SESSION IX

NEW INSIGHTS INTO CELLULAR FUNCTIONS

Wednesday (September 15, 2021; 14:15 – 17:45)
Thursday (September 16, 2021; 15:30 – 16:10)

Chair:

Prof. Mariusz Ratajczak
Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA
Department of Regenerative Medicine, Center for Preclinical Research and Technology, Medical University of Warsaw, Warszawa, Poland

Prof. Jakub Wlodarczyk
Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology, Polish Academy of Science, Warsaw, Poland.

DETAILED SESSION IX SCHEDULE

Opening lectures (Wednesday; September 15, 2021; 14:15 – 16:15; *virtual stream B*)

S9.L1 EXTRACELLULAR MEMBRANE VESICLES-MEDIATED ANGIOGENESIS. **G. Camussi** (Department of Medical Sciences, University of Torino, Torino, Italy).

S9.L2 VERY SMALL EMBRYONIC-LIKE STEM CELLS (VSELS) - AN UPDATE AND FUTURE DIRECTIONS. **M. Kucia** (Medical University of Warsaw, Warsaw, Poland).

S9.L3 CONTROL OF NEURAL STEM CELL FATE DETERMINATION IN HUNTINGTON'S DISEASE BY ADENOSINE TRIPHOSPHATE (ATP) AND SPONTANEOUS CALCIUM OSCILLATIONS. **T. Glaser[1], H. Shimojo[2], D. Elisa Ribeiro[1], J. Correa-Velloso[1], A. Oliveira-Giacomelli[1], C. Lameu[1], Y.D. Teng[2], R. Kageyama[3], H. Ulrich[1].** ([1]Department of Biochemistry, IQ, University of Sao Paulo, Sao Paulo, Brazil, [2]Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, Division of SCI Research, Veterans Affairs Boston Healthcare System, Boston, MA, USA, [3]Institute for Virus Research, Kyoto University, Kyoto, Japan).

S9.L4 AN OVERVIEW OF NOVEL UNCONVENTIONAL MECHANISMS OF HEMATOPOIETIC DEVELOPMENT AND REGULATORS OF HEMATOPOIESIS – A ROADMAP FOR FUTURE INVESTIGATIONS. **M.Z. Ratajczak** (Medical University of Warsaw, Warsaw, Poland).

Oral presentations (Wednesday; September 15, 2021; 16:35 – 17:45; *virtual stream B*)

S9.L5 THE ROLE OF P2X4 PURINERGIC RECEPTOR IN HEMATOPOIETIC STEM/PROGENITOR CELLS TRAFFICKING. **M. Adamiak[1], M. Kucia[1,2], M.Z. Ratajczak[1,2]** ([1]Department of Regenerative Medicine Medical University of Warsaw, Poland, [2]Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA).

S9.L6 THE ROLE OF NOX2-ROS-NLRP3 INFLAMMASOME AXIS IN HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPCS) TRAFFICKING. **M. Kucia[1,2], K. Bujko[1], M. Adamiak[1], V. Chumak[1], J. Ratajczak[2], M.Z. Ratajczak[1]** ([1]Department of Regenerative Medicine, Center for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland, [2]Stem Cell Institute, University of Louisville Brown Cancer Center, Louisville, KY, USA).

S9.L7 BONE MARROW-DERIVED VSELS ENGRAFT AS LUNG PROGENITORS AFTER BLEOMYCIN-INDUCED LUNG INJURY. **A.K. Ciechanowicz[1], K. Sielatycka[2], M. Cymer[1], M.S. Uszynska[3], K. Bujko[1], M.Z. Ratajczak[1,3], D.S. Krause[4], M. Kucia[1,3]** ([1]Department of Regenerative Medicine, Center for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland, [2]Institute of Biology, Faculty of Exact and Natural Sciences, University of Szczecin, Szczecin, Poland, [3]Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA, [4]Department of Laboratory Medicine, Cell Biology and Pathology and the Yale Stem Cell Center, Yale University School of Medicine, New Haven, USA).

