MEDICAL UNIVERSITY OF WARSAW

5th Baltic Stem Cell Meeting

(BSCM)

Conference Book

21-23 October 2022
Warsaw, Poland

Medical University of Warsaw
Didactic Center
ul. Księcia Trojdena 2a, 02-019 Warsaw

Official website:
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List of Speakers:

Prof. Grzegorz Basak (Medical University of Warsaw, Poland),
Prof. Deepa Bhartiya (Epigeneres Biotech Pvt Ltd; ICMR-NIRRCH, India),
Prof. Katarzyna Brzeźniakiewicz-Janus (University of Zielona Góra, Hospital Gorzów Wlkp., Poland),
Msc. Kamila Bujko (Medical University of Warsaw, Poland),
Prof. Giovanni Camussi (University of Turin, Italy),
Dr Andrzej K Ciechanowicz (Medical University of Warsaw, Poland),
Prof. Rober Peter Gale (Imperial College of Science, Technology and Medicine London, UK; Sun Yat-sen Cancer Centre Gunangzhou, China)**,**
Prof. Philip Henon (CellProthera, France),
Prof. Wiesław Jędrzejczak (Medical University of Warsaw, Poland),
Prof. Bożena Kamińska (Nencki Institute of Experimental Biology, Poland),
Prof. Yupo Ma (Renaissance School of Medicine at Stony Brook University, USA),
Prof. Magdalena Kucia (Medical University of Warsaw, Poland),
Prof. Marek Łos (Silesian University of Technology, Poland),
Prof. Marcin Moniuszko (Medical University of Bialystok, Poland),
Prof. Manuela Monti (University of Pavia, Italy),
Dr Kshama Pansare (Epigeneres Biotech Pvt Ltd, India),
Msc. Joanna Płaczkowska (Polish Stem Cells Bank, FamiCord Group),
Prof. Janina Ratajczak (University of Louisville, USA),
Prof. Mariusz Z Ratajczak (University of Louisville, USA; Medical University of Warsaw, Poland),
Dr. Artur Reginia (Pomeranian Medical University in Szczecin, Poland),
Prof. David Smadja (Université de Paris / Hopital Europeen Georges Pompidou, France),
Dr Małgorzata Czystowska-Kuźmicz (Medical University of Warsaw, Poland)**,**
Prof. Torsten Tonn (Technical University Dresden, Germany),
Prof. Henning Urlich (University of Sao Paulo, Brasil),

5th Baltic Stem Cell Meeting
Conference Program

Friday, 21 October 2022		
Opening Session		
12:00-13:30	**Registration**	
13:30-14:00	**Opening Ceremony**	
Plenary Lectures **Chairmen:**	***Prof. Donald M Miller and Prof. Igor Resnick***	
14:00-14:30	**Prof. Robert Peter Gale** (Centre for Haematology Imperial College of Science, Technology and Medicine, London UK/ Department of Hematologic Oncology Sun Yat-sen Cancer Centre, Gunangzhou, China)	***Man or elephant need more stem cells?***
14:30-15:00	**Prof. Mariusz Z Ratajczak** (University of Louisville, USA, Medical University of Warsaw, Poland)	***New developments in experimental hematology***
15:15-15:45	*Coffee Break*	
Session: VSELs I **Chairmen:**	***Prof. Mariusz Z Ratajczak and Prof. David Smadja***	
15:45-16:05	**Prof. Deepa Bhartiya** (Epigeneres Biotech Pvt Ltd, Mumbai / ICMR-NIRRCH, Mumbai, India)	***Endocrine insults disrupt VSELs functions in mice reproductive organs and initiate various pathologies including cancer***
16:10-16:30	**Prof. David Smadja** (Université Paris Descartes / Hopital Europeen Georges Pompidou, France)	***Endothelial stem cells and VSEL: past, present and future***
16:35-16:55	**Prof. Manuela Monti** (University of Pavia, Italy)	***Differentiation potentials of Very small embryonic-like stem cells isolated from human umbilical cord blood***
17:00-17:30	*Coffee Break*	
Session: Stem Cell in Neurology **Chairmen:**	***Prof. Bożena Kamińska and Prof. Henning Ulrich***	
17:30-17:50	**Prof. Bożena Kamińska** (Nencki Institute of Experimental Biology, Poland)	***Targeting tumor stem cells as a therapeutic strategy in glioblastoma***
17:55-18:15	**Prof. Henning Ulrich** (Department of Biochemistry, University of São Paulo, Brasil)	***Purinergic signaling in neurogenesis and Huntington's disease***
18:20-18:35	**Dr. Artur Reginia** (Department of Psychiatry, Pomeranian Medical University in Szczecin, Poland)	***Will regenerative medicine change psychiatry?***
18:40-19:40	***Welcome reception***	

