBS. Upon reaching the third passage, samples run in triplicates were subjected to short- (24 h) and long-term (10 days) treatment with 10 ng/ml human recombinant FGF8. 2D DIGE analysis was conducted using 24-cm strips in the narrow 4–7 pH range for the first dimension and SDS-PAGE (8–16%) for the second. Protein spots were subsequently identified by MALDI-TOF/TOF-MS.

Results and Discussion. 20 protein spots identified by MALDI-TOF/TOF-MS with high confidence were found to be differentially expressed among treatment groups as compared to untreated controls. These belonged to clusters of gluconeogenesis (triosephosphate isomerase), cellular structure and motility, inflammation, protein biosynthesis, trafficking and degradation, cell cycle and DNA repair, osteogenic differentiation, cytokinesis and vesicle trafficking.

Conclusions. In summary, the proteomic approach has yielded novel information about the effect of FGF8 on cells of adult dental pulp with identified proteins being linked to regulation of actin cytoskeletal rearrangements, migration, osteogenic differentiation, neuronal cell function, consistent with what has been reported about the role of FGF8 in the context of embryonic tooth development and the neural crest origin of dental pulp cells. FGF8 is likely to be able to instigate pulp cell migration and odontogenic differentiation in response to pulp injury in an appropriate model organism, however further studies need to be conducted to evaluate its therapeutic potential.

ADULT NEURAL CREST-DERIVED MULTIPOTENT STEM CELLS FROM HAIR FOLLICLE: PHENOTYPE, FUNCTIONAL PROPERTIES AND PROSPECTS FOR USE IN REGENERATIVE MEDICINE

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Introduction. Neural crest is a transient structure during embryonic development of vertebrates which gives rise to peripheral nervous system; melanocytes; cartilage, bone and connective tissue in head; some endocrine cells, etc. Several tissues and organs of adult mammals contain adult neural crest-derived multipotent stem cells (NC-MSCs) and they have great potential to use in regenerative medicine.

Aim. To obtain the NC-MSCs from human hair follicle, characterize their morphological and functional properties; based on our positive results in animal models to conduct pilot human studies for treatment of spinal cord injuries (SCI), degeneration of intervertebral disks (IVD) and calvarial bone defects.

Materials and Methods. All procedures were performed with the voluntary informed consent of patients and in accordance with the current legislation of Ukraine. NC-MSCs were obtained from hair follicle by explant method and expanded by our original protocol. Cultured NC-MSCs were characterized by qPCR, immunocytochemistry, flow cytometry, cytogenetic and functional assays. Cytokines and growth factors produced by NC-MSCs were determined by ELISA and Bio-Rad multiplex assay. Patients subjected to non-effective standard drug therapies, surgery approaches or neuro-rehabilitation have received the cell therapy treatment or transplantation tissue-engineered (TE) bone equivalent. Three patients with contusion SCI received single combined paravertebral and intrathecal injections ($20 \cdot 10^6$ cells per injection/patient). Ten patients with IVD degeneration, herniation and pain syndrome received only paravertebral cell injection ($10 \cdot 10^6$ cells per patient). Three patients with critical sized calvarial bone defects had the transplantation of TE bone equivalent. Minimal follow-up period was 1 year.

Results and Discussion. Adult NC-MSCs were successfully isolated and expanded from all patients. NC-MSCs have stable karyotype, $Sox2^+$ $Sox10^+$ $Nestin^+$ $CD73^+$ $CD90^+$ $CD105^+$ $CD140a^+$ $CD140b^+$ $CD146^+$ $CD166^+$ $CD271^+$ $CD349^+$ $CD34^-$ $CD45^-$ $CD56^-$ HLA-DR^- phenotype and were able to self-renewal and directed differentiation *in vitro* into neurons, Schwann cells, melanocytes, osteoblasts, chondrocytes and adipocytes. NC-MSCs secreted the following proteins: NGF, BDNF, NT-3, NT-4/5, IL-1ra, IL-10, bFGF, VEGF, and GM-CSF. Two patients with lumbar SCI caused by shrapnel improved from ASIA C to ASIA D after cell therapy. One patient with thoracic SCI caused by bullet improved from ASIA B to ASIA C after treatment. Treated patients showed the restoration of sensitivity as well as the emergence of active movements and strength grown in paretic limbs. Ten patients with IVD degeneration showed a significant improvement in pain syndrome and reduction in size of a protrusion/extrusion. In three patients, the calvarial bone defects were successfully restored with TE bone equivalent.

Conclusion. NC-MSCs can be efficiently obtained and expanded from adult human hair follicle. Our preliminary results have shown significant therapeutic and regenerative potential of this cell type.