S9.L8 EXENDIN-4 AFFECTS METABOLIC AND SECRETORY ACTIVITIES OF HUMAN DERMAL FIBROBLASTS CULTURED IN A HYPERGLYCEMIC ENVIRONMENT. **M. Wolak[1], E. Bojanowska[1]** ([1]Department of Behavioral Pathophysiology, Medical University of Lodz, Lodz, Poland).

Session summary

Poster session (Thursday, September 16, 2021; 15:30 – 16:10; *virtual stream D*)

S9.P1 DIRECT EFFECT OF VITAMIN C ON CELL CYCLE AND APOPTOSIS GENE AND PROTEIN EXPRESSION IN CANCER CELLS. **N. Respekta, E.L Gregoraszczuk** (Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research , Jagiellonian University in Krakow, Krakow, Poland).

S9.P2 RELEASE OF INTERLEUKIN-6 IS DEPENDENT ON $\alpha 2\beta 1$ INTEGRIN. **M. Galdyszynska, P. Radwanska J. Drobnik** (Department of Pathophysiology, Medical University of Lodz, Lodz, Poland).

S9.P3 miR-138-5p AS A PREDICTIVE FACTOR OF SEVERE ORAL MUCOSITIS IN PATIENTS WITH HEAD AND NECK CANCER UNDERGOING INTENSITY-MODULATED RADIATION THERAPY. **R. Mlak[1], I. Homa-Mlak[1], T. Powrozek[1], M. Mazurek[1], A. Brzozowska[2], T. Malecka-Massalska[1]** ([1]Department of Human Physiology, Medical University of Lublin, Lublin, Poland, [2]II Department of Radiotherapy, Center of Oncology of the Lublin Region St. John of Dukla, Lublin, Poland).

S9.P4 THE ASSESSMENT OF THE PROLIFERATIVE POTENTIAL OF BRONCHOALVEOLAR STEM CELLS AND ALVEOLAR TYPE 2 CELLS ISOLATED AT VARIOUS STAGES OF BLEOMYCIN-INDUCED LUNG INJURY. **A.K. Ciechanowicz[1], E. Suchocki[1], S. Leszczak[1], W.X. Lay[1], J. Prado Paulino[1], C. Leszczak[1], M. Kucia[1,2], D.S. Krause[3]** ([1]Department of Regenerative Medicine, Center for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland, [2]Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA, [3]Departments of Laboratory Medicine, Cell Biology and Pathology and the Yale Stem Cell Center, Yale University School of Medicine, New Haven, USA).

S9.L1

EXTRACELLULAR MEMBRANE VESICLES-MEDIATED ANGIOGENESIS

G. CAMUSSI

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Growing evidence indicates that membrane vesicles actively released from cells may act as autocrine and paracrine mediators in the angiogenic processes. These vesicles which include exosomes and microvesicles, can interact with neighboring cells and/or with distant cells and activate the angiogenic process through the transfer of encapsulated transcriptional regulators that may induce epigenetic changes in the recipient cells. These membrane vesicles gained a place amongst the vast group of angiogenic mediators and have been involved in physiological and pathological conditions of angiogenesis. The basic mechanism involved is the activation of an angiogenic program in quiescent endothelial cells. Many types of cells release angiogenic membrane vesicles such as stem/progenitor cells, inflammatory cells, activated endothelial cells and tumor cells. The angiogenic pathways activated in the recipient cells depend on the cell of origin, on the cargo of vesicles and on the state in which the vesicles are secreted. We recently investigated the mechanisms of action and the angiogenic capability of membrane vesicles derived from serum and their possible therapeutic use in regenerative medicine.