Saturday, 22 October 2022

Session: CAR-T cells

Chairmen: *Prof. Yupo Ma and Prof. Wiesław Jędrzejczak*

9:30-9:50	**Prof. Wiesław Jędrzejczak** (Department of Hematology, Transplantation and Internal Medicine, Medical University of Warsaw, Poland)	***Baltic stem cell donors***
9:55-10:15	**Prof. Yupo Ma** (Department of Pathology, Renaissance School of Medicine at Stony Brook University, USA)	***Novel approach to treat autoimmune disorders including systemic lupus erythematosus, by targeting the "root cause", B cells and Plasma cells, using BCMA-CD19 Compound CAR***
10:20-10:40	**Prof. Torsten Tonn** (Chair Transfusion Medicine, Technische Universität Dresden, Dresden, Germany)	***NK cells as carriers for chimeric antigen receptor (CAR) for targeted cancer immunotherapies***
10:45-11:05	**Prof. Grzegorz Basak** (Department of Hematology, Transplantation and Internal Medicine, Medical University of Warsaw, Poland)	***Today and tomorrow: CAR T in everyday clinical practice***
11:10-11:40	*Coffee Break*	

Session: Covid-19

Chairmen: *Prof. David Smadja and Prof. Deepa Bhartiya*

11:40-12:00	**Prof. David Smadja** (Université Paris Descartes / Hopital Europeen Georges Pompidou, France)	***Trust, Fake news and Covid-19: how public communication have build a new area***
12:05-12:25	**Prof. Magdalena Kucia** (Laboratory of Regenerative Medicine, Medical University of Warsaw)	***Stem cells and Covid-19***
12:30-12:50	**Prof. Katarzyna Brzeźniakiewicz-Janus** (Department of Hematology, University of Zielona Gora, Hospital Gorzow Wlkp., Poland)	***Treat or not to treat hematological patients during Covid-19 infection***
12:55-13:55	*Lunch Break*	

Session: VSELs II

Chairmen: *Prof. Janina Ratajczak and Prof. Katarzyna Brzeźniakiewicz-Janus*

13:55-14:15	**Prof. Magdalena Kucia** (Laboratory of Regenerative Medicine, Medical University of Warsaw, Poland)	***Developmental origin of VSELs***
14:20-14:40	**Prof. Janina Ratajczak** (University of Louisville, USA)	***Mystery population of small cells in BM and its potential relationship to VSELs***
14:45-15:05	**Kamila Bujko** (Laboratory of Regenerative Medicine, Medical University of Warsaw, Poland)	***Troubleshooting of VSELs sorting***
15:10-15:40	*Coffee Break*	

Session: New advances in regenerative medicine I

Chairmen: *Prof. Marek Łos and Prof. Philip Henon*

15:40-16:00	**Prof. Philip Henon** (CellProthera, Mulhouse, France)	***Progresses in CD34+ cells knowledge and therapeutic use: from hematopoietic stem cells to VSELs***
16:05-16:25	**Dr Andrzej K Ciechanowicz** (Laboratory of Regenerative Medicine, Medical University of Warsaw)	***Bone marrow-derived VSELs engraft as lungs progenitors after Bleomycin-induced lung injury***
16:30-16:50	**Prof. Marek Łos** (Biotechnology Center, at Silesian University of Technology, Gliwice, Poland)	***Novel biomaterials for tissue Engineering with the use of Transdifferentiation***
16:55-17:15	**Joanna Płaczkowska** (Polish Stem Cells Bank (PBKM)/ FamiCord Group)	***Polish Stem Cell Bank (FamiCord Group) - From cell culture to ATMP manufacturing***
17:20-17:40	**Dr Felicitas Mungenast** (TissueGnostics GmbH, Vienna, Austria)	***Applications of Tissue Cytometry – Immunophenotyping, Spatial Analysis and Tissue Classification***
17:45-19:00	***Poster session***	

Sunday, 23 October 2022		
9:30-10:00	*Coffee*	
Session: New advances in regenerative medicine II **Chairmen:**	***Prof. Manuela Monti and Prof. Marcin Moniuszko***	
10:00-10:20	**Prof. Giovanni Camussi** (University of Turin, Italy)	***Stem cell derived extracellular vesicles and kidney injury recovery***
10:25-10:45	**Prof. Marcin Moniuszko** (Medical University of Bialystok, Poland)	***Can stem cells reverse airway remodeling?***
10:50-11:10	**Dr Małgorzata Czystowska-Kuźmicz** (Department of Biochemistry, First Faculty of Medicine, Medical University of Warsaw)	***Extracellular vesicles as modern tools in diagnostics and therapy***
11:15-11:35	**Dr Kshama Pansare** (Epigeneres Biotech Pvt Ltd, Mumbai, India)	***Nano-formulation of Extremely Active Resveratrol (XAR) restores VSELs function in chemoablated mice gonads***
11:40-12:10	*Coffee Break*	
Session: Oral presentations – best selected abstracts **Chairmen:**	***Prof. Henning Ulrich and Prof. Mariusz Z Ratajczak***	
12:10-13:30	**Susanne Leszczak**	***Preliminary studies on identification of proteome differences between Alveolar type 2 cells and Bronchoalveolar stem cells***
	Dr Andrzej K Ciechanowicz	***The evaluation of the proliferative potential of bronchoalveolar stem cells and alveolar type 2 cells isolated at various stages of bleomycin-induced lung injured***
	Dr Vira Chumak	***Proteomic analysis of murine bone marrow VSELs at steady-state conditions and after in vivo stimulation by nicotinamide and follicle-stimulating hormone***
	Dr Lilya Lehka	***Potential role of unconventional myosin VI in myogenesis and muscle functioning***
	Dr Marek Konop	***The regenerative potential of keratin-butyrate dressing on skin wound healing in diabetic rats***
	Dr Alicja C. Głuszko	***Lipidomic profiling of small extracellular vesicles from head and neck squamous cell carcinoma cells uncovers signatures for tumor hypoxia***
13:30-14:00	*Snack Break*	
14:00-14:30	**Closing Ceremony**	
VSELs workshop at Laboratory of Regenerative Medicine, Medical University of Warsaw*		
14:30-18:30	Dr Vira Chumak Msc Kamila Bujko Dr Andrzej K. Ciechanowicz	*Flow cymetry and cell sorting* *Mass spectrometry*