TISSUE-ENGINEERED BONE FOR TREATMENT OF COMBAT RELATED LIMB INJURIES

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Introduction. Combat-related bone defects are manifested in 10–12% of casualties, and 30% of these defects are complicated with osteomyelitis. The greatest difficulties arise in the treatment of diaphyseal bone defects more than 5 cm and metaepiphyseal defects that exceed 15–20 cm^3. Treatment of critical sized bone defects is an actual clinical challenge. The "gold standard" in this case is autologous bone grafting. But disadvantage of this method is associated with limited donor bone resources.

Aim. Based on our preliminary positive clinical results with use of cultured bone marrow-derived multipotent mesenchymal stem/stromal cells (BM-MSCs) in trau-

matology, our aim was to develop 3D tissue-engineered bone equivalent (3D-TEBE) transplantation technology for restoration of critical sized bone defects.

Materials and Methods. To fabricate 3D-TEBE we used devitalized allogeneic bone (blocks and chips) seeded with cultured autologous cells: BM-MSCs in mix with periosteum progenitor cells (PPCs) and endothelial progenitor cells (EPCs). Quality/identity of cell cultures was assured by flow cytometry (cell phenotype), cytogenetic analysis (GTG-banding), donor and cell cultures infection screening (IFA, PCR), functional analysis (cell kinetics, CFU analysis, multilineage differentiation assay, cell senescence assay). 3D-TEBE transplantation was performed in 47 combat-injured with 49 bone defects. New bone formation was assessed by the radiographic examination. Treatment was based on local clinical protocol approved by Ministry of Health of Ukraine.

Results and Discussion. Patients were included in a treatment program 8–19 months after injury, provided the ineffectiveness of conventional surgery methods. All cell cultures had a normal karyotype and phenotype, differentiation potential and functional properties, 30% CFU frequency and hadn't any signs of cell senescence. FDA/PI staining, MTT-assay and histological analysis of 3D-TEBE samples showed their regular seeding with viable cells. Histological analysis of 3D-TEBE biopsies 3 months after transplantation taken within adoptive resection surgery showed immature bone tissue formation. Restoration of all bone defects was observed after 5–6 months (by radiographic examination).

Conclusions. The developed biotechnology of 3D-TEBE transplantation allows restoring the bone integrity, forming new bone tissue in a site of bone defect, and significantly reducing the rehabilitation period of a patient.

RESULTS OF MONITORING GENETIC STABILITY OF MESENCHYMAL STEM CELLS IN THE SMARTCELL BIOTECHNOLOGY COMPANY

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Introduction. Due to the active development of cellular technologies, the study of the genetic stability of human stem cells (SCs) is receiving increasing attention. Since the amount of SCs isolated from the body is too small to be used for medicinal purposes, they should be expanded in culture. In the course of culturing, *de novo*-induced chromosomal abnormalities are possible, giving rise to abnormal cell lines. Accumulation of such abnormalities can lead to malignant transformation of cells.

Aim. To assess the chromosomal variability of mesenchymal stem cells (MSCs), depending on the source of cell production and the duration of their cultivation.

Materials and Methods. MSCs were isolated from bone marrow and adipose tissue. Their genetic stability was evaluated by the method of karyotyping using specialized software. G-staining in metaphase plates was provided for cytogenetic analysis.

Results. We conducted a cytogenetic analysis of a number of MSC cultures originating from bone marrow and adipose tissue starting from the 2^{nd} and ending with the 10^{th} passage. In all samples under study, neither metaphase plates with an altered chromosome number nor structural changes in the chromosomes were detected upon cell culture.

Conclusions. The results of our studies showed that the methods used for MSC isolation, as well as the conditions for their cultivation, preserve the normal karyotype of the cells at the early passages.

MOLECULAR PROFILE FEATURES OF PROSTATE CANCER CELLS WITH THE $CD44^+/CD24^-$ PHENOTYPE

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Introduction. The clinical significance of the molecular heterogeneity of prostate cancer (PC) is actively discussed in recent publications due to the important role of cancer stem cells (CSC) in the disease course. The presence of these cells is a determining factor for the growth and progression of the tumor, its metastatic activity, and also sensitivity to therapy. In this regard, the study of the molecular profile of cells with the CSC phenotype is an urgent issue, the solution of which will help to deepen knowledge about the biology of tumor growth.

Aim. To investigate the features of the molecular profile of PC cell lines, depending on the expression of CSC markers (CD24 and CD44).

Materials and Methods. The study was conducted on human PC cell lines (DU-145 and LNCaP). Expression of CSC markers (CD24 and CD44), steroid hormones receptors (testosterone (AR), estrogen α (ERα), progesterone (PR) and epidermal growth factor — (HER2/neu)), E-cadherin and Ki-67 was determined by the immunocytochemical method. The evaluation of the results was performed using optical microscopy by H-Score method. The marker expression levels were considered to be high at the values from 201 to 300 points of the H-Score, average — from 101 to 200 points of the H-Score, and low at the range from 0 to 100 points of the H-Score. The invasive activity of the cells was examined using a standard invasive test according to the manufacturer's instructions (BD Biociences). STATISTICA 6.0 software was used to process the results.