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S9.L2

VERY SMALL EMBRYONIC-LIKE STEM CELLS (VSELS) - AN UPDATE AND FUTURE DIRECTIONS

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Regenerative medicine is looking for a pluripotent/multipotent stem cell able to differentiate across germ layers and be safely employed in therapy. Unfortunately, with the exception of hematopoietic stem cells (HSCs) for hematological applications, the current clinical results with stem cells are somewhat disappointing. The potential clinical applications of the more primitive embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have so far been discouraging, as both have exhibited several problems, including genomic instability, a risk of teratoma formation, and the possibility of rejection. Therefore, the only safe stem cells that have so far been employed in regenerative medicine are monopotent stem cells, such as the abovementioned HSCs or mesenchymal stem cells (MSCs) isolated from postnatal tissues. However, their monopotency, and therefore limited differentiation potential, is a barrier to their broader application in the clinic. Interestingly, results have accumulated indicating that adult tissues contain rare, early-development stem cells known as very small embryonic-like stem cells (VSELs), which can differentiate into cells from more than one germ layer. Results from at least 40 independent laboratories indicate that adult tissues contain rare, early-development stem cells known as very small embryonic-like stem cells (VSELs), which can differentiate into cells from more than one germ layer. It has been proposed that VSELs originate from cells related to the germline, are deposited in developing organs during embryogenesis, and play a role as a backup population for monopotent tissue-committed stem cells. VSELs are quiescent but are activated during stress situations and mobilized into the circulation. The number of these cells decreases with age. Overall, the presence of these early-development cells in postnatal tissues challenges the accepted hierarchy within the adult stem cell compartment in bone marrow. Further research on these cells may provide a path forward to application of these cells in regenerative medicine that perhaps may solve several problems inherent in the use of controversial embryonic stem cells (ESCs) and somehow problematic induced pluripotent stem cells (iPSCs).

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S9.L3

CONTROL OF NEURAL STEM CELL FATE DETERMINATION IN HUNTINGTON'S DISEASE BY ADENOSINE TRIPHOSPHATE (ATP) AND SPONTANEOUS CALCIUM OSCILLATIONS

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Huntington's disease (HD) is an autosomal dominant inherited disease caused by at least 35 repetitions of the N-terminal CAG trinucleotide (glutamine) in the Huntington's gene (Htt). We used as *in vitro* disease models induced pluripotent iPS cells obtained from HD patients and Htt-gene edited embryonic stem cells, which were induced to neuronal differentiation into GABAergic neurons. Calcium oscillations were tracked by real-time fluorescence and luminescence microscopy to analyse the correlative relationship between calcium transient activity and rhythmic proneuronal transcription factor expression in embryonic stem cells after stable transfection with ASCL-1 or neurogenin-2 promoter-protein fused to the luciferase reporter gene. We show that pharmacological activity manipulation of P2Y2 and P2X7 purinergic receptors induced a two-step process of neuronal differentiation. *In vitro* models of Huntington's disease (HD) showed increased basal intracellular calcium concentration together with augmented apoptosis rates and lacked spike-like calcium oscillations and P2Y2 receptor activity, agreeing with deficiency of ASCL-1 expression activation and GABAergic differentiation. Our results suggest that HD may have developmental origins based on inefficient GABAergic differentiation, shedding new light on the mechanisms underlying neurogenesis of inhibitory neurons.

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S9.L4

AN OVERVIEW OF NOVEL UNCONVENTIONAL MECHANISMS OF HEMATOPOIETIC DEVELOPMENT AND REGULATORS OF HEMATOPOIESIS – A ROADMAP FOR FUTURE INVESTIGATIONS