Selected Abstracts for *Oral* and *Poster* Session

Abstract 001 – oral presentation

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

Andrzej K. Ciechanowicz[1], Erika Suchocki[1], Susanne Leszczak[1], Wen Xin Lay[1], Christian Leszczak[1], and Diane S. Krause[2]

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Introduction: The aim of the experiment was to assess the physiological ability of Alveolar Type 2 (AT2) cells and Bronchoalveolar Stem Cells (BASCs) to proliferate and self-renew in response to bleomycin-induced lung injury. The studies address gaps in our knowledge of lung regeneration under pathological conditions. AT2 and BASCs are rare subpopulations of lung cells that are directly responsible for the regeneration of small airways and alveoli. AT2 cells and BASCs are vulnerable to damage, which is the reason for slow and often inadequate lung regeneration. Therefore, it is necessary to develop an effective methodology for their isolation from damaged lungs, multiplication and their in vitro differentiation, and then implantation into the damaged organ. Progressive lung damage may affect the proliferative potential and differentiation of lung stem cells in various ways. Determination of the stage of lung injury, in which the lung stem cells have the highest potential for differentiation and proliferation, can directly contribute to the development of the method of in vitro generation of pulmonary stem cells for later transplantation into the lungs for repair.

Materials and Methods: Studies were conducted on healthy 6-8 week old C57BL/6J mice. On 3, 5, 7, 10, 12 and 14 days after bleomycin administration, the animals were sacrificed by cervical dislocation.

From isolated lungs the AT2 (CD31-, CD45-, Ep-CAM+, Sca-1-) and BASCs (CD31-, CD45-, Ep-CAM+, Sca-1+) were FACS sorted. Those populations were quantified and sorted BASCs and AT2 cells were used for organoid. Organoids were established from 3 000 AT2 cells or 3 000 BASCs and 100 000 MLG cells. Organoids were cultured for 21 days. Then, organoids were stained and analyzed with the use of flow cytometry.

Results and Discussion: 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 bleomycin administration have the highest proliferative potential. To confirm this hypothesis, organoid cultures were established (from each collection point and from each cell type - AT2 and BASCs). Flow cytometric analysis of organoid culture confirmed that AT2 cells and BASCs isolated on the 3rd day from bleomycin administration were characterized by the highest proliferative potential. In addition, it was confirmed by TTF1 and pro-SPC protein expression in lung tissue.

Conclusion

• Bleomycin-induced lung injury do influence on proliferative and self-renawal potential of AT2 and BASCs

• AT2 cells and BASCs in the first phase of lung injury present the highest proliferative potential and self-renewal ability.

*This work was supported by the Miniatura NCN grant 2017/01/X/NZ3/00250.

Abstract 002 – oral presentation

PRELIMINARY STUDIES ON IDENTIFICATION OF PROTEOME DIFFRENCES BETWEEN ALVEOLAR TYPE 2 CELLS AND BRONCHOALVEOLAR STEM CELLS

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Introduction:
The termination point of the mouse airways is the bronchoalveolar duct junction (BADJ), where the cells shift from mainly consisting of club cells and ciliated cells, to alveolar type 1 and 2 cells. Studies show this area houses the bronchoalveolar stem cells (BASC) [1-5], a potentially crucial component of the lung regeneration process [6-7]. The BASC cells, whilst usually in a quiescent state, become activated and proliferate in response to alveolar injury in vivo [4,8]. However, the lack of a specific marker gene for the BASCs has led to extensive debate on the topic of the cell indeed being a distinctive stem cell population, or just a subset of the AT2 cell, with which it shares many markers as well as functions [1-2,5]. Concluding, methods other than cell surface marker differences should be explored to definitely distinguish BASC cells from AT2 cells.

Aim of the study:
The aim of this project is to identify distinctive marker proteins characteristic for a BASC and AT2 cells populations isolated from the lungs of mice.