Results. The study of CD24 and CD44 expression demonstrated that the PC cell lines differ in expression of CSC markers. DU-145 cells were positive for the CSC phenotype ($CD44^+/CD24^-$), while LNCaP cells had low levels of CD44 (65 ± 12.3 points of the H-Score) and did not express CD24. We established that DU-145 cells with CSC phenotype exhibit a high level of testosterone receptor (250 ± 14.6 points of H-Score), low level of HER2/neu (60.3 ± 14.6 points of H-Score), and have lack of ER, PR, and E-cadherin expression.

In the cells of LNCaP line, negative for the CSC phenotype, we observed a high level of testosterone expression (272.6 ± 10.3 points of the H-Score), the average PR expression (145.7 ± 11.0 points of the H-Score), the absence of HER2/neu, and the average level of E-cadherin (187.7 ± 16.0 points of the H-Score).

Cells with the $CD44^+/CD24^-$ phenotype of DU-145 cell line were characterized by a high proliferative (Ki-67 levels considered to be 265.0 ± 20.5 points of the H-Score) and invasive activity (0.37/1000 cells) compared to the LNCaP cells (Ki-67 level amounted 174.0 ± 24.6 points for H-Score, the invasive activity was 0.076/1000 cells, respectively).

Conclusions. The association of the presence of CSC markers with a high proliferative and invasive activity

and a decrease in the adhesion properties of PC cells has been established. The findings point to the need for a more in-depth study of the role of CSC in the biology of cell growth.

ENDOMETRIAL STROMAL CELLS: ISOLATION, EXPANSION, MORPHOLOGICAL AND FUNCTIONAL PROPERTIES

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Introduction. The endometrium is a unique structure that is able to complete self-renewal over a month cycle, and undergoes these changes over 400 times during woman reproductive age. A significant regenerative potential is due to the presence of stem cells in the endometrium, such as mesenchymal, epithelial and endothelial progenitor cells. That is why endometrium is a promising object for MSCs isolation and *in vitro* short-term expansion for their further exploration and use in assisted reproductive technologies, reproductology and regenerative medicine.

Aim. To study the biological properties of human endometrial stromal cells *in vitro*.

Materials and Methods. The endometrium samples (n = 10) were obtained by biopsy at the first phase of the menstrual cycle from women with endometrial hypoplasia. In all cases, a voluntary written informed consent was obtained from the patients. Endometrial fragments were dissociated by enzymatic treatment for 1 h in 0.05% collagenase IA and 0.05% pronase and 2% FBS. The cells were cultured in DMEM:F12 supplemented with 10% FBS, 2 мМ *L*-glutamine and 1 ng/ml FGF-2 in a multi-gas incubator at 5% CO_2 and 5% O_2. For the phenotype determination, the ability to directed adipogenic, osteogenic and chondrogenic differentiation assays, CFU-test, karyotype stability, the P3-cells have been used. The cell secretome was assessed by BioRad Multiplex immunoassay kit.

Results and Discussion. Primary population of endometrial cells was heterogeneous and contained cells with fibroblast-like and epithelial-like morphology, but at P3 the majority of cell population had fibroblast-like morphology. The cells possessed MSCs typical phenotype $CD90^+CD105^+CD73^+CD34^-CD45^-HLA\text{-}DR^-$ assessed by flow cytometry. Positive expression of $CD146^+CD166^+CD49f^+CD140a^+CD140b^+nestin^+$ was determined. Cell doubling time is 29.6 ± 1.3 h. They were capable of direct osteogenic and adipogenic differentiation. The cells showed 35.7% colony forming efficiency and a tendency to 3D spheroid formation. The GTG-banding assay confirmed the stability of eMSC karyotype during long-term culturing (up to P8). After 48 h incubation period in serum-free medium eMSC secreted anti-inflammatory IL-1ra (74.6 ± 9.5 pg/ml), as well as IL-6 (29.8 ± 8.3 pg/ml), IL-8 (138.5 ± 33.3 pg/ml) and IFNγ (55.9 ± 3.8 pg/ml), angiogenic factors VEGF (92.2 ± 19.8 pg/ml), GM-CSF (133.2 ± 5.1 pg/ml) and FGF-2 (17.8 ± 4.3 pg/ml), chemokines IP-10 (39.9 ± 3.3 pg/ml) and MCP-1 (41.1 ± 6.7 pg/ml).

Conclusion. Thus, obtained endometrial stromal cells meet minimal ISCT criteria for MSCs, such as adherence to plastic in standard culture conditions, expression of typical phenotype markers and ability for the directed differentiation *in vitro*. eMSCs are stable during long-term culturing. They also produce a range of cytokines, chemokines and growth factors. Proliferative potential, karyotype stability and secretome profile make them a perspective object for the use in the regenerative medicine.