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Hematopoietic stem cells (HSCs) are the best-characterized stem cells in adult tissues. Nevertheless, as of today, many open questions remain. First, what is the phenotype of the most primitive "pre-HSC" able to undergo asymmetric divisions during *ex vivo* expansion that gives rise to HSC for all hemato-lymphopoietic lineages. Next, most routine *in vitro* assays designed to study HSC specification into hematopoietic progenitor cells (HPCs) for major hematopoietic lineages are based on a limited number of peptide-based growth factors and cytokines, neglecting the involvement of several other regulators that are endowed with hematopoietic activity. Examples include many hormones, such as pituitary gonadotropins, gonadal sex hormones, IGF-1, and thyroid hormones, as well as bioactive phosphosphingolipids and extracellular nucleotides (EXNs). Moreover, in addition to regulation by stromal-derived factor 1 (SDF-1), trafficking of these cells during mobilization or homing after transplantation is also regulated by bioactive phosphosphingolipids, EXNs, and three ancient proteolytic cascades, the complement cascade (ComC), the coagulation cascade (CoA), and the fibrinolytic cascade (FibC). Finally, it has emerged that bone marrow responds by "sterile inflammation" to signals sent from damaged organs and tissues, systemic stress, strenuous exercise, gut microbiota, and the administration of certain drugs. This review will address the involvement of these unconventional regulators and present a broader picture of hematopoiesis and address a novel proposed stem cell hierarchy in BM microenvironment including presence of very small embryonic like stem cells (VSELs). These cells can differentiate into cells from more than one germ layer and results from at least 40 independent laboratories confirmed a presence of these cells in postnatal tissues including BM.

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S9.L5

THE ROLE OF P2X4 PURINERGIC RECEPTOR IN HEMATOPOIETIC STEM/PROGENITOR CELLS TRAFFICKING

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Our previous work we demonstrated that eATP activates P2X7 ion channel receptor in HSPCs and its deficiency impairs stem cell trafficking. Evidence suggest that P2X4 receptor in addition to P2X7, are also highly expressed on hematopoietic stem progenitor cells among P2X family and also is more sensitive to eATP and signals much faster. We have hypothesized that extracellular ATP activity on BM homing of HSPCs are dependent on P2X4 receptor. *In vivo* transplantations were performed with normal BM cells into irradiated mice or cells exposed to PSB12054–P2X4 receptor antagonist. Homing was evaluated by enumerating 24 hours after transplantation labeled cells, 12 days CFU-S and CFU-GM and hematological recovery. Results: Inhibition of P2X4 receptor both *in vivo* and *in vitro* negatively affected homing of HSPCs. HSPCs from control mice engrafted better than WT cells treated with PSB12054 what indicates involvement of P2X4 receptor in transplanted HSPCs. We noticed that P2X4 receptor similarly as P2X7 promotes trafficking of HSPCs, as its deficiency leads defective homing and engraftment of HSPCs after transplantation into myeloablated hosts. Moreover, the perturbation of P2X4 expression in BM of recipient mice also resulted in impaired homing, what corroborated with decrease of SDF-1 expression in BM microenvironment. Thus, our data sheds more light and confirm postulated cooperative dependence of both receptors in response to eATP signaling.

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S9.L6

THE ROLE OF NOX2-ROS-NLRP3 INFLAMMASOME AXIS IN HEMATOPOIETIC STEM/PROGENITOR CELLS TRAFFICKING

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Nox2 or nicotinamide adenine dinucleotide phosphate (NADPH) oxidase forms reactive oxygen species (ROS) are involved in several physiological and pathological processes of hematopoiesis. To support this, it has been reported that ROS are involved in mobilization of hematopoietic stem/progenitor cells (HSPCs) from bone marrow (BM) into peripheral blood (PB). Based on this and to learn more on the role of Nox2 in this process, we performed mobilization studies in Nox2-KO mice and noticed that these animals are poor G-CSF and AMD3100 mobilizers. Moreover, Nox-2 deficient BMMNC show defective homing and engraftment after transplantation into normal syngeneic recipients. To explain this, Nox2 as a source of ROS is involved in the activation of NLR family pyrin domain containing 3 (Nlrp3) inflammasome that by the release of extracellular adenosine triphosophate (eATP) promotes recruitment of CXCR4 receptor into membrane lipid rafts (MLRs) to enhance the responsiveness of HSPCs to stromal-derived factor-1 (SDF-1) gradient. Nox2 also sensitizes these cells responsiveness to other BM chemoattractants such as sphingosine-1 phosphate (S1P) and extracellular adenosine triphosphate (eATP). We report that Nox2 deficient HSPCs show defective migration to chemoattractants and do not form MLRs efficiently. This confirms the role of Nox2 as the ROS source that in Nlrp3 inflammasome-dependent manner enhances cell migration by promoting the formation of MLRs.