Material and methods: The study was conducted on 24 healthy wild type (C57BL/6Clzd) mice. From isolated lungs we FACS sorted populations of AT2 cells (CD31neg, CD45 neg, Ep-CAM pos, Sca-1neg) and BASC (gating strategy: CD31neg, CD45 neg, Ep-CAM pos, Sca-1 pos). Those cells were lyzed/digested and analyzed with the use of nano-UHPLC coupled to ESI-Q-TOF MS. Mass spectra were analyzed with the use of DataAnalysis program and identified with the MASCOT server and SwissProt database. Identified proteins were annotated to signaling pathways and biological processes with the use of Uniprot.org.

Results:
During proteomic analysis, we identified 1076 proteins, among which 60 were characteristic and observed only among bronchoalveolar stem cells, 389 were observed only in alveolar type 2 cells, and 626 in both cell populations. Those two populations differed from each other in many of signaling pathways. The highest number of diverse proteins were involved in cell metabolism.

Conclusions:
We have identified 515 proteins that might serve as potential biomarkers differentiating BASCs from AT2 cells.

Funding:
This study was supported by the funding from Medical University of Warsaw (2W10/1/M/MG/E.NED/20)

[1] Lung regeneration by multipotent stem cells residing at the bronchoalveolar-duct junction, 2019, Qiaozhen Liu. [2] Bronchoalveolar stem cells are a main source for regeneration of distal lung epithelia in vivo, 2019, Isabelle Salwig. [3] C-Myc regulates self-renewal in bronchoalveolar stem cells, 2011, Jie Dong. [4] Identification of bronchioalveolar stem cells in normal lung and lung cancer, 2005, Carla F. Bender Kim [5] BASC-ing in the glow – bronchioalveolar stem cells get their place in the lung, 2019, Maria C Basil & Edward E Morrisey. [6] XB130 promotes bronchioalveolar stem cells and Club cell proliferation in airway epithelial repair and regeneration, 2015, Hiroaki Toba. [7] A Gata6-Wnt pathway required for epithelial stem cell development and airway regeneration, 2008, Yuzhen Zhang. [8] Cellular kinetics and modelling of bronchioalveolar stem cell response during lung regeneration, 2008, RD Nolen-Walston.

Abstract 003 – oral presentation

PROTEOMIC ANALYSIS OF MURINE BONE MARROW VSELS AT STEADY-STATE CONDITIONS AND AFTER IN VIVO STIMULATION BY NICOTINAMIDE AND FOLLICLE-STIMULATING HORMONE

Vira Chumak[1], Katarzyna Sielatycka[2,3], Andrzej Ciechanowicz[1], Kamila Bujko[1], Mariusz Z Ratajczak[1,5] and Magdalena Kucia[1,4,5]

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Introduction.
Very small embryonic-like stem cells (VSELs) are a dormant population of development early stem cells deposited in adult tissues that as demonstrated contribute to tissue/organ repair and regeneration. In the current report, we performed proteomic analysis of VSELs purified from murine bone marrow (BM) after repeated injections of nicotinamide (NAM) + Follicle-Stimulating Hormone (FSH) – an effective combination to expand these cells. We have identified several GO biological processes regulating development, organogenesis, gene expression, signal transduction, Wnt signaling, DNA repair etc. We report that VSELs express a unique panel of proteins that only partially overlapped with the proteome of BM – derived hematopoietic stem cells (HSCs) and hematopoietic mononuclear cells (MNCs) and respond to FSH+NAM stimulation by expressing proteins involved in the development of all three germ layers.
Materials and Methods.
C57BL/6 male mice were injected by combination of FSH+NAM for 10 days. After than bone marrow VSELs were isolated and prepared for proteomic analysis. By employing the Gene Ontology (GO) resources, we have performed a combination of standard GO annotations (GO-CAM) to produce a network between BM steady-state conditions VSELs (SSC) and FSH+NAM expanded VSELs (FSH+NAM).
Results.
We noticed that up to 200 annotated proteins were common for both experimental groups SSC-VSELs and FSH+NAM VSELs. We found that 20 proteins were down-regulated and 7 proteins up-regulated in a statistically significant manner in FSH+NAM VSELs compared to SSC-VSELs. The most enriched GO biological pathways belonged to regulation of gene transcription, multicellular organism development, cell cycle and division, cytoskeleton organization, protein maturation, transport, and immunity. Among the down-regulated proteins we found NHL repeat-containing protein 2 (NHLC2) (60 fold), microtubule-actin cross-linking factor 1 (MACF1) (29 fold), F-box only protein 9 (FBX9) (24 fold), and several histones. The group of up-regulated proteins includes proteins involved in early development, such as CUB and zona pellucida-like domain-containing protein 1 (CUZD1), Protein Daple (DAPLE), core-binding factor subunit beta (Cbfβ), and condensin-2 complex subunit G2 (CNDG2). The most up-regulated protein in expanded VSELs was the centrosomal protein of 135 kDa (CP135) required for centriole biogenesis.
Conclusion.
VSELs proliferate and differentiate in response to NAM + FSH stimulation and express genes involved in organogenesis and development, signal transduction, Wnt and insulin signaling, cytoskeleton organization, cell adhesion, inhibiting apoptosis, protein transport and stabilization, DNA repair, immune response, and regulation of circadian rhythm.