S9.L7

BONE MARROW-DERIVED VSELS ENGRAFT AS LUNG PROGENITORS AFTER BLEOMYCIN-INDUCED LUNG INJURY

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Alveolar type 2 (AT2) cells and bronchioalveolar stem cells (BASC) perform critical regenerative functions in response to lung injury. Published data show that non-hematopoietic bone marrow-derived very small embryonic-like stem cells (VSELs), can differentiate *in vivo* into surfactant protein C (SPC)-producing AT2 cells in the lung. Here we test directly whether VSEL derived BASC and AT2 cells function to produce differentiated progeny. With the use of a reporter mice in which the H2B-GFP fusion protein is driven from the murine SPC promoter, we tested whether bone marrow-derived VSELs or non-VSEL/non-hematopoietic stem cells (n-VSEL/n-HSCs) are capable of engrafting into BASCs and AT2 cells that function as lung progenitor cells. Immediately following bleomycin administration, WT recipient mice underwent intravenous administration of VSELs or n-VSEL/n-HSCs from SPC-H2B-GFP mice. GFP+ AT2 and BASC were isolated and tested for progenitor activity using *in vitro* organoid assays. After 21 days *in vivo*, we observed differentiation of VSELs but not n-VSEL/n-HSCs into phenotypic AT2 and BASC consistent with previous data in irradiated recipients. Subsequent *in vitro* organoid assays revealed that VSEL-derived AT2 and BASC maintained physiological potential for differentiation and self-renewal. These findings prove that VSELs produce functional BASC and AT2 cells, and may open new avenues using VSELs to develop effective cell therapy approaches for patients with lung injury.

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S9.L8

EXENDIN-4 AFFECTS METABOLIC AND SECRETORY ACTIVITIES OF HUMAN DERMAL FIBROBLASTS CULTURED IN A HYPERGLYCEMIC ENVIRONMENT

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Treatment of hard-healing wounds in diabetic individuals still remains a challenging medical problem. Exendin-4, a glucagon-like peptide-1 receptor agonist with antidiabetic properties, was found to have beneficial effects on wound healing in rodents but, to date, there have been no reports on the possible exendin-4 effects on human dermal fibroblasts. Therefore, we have examined the effects of this drug on the metabolic and secretory activities of human skin fibroblasts. We used a commercial human fibroblast cell line CLTH Dermal Fibroblasts incubated in the high-glucose (5 g/l, i.e., 25 mmol/l) Dulbecco's Modified Eagle's Medium (DMEM) in a humidified atmosphere with 5% CO_2 at 37°C in the presence of 0–100 nmol exendin-4 for 3 days. The fibroblast metabolic activity was measured using MTT method. The secretory activity was assessed regarding the matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) secretion and gene expression by immunoenzymatic methods and a real-time PCR reaction, respectively. Collagen type I and glycosaminoglycan (GAG) contents in fibroblast colonies were examined using ELISA method. At a concentration of 20 nmol, exendin-4 inhibited the fibroblast metabolic activity but had no effects when used at higher concentrations. The drug did not affect significantly the MMP-9 content and MMP-9 gene expression in fibroblast colonies. On the other hand, it increased both TIMP-1 content and gene expression when used at lower concentrations. Also, at the same doses, exendin-4 markedly increased GAG content without affecting collagen production. To conclude, exendin-4 augmented the production of TIMP-1, an important pro-healing protein, and GAG, a basic constituent of the extracellular matrix. Hence, the drug has a potential to improve wound healing process in diabetic human subjects.

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S9.P1

DIRECT EFFECT OF VITAMIN C ON CELL CYCLE AND APOPTOSIS GENE AND PROTEIN EXPRESSION IN CANCER CELLS

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Vitamin C regulates many physiological processes. Protects against immune system deficiencies, cardiovascular disease, prenatal health problems, and eye disease. It is also scavenger of free radicals in biological systems participating in the first line of antioxidant defence, protecting lipid membranes, and proteins from oxidative damage. Vitamin C has a controversial history in cancer treatment; however, there are reports described that ascorbate, given in pharmacologic doses as effective in treating some cancers and in improving patient well-being. In the present study, we determined the effect of high physiological or pharmacological dose of vitamin C on selected parameters in ovarian cancer. Ovarian epithelial (OVCAR-3) and granulosa (KGN) cancer cells were incubated for 1 hour with vitamin C in high physiological (0.1 mM) or pharmacologic concentrations (0.5, 1, 10, 20 mM). Cells proliferation, membrane cell permeability, caspase-3 activity, cell morphology, UCP-2, CYCS as marker of oncosis, and BECN1, ATG5/7 gene expressions as markers of autophagy were measured. In both types of cells, an inhibitory effect of vitamin C on cell proliferation corresponding with inhibitory effect on cyclin A and CDK2 protein expression and stimulatory effect on membrane cell permeability was noted. A stimulatory effect on caspase-3 activity in KGN cells, while no effect on caspase-3 activity in OVCAR-3 cells suggested cell-specific apoptotic action of vitamin C. Morphological observations and data concerning oncosis and autophagy gene expression showed various types of cell deaths, including autophagy, oncosis and apoptosis in OVCAR-3 cells, and near-entirely apoptosis in KGN cells. Our study filling the gap in research on the mechanism of vitamin C action in ovarian cancer suggesting direct effect on cell cycle and apoptosis. Moreover, points to action of vitamin C as PARP inhibitor.

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S9.P2

RELEASE OF INTERLEUKIN-6 IS DEPENDENT ON A2B1 INTEGRIN

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Cardiac fibrosis, which determines stiffness of the heart wall, is a complex phenomenon. Cardiac fibroblasts constitute about 70% of cardiac cells and determine extracellular matrix metabolism. Moreover, cardiac fibroblast sense not only the biochemical stimuli but also the physical changes in the environment which may lead to release of cytokines, interleukin-6 (IL-6) including. The aim of the present study was to examine whether the stiffness of the environment of cardiac fibroblasts can influence the release of IL-6 and soluble IL-6 receptor (sIL-6). Moreover, it verify the role of integrin $\alpha 2\beta 1$ activation and its intracellular signaling in these processes. The research was conducted using stable cardiac fibroblast cell line, cultured on the polyacrylamide gels with different stiffness (soft gel elasticity 2.23 ± 0.8 kPa and stiff gel elasticity 8.28 ± 1.06 kPa), as well as on integrin $\alpha 2\beta 1$ knockout animals (homozygous Itga2tm1.1Tkun/tm1.1Tkun) and wild-type mice. Cardiac fibroblasts settled on the soft gel demonstrated increase in expression of the $\alpha 2$ integrin subunit on both gene and protein level and subsequent higher release of IL-6 and sIL-6 in those cells. The inhibition of $\alpha 2$ integrin subunit by means of siRNA or administration of TC-I 15 ($\alpha 2\beta 1$ integrin inhibitor) decreased the release of IL-6. Administration of Src inhibitor increase release of IL-6 in cells cultured on soft gel. Both heart and serum of integrin $\alpha 2\beta 1$ knockout animals exhibit markedly lower levels of IL-6 in comparison to wild type animals. No changes in sIL-6 level were observed in animals. Our data suggest that release of IL-6 and sIL-6 in cardiac fibroblast is related to changes in physical properties of the cell environment. Moreover, integrin $\alpha 2\beta 1$ exerts a regulatory effect on IL-6 release *via* Src signaling.