Abstract 004 – oral presentation

POTENTIAL ROLE OF UNCONVENTIONAL MYOSIN VI IN MYOGENESIS AND MUSCLE FUNCTIONING

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Introduction.
Skeletal muscle stem cells (satellite cells) differentiate into myoblasts, which in turn fuse into myotubes and are considered to play a crucial role in muscle repair and remodeling. While studies on muscle function are intensively performed, new players involved in the regulation of myogenesis and muscle development remain enigmatic. One such player is unconventional myosin VI (MVI).
Materials and Methods.
In the current study, the following technics were used: fluorescent and confocal microscopy, western blot analysis, real-time qPCR, and flow cytometry.
Results.
We have shown that the mechanisms controlling cytoskeleton organization and myoblast fusion are dysregulated in the absence of MVI. This resulted in the formation of aberrant myotubes. Interestingly, the observed morphological changes were accompanied by the increased level of reactive oxygen species (ROS) both in myoblasts and forming myotubes isolated from mice with MVI knockout. In the lack of MVI, the outbreak of ROS led to a decrease in GSH/GSSG ratio. The activity of catalase in MVI-KO myogenic cells was also significantly reduced. Analysis of mitochondria status indicated the decrease of mitochondrial integrity in MVI-KO myogenic cells with respect to control ones. The reduction in the GSH/GSSG ratio and catalase activity was also noticed in the hindlimb muscles isolated from newborn MVI-KO mice. A decrease in the amount of IκB protein (a specific inhibitor of NFκB) was observed, which correlated with an increase in the level of the transcription factor NF-κB in the hind limb skeletal muscles of mice with the lack of MVI compared to wild-type animals. Additionally, this phenomenon was accompanied by a statistically significant increase in the levels of interleukin-6, a well-known proinflammatory cytokine. The biggest changes were observed in the hindlimb skeletal muscles of newborn SV mice.
Conclusion.
Obtained results not only provided new information on the involvement of MVI in modulating the redox state of myogenic cells and skeletal muscle but also deepened the knowledge of possible mechanisms involved in muscle pathologies related to the disruption of myogenic cell differentiation and muscle regeneration.

Abstract 005 – oral presentation

THE REGENERATIVE POTENTIAL OF KERATIN-BUTYRATE DRESSIN ON SKIN WOUND HEALING IN DIABETIC RATS

Marek Konop[1], Mateusz Rybka[1], Mateusz Szudzik[1], Łukasz Mazurek[1], Robert A. Schwartz[2], Anna K. Laskowska[3], Joanna Czuwara[4], Marcin Ufnal[1]

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Chronic non-healing wounds are the major medical problem around the world. There is a continuing search for new materials that will improve wound healing. In those context keratin-based materials have shown to be a promising choice due to their intrinsic biocompatibility, biodegradability and natural abundance.

The study aimed to evaluate the effect of keratin-butyrate powder (FKDP+0.1%NaBu) in a full-thickness skin wound model in diabetic rats. Physico-chemical assessment showed that obtained dressing possesses a heterogeneous structure and butyrate was slowly released into the wound. Moreover, the obtained dressing is nontoxic and supports cell growth. In vivo results showed keratin-butyrate dressing accelerates wound healing on days 4 and 7 post-injury ($p<0.05$). Histopathological and immunofluorescence examination revealed that applied dressing stimulated macrophages infiltration which favors tissue remodeling and regeneration. The highest mRNA expression level of interleukin 1β (IL-1β) was observed during the first two weeks in the control wound compared to FKDP+0.1%NaBu treated wounds, which was significantly decreased. In keratin-butyrate treated wounds enhanced mRNA expression of keratin 16 and 17 ($p < 0.05$) was observed on days 14, 21, and 28 post-injury.

The obtained results suggest that Na-Bu supports cutaneous wound healing in diabetic rats. Our data also show that FKDP+0.1%NaBu might be crucial for the activation of KRT16, KRT17, and down-regulation of IL-1β during wound healing in diabetic conditions.

Abstract 006 – oral presentation

LIPIDOMIC PROFILING OF SMALL EXTRACELLULAR VESICLES FROM HEAD AND NECK SQUAMOUS CELL CARCINOMA CELLS UNCOVERS SIGNATURES FOR TUMOR HYPOXIA

Alicja C. Głuszko[1], Andrzej Ciechanowicz[2], Mirosław J. Szczepański[1], Nils Ludwig[3]

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Introduction:
Molecular and functional alterations in head and neck squamous cell carcinomas (HNSCCs) are a result of commonly observed tissue hypoxia. In our previous study we demonstrated the influence of hypoxic conditions on increased release and proteomic profile of tumor-derived small extracellular vesicles (sEVs). The aim of this study was to characterize the lipidomic content of sEVs under normoxic and hypoxic conditions.

Materials and Methods:
HNSCC cells (PCI-30) and normal control cells (HaCaT keratinocytes) were exposed to 21 % (normoxia) and 1 % (hypoxia) oxygen supply. sEVs were isolated from supernatants using size exclusion chromatography (SEC) and characterized by nanoparticle tracking analysis, electron microscopy, immunoblotting, and high-resolution mass spectrometry. Gene expression levels based on RNA-seq data from HNSCC patients and clinical characteristics were obtained from the Cancer Genome Atlas (TCGA). Expression profiles of lipidomic signatures were compared between a total of 522 cases of primary HNSCC and 44 normal control samples.

Results:
Isolated sEVs ranged in size from 125–135 nm and carried CD63 and CD9 but not Grp94. The lipidome cargo components of sEVs isolated from HNSCC cells cultured under hypoxic conditions differed from normoxic sEVs in 84 % of total detected lipids and in 82 % in comparison to hypoxic HaCaT-derived sEVs. The most dysregulated group of lipids was Glycerophospholipids (GP) with Glycerophosphates (PA) being most upregulated. The least dysregulated was Fatty Acyls (FA). This data was validated on the transcriptome level using the TCGA HNSCC cohort. Specifically, GP-regulating genes were significantly up- (LPCAT1) and

downregulated (GDPD3) in HNSCC, significantly correlated with tumor stage (PLAG2G10, GDPD3); overall survival rate (PLA2G10, LYPLA2, LPCAT1, LPCAT2, DGKG, PPAP2B, JMJD7-PLA2G4B, CHKB, PNPLA6, ENPP3, GDPD3), and hypoxia and radiation gene signatures (PLA2G10, LYPLA2, LPCAT1, LPCAT2, DGKG, PPAP2B, JMJD7-PLA2G4B, PNPLA6, GDPD3).

Conclusion:
Tumor hypoxia was reflected in lipid profiles of sEVs, therefore, sEVs emerge as potential clinical biomarkers for hypoxic conditions and lipid metabolism in HNSCC.

Abstract 007 – poster presentation

ROLE OF PURINERGIC RECEPTOR P2X7 IN GLIOBLASTOMA AGRESSIVNES

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Introduction:
P2X7 is an ionotropic nucleotide receptor that acts as a cation permeable channel upon ATP stimulation. This receptor can also form a large transmembrane pore or transmit an ATP dependent signal without creating a channel at all. P2X7 receptors control many physiological and pathological cellular processes, and their increased expression is often associated with tumor progression. Since nucleotides are important signaling molecules in the central nervous system, P2X7 also plays an important but ambiguous role in glioblastoma biology.

Materials and Methods:
Therefore, our research aimed to investigate the expression and function of the P2X7 receptor in three human glioblastoma cell lines (U-138, U-251, LN-229) and in one rat glioma cell line (C6). We performed an in vitro and in vivo assays.

Results:
Although the receptor mRNA and protein were detected in all the studied cells, we found profound differences in their level. In U-138 human cell line, the receptor seemed to be inactive, while in U-251 human and C6 rat cell line its activation resulted in calcium influx and large pore formation. The viability of studied cells upon the administration of specific P2X7 agonist – BzATP – was not affected for U-138 and U-251, whereas for C6 cells a stimulatory effect was observed. This process is accompanied by an increase of prosurvival proteins expression (CD133, HSPA1, HSPA5) as well as an increase in phosphorylation of kinases influencing the progress of the cell cycle (Akt and p38 MAPK). It was also shown that P2X7 activation promoted cell adhesion, mitochondria depolarization, and overproduction of reactive oxygen species in C6 cells in vitro. The effect of the P2X7 receptor on the growth of C6 glioma tumors in vivo was also investigated. These results are in the line with the majority of the data obtained in vitro. The administration of BBG, a P2X7 inhibitor, effectively inhibited growth of the tumor mass and tumor development, reduced the amount of ATP with a simultaneous decrease of cancer-associated pro-survival protein expression. A decreased level of negative prognostic cancer markers (CD133, HSPA1, HSPA5, Akt, p38 MAPK, NOS-2) and proteins related to the epithelial-mesenchymal transition (N-cadherin, vimentin, β-catenin) were noted. It has also been shown that the P2X7 receptor may be involved in shaping the glioblastoma tumor microenvironment by modulating the immune response and regulating the level of inflammatory markers.

Conclusion:
These data bring some new insight into P2X7 influence on the biology of glioma. For the first time, the results showing the receptor-promoting effect on the proliferation of glioma cells in vitro were shown in correlation with the growth of neoplastic tumors in vivo. Moreover, the cell signaling pathways were investigated to elucidate the molecular mechanisms activated by P2X7 receptor in glioblastoma cells as well as the receptor engagement in shaping of glioma tumor microenvironment through modulation of inflammation marker profile.