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S9.P3

MIR-138-5P AS A PREDICTIVE FACTOR OF SEVERE ORAL MUCOSITIS IN PATIENTS WITH HEAD AND NECK CANCER UNDERGOING INTENSITY - MODULATED RADIATION THERAPY

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Globally, the incidence of head and neck cancers (HNCs) is on the 6th location across all malignant neoplasms. Radiotherapy (RT), widely used in the treatment of HNCs, causes numerous troublesome side effects. The most common and most serious complication of RT is oral mucositis (OM). Severe OM (grade ≥3) often forces the reduction of radiation doses or leads to treatment discontinuation, which significantly reduces its effectiveness. Despite, that the introduction of the intensity-modulated radiation therapy (IMRT) technique led to the significant reduction of radiation reactions, most patients still develop OM. Interestingly, it was observed, that even patients receiving the same dose of radiation may develop various degrees of OM severity (probably due to patient variability at the molecular level). miR-138 belongs to the group of small, non-coding RNAs responsible for the regulation of many different genes, including this involved in DNA repair mechanisms (e.g. *ERCC1*, *ERCC2*, *PARP2*). The study material was peripheral blood serum obtained from 36 patients with pathomorphological diagnoses of HNC, subjected to IMRT. The expression level of miR-138-5p was assessed by the RT-PCR method and commercially available probes. The study group was dominated by men (86%). The median age of the patients was 63 years. According to the 7th edition of TNM, the dominant features were: T3 (53%), N + (67%) and M0 (100%). We noted, that patients with severe OM (>grade 3) after 4, 5, 6, and 7 weeks of RT had significantly higher levels of pre-treatment expression of miR-138-5p. Pre-treatment miR-138-5p expression demonstrated to have a significant predictive value for weeks 4th to 7th of OM evaluation. The sensitivity and specificity of this biomarker were, respectively, in the 4th week: 100% and 86% ($p < 0.0001$), in the 5th week: 86% and 79% ($p = 0.0012$), in the 6th week: 100% and 86% ($p < 0.0001$) and in the 7th week: 83% and 92% ($p < 0.0001$). miR-138-5p is a promising biomarker, that may serve as a useful tool in the prediction of more severe OM in patients with HNCs undergoing IMRT.

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S9.P4

THE ASSESSMENT OF THE PROLIFERATIVE POTENTIAL OF BRONCHOALVEOLAR STEM CELLS AND ALVEOLAR TYPE 2 CELLS ISOLATED AT VARIOUS STAGES OF BLEOMYCIN-INDUCED LUNG INJURY

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Alveolar type 2 (AT2) and bronchoalveolar stem cells (BASC) are rare subpopulations of lung cells that are directly responsible for the regeneration of small airways and alveoli, which are vulnerable to injury. The aim of the experiment is to assess the physiological ability of AT2 cells and BASCs to proliferate and self-renew in response to bleomycin-induced lung injury. Studies were conducted on healthy 6-8 week old C57BL/6J mice. Animals were administrated intratracheally with bleomycin (2.5 mg per kg. b.w.) suspended in 50 µl of saline. On 0, 3, 5, 7, 10, 12 and 14 days after bleomycin administration animals were sacrificed. To evaluate our hypothesis we conducted flow cytometry analysis, AT2 and BASC FACS sorting, organoid assay, western blot analysis (WB) and full proteome analysis. FACS analysis showed a significant increase on the 5th day of the amount of AT2 and BASCs sorted. This indicates that cells up to day 4 from lung injury have the highest proliferative potential. To confirm this hypothesis, organoid cultures were established. Flow cytometric analysis confirmed the hypothesis that AT2 cells and BASCs isolated on the 3rd day from injury have the highest proliferative potential. WB analysis showed an increase in expression of TTF1 and pro-SPC proteins in group from day 7. The literature states that the newly formed AT2 and BASC cells begin to present characteristic TTF1 and pro-SPC proteins only from 2–3 days, what confirms our results. In proteome analysis we identified 5739 proteins, and observed up-regulation of proteins responsible for cell proliferation and differentiation process in 3rd day after injury what confirms our hypothesis.

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