Abstract 008 – poster presentation

THE NOVEL EVIDENCE THAT NOX2-ROS-NLRP3 INFLAMMASOME AXIS PLAYS A PIVOTAL ROLE IN TRAFFICKING OF HEMATOPOIETIC STEM PROGENITOR CELLS (HSPCS)

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Introduction:
NADPH oxidase 2 (Nox2) is a superoxide-generating enzyme that forms reactive oxygen species (ROS) to regulate the redox state involved in the self-renewal of hematopoietic stem/progenitor cells (HSPCs). ROS are potent activators of intracellular pattern recognition receptor known as Nlrp3 inflammasome, that regulates several aspects of HSPCs biology including their trafficking. Our previous research demonstrated a novel role of Nlrp3 inflammasome in migration, mobilization, homing and engraftment of HSPCs. Based on a role of Nox2 in activating Nlrp3 inflammasome we hypothesized that Nox2-ROS-Nlrp3 inflammasome axis in involved in trafficking of HSPCs.

Materials and Methods:
To shed more light on a role of Nox-2 as activator of Nlrp3 inflammasome in hematopoiesis we performed G-CSF and AMD3100 mobilization studies in Nox-2-KO animals. In parallel, we studied Transwell chemotaxis studies of Nox-2 deficient cells to bone marrow (BM) chemoattractants including SDF-1, S1P and eATP. We also studied homing and engraftment of Nox-2-KO cells in BM of wild type (WT) animals as well as homing and engraftment o WT BM cells in Nox-2-KO recipients. Nox-2-KO were also sublethal irradiated and we followed recovery of peripheral blood counts in these animals. The Nlrp3 inflammasome activity was evaluated by Glow assay.

Results:
We noticed that Nox2-KO animals have a profound defect in the trafficking of HSPCs as evidenced by impaired chemotaxis to BM chemoattractants and decrease in pharmacological mobilization. We also obsereved defect in homing and engraftment after transplantation as well as delayed hematological recovery from sublethal irradiation in Nox-2-KO animals. We also learned that Nox2-KO mice have almost completely abrogated activation of Nlrp3 inflammasome as assayed by immunofluorescence activation assay.

Conclusions:
We provide for the first time evidence that Nox2-ROS-Nlrp3 inflammasome axis operates in normal HSPCs and is crucial for regulating trafficking and post-irradiation recovery of these cells.

Abstract 009 – poster presentation

THE NOVEL EVIDENCE THAT P2X1 PURINERGIC RECEPTOR-NLRP3 INFLAMMASOME AXIS ORCHESTRATES OPTIMAL TRAFFICKING OF HEMATOPOIETIC STEM PROGENITOR CELLS (HSPCS)

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Introduction:
Extracellular adenosine triphosphate (eATP) and P2X family of purinergic receptors were for many years understudied and not fully appreciated as regulators of hematopoiesis. The P2X family of receptors consists of seven purinergic receptors (P2X1-7) where P2X1 receptor is the most sensitive to eATP. This prompted us to investigate the potential novel role of P2X1 in trafficking of HSPCs. We also become interested if P2X1 receptor mediated trafficking of HSPCs, occurs also similarly as P2X4 and P2X7, in Nlrp3 inflammasome-dependent manner.

Materials and Methods:
We employed mice with perturbed expression of P2X1 receptor and evaluated migration of HSPCs to major bone marrow (BM) chemoattractant including SDF-1, S1P and eATP. Subsequently we evaluated a role of P2X1 in
G-CSF and AMD3100 mediated mobilization of HSPCs as well as homing and engraftment of these cells in wild type (WT) animals. Finally, we evaluated effect of P2X1 receptor stimulation of activation of Nlrp3 inflammasome by employing Glow assay.

Results:
We report for a first time that ion-gated P2X1 purinergic receptor ids a novel regulator of migration, mobilization, homing and engraftment of HSPCs. We also demonstrate that this most sensitive receptor from P2X receptor family activates in HSPCs Nlrp3 inflammsome as mediator as of its biological function.

Conclusions:
The silent novel observation of this report is that the P2X1 purinergic receptor is involved in regulating migration, mobilization, homing, and engraftment of HSPCs. Moreover, activation of the P2X1 receptor in hematopoietic cells activated intracellular innate immunity pattern recognition receptor - Nlrp3 inflammasome, that as demonstrated by, is required for optimal trafficking of these cells out of BM into PB and back from PB into BM microenvironment. Thus, our data again confirms an involvement of purinergic signaling and elements of innate immunity, including Nlrp3 inflammasome in modulating the migration of HSPCs.

Abstract 010 – poster presentation

EXTRACELLULAR ADENOSINE INHIBITS HOMING AND ENGRAFTMENT OF HEMATOPOIETIC STEM CELLS

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Introduction.
Extracellular adenosine (eAdo) is a metabolite of extracellular adenosine triphosphate (eATP) and an important mediator of purinergic signaling that activates a family of G-protein coupled P1 purinergic receptors: A1, A2A, A2B and A3. We have previously shown that eAdo inhibits egress of HSPCs from BM into PB in response to pharmacological mobilization after administration of granulocyte colony stimulating factor (G-CSF)- and CXCR4 receptor antagonist AMD3100. We have also postulated that this effect on migration of HSPCs is mediated by inhibition of Nlrp3 inflammasome that occurs in heme oxygenase-1 (HO-1)-dependent manner. Since murine and human HSPCs express highly two P1 receptors – A2A and A2B, we decided to investigate which P1 receptor is responsible for defective migration of cells as well as we addressed a role of eAdo in homing and engraftment of HSPCs after transplantation.

Materials and Methods.
6–8 weeks old C57BL/6 J mice were injected with G-CSF (45 μg/kg daily) for 4 days by subcutaneous injection or AMD3100 (1 mg/kg once) for 1 day or adenosine receptor P1 antagonists, A2A inhibitor (SCH442416; 3 mg/kg daily) for 7 days or A2B inhibitor (PSB1115; 3 mg/kg daily) for 7 days via intraperitoneal injection. Peripheral blood (PB) and bone marrow (BM) samples were collected from all groups of mice. Then migration, mobilization, BMMNCs transplantation and proteomic analyses were performed.

Results.
We observed that inhibition of A2B but not A2A receptor improves pharmacological mobilization of HSPCs from BM into PB. We also demonstrated that eAdo inhibits in A2Bdependent manner homing and engraftment of murine HSPCs. eAdo stimulates murine BMMNC to induce phosphorylation of NF-kBp65, expression of Nrf2 transcription factor and cAMP that all are involved in upregulation of HO-1. This data explains at molecular level eAdo mediated inhibition of trafficking of hematopoietic cells due to upregulation of HO-1 via A2B receptor. Studying the proteomics profile of murine HSPCs we find that A2B inhibition led to up-regulation of APC, transcriptional factor SOX6 and NRBF-2. Such proteomics signature suggests that A2B inhibition promotes migration and proliferation of HSPCs.

Conclusion.
The modification of purinergic signaling in HSPCs and BM microenvironment by inhibiting negative effects of eAdo could be an important step in facilitating homing and engraftment of transplanted HSPCs.

Abstract 011 – poster presentation

INHIBITION OF PURINERGIC P2X4 AND P2X7 RECEPTORS DOWN-REGULATED THE EXPRESSION OF CANCER STEM CELL MARKERS IN HUMAN LEUKEMIA CELLS IN VITRO

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Introduction.
Leukemic stem cells (LSCs) are cells with a self-renewal capacity to repopulate leukemia (H. Han et al., 2021). In addition, quiescent or slow-cycling LSCs may survive therapeutic intervention and result in recurrence. In the case of human leukemia, a combination of CD34, CD38, and IL3Rα has enabled the prospective isolation of leukemia stem cells (J. E. Visvader et al., 2012). The leukemic niche also characterized by the expression of other cell surface markers such as CD133, CD96, CD44, CD47, Tie2, and N-cadherin. The bone marrow (BM) microenvironment can initiate the changes in potential leukemogenic cells and can induce the development of myelodysplasia and leukemia. One of the most important signaling pathways in BM is purinergic signaling via P1, P2X, and P2Y receptor families. In this study, we focused on the influence of two

purinergic receptors – P2X4 and P2X7 on the expression of a few selected cell surface markers. Despite the LSCs arising from hematopoietic stem cells, we study the leukemic cell phenotype change in the in vitro condition using acute amyloid leukemia cell lines: HL-60 and Hel 92.1.7.

Materials and Methods.
HL-60 and Hel 92.1.7 cells were cultured in IMDM and RPMI medium respectively with 10% of FBS at 37°C and 5% CO2 with saturating humidity. 0.5x106 cells were seeded in 12 well plates and treated with P2X receptor agonists 100 μM ATP and 100 μM Bz-ATP or antagonists 10 μM PSB-12054 (P2X4 inhibitor) and 10 μM A740003 (P2X4 inhibitor) for 14 days. The fresh portion of medium with appropriate amount of substances mentioned above were added every 48 hours. At the end of the experiment, the cells were collected, stained with fluorescence labeled antibodies and analyzed using BD FACSVerse flow cytometer.

Results.
We performed a long-term stimulation and inhibition of P2X4 and P2X7 receptors in HL-60 and Hel 92.1.7 AML cells. We observed that P2X4 inhibition significantly down-regulated the CD133 and CD96 cell surface marker expression in both studied cell lines. An interesting, similar effect was noted for 100 μM ATP. We noted additionally the decreased number of cells with phenotype CD34+/CD38-/CD123+ after the long-term P2X4 inhibition. The Bz-ATP treatment caused a slight increase in CD133 and CD96 levels compared to control cells. The P2X7 inhibition did not affect CD133 expression but down-regulated the level of the CD96 marker.

Conclusion.
The inhibition of P2X4 purinergic receptors significantly down-regulated the expression of CD133 and CD96 cell surface markers in human HL-60 and Hel 92.1.7 AML cell lines. Whereas, the inhibition of P2X7 receptor changed the expression of CD96 marker only.

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Abstract 012 – poster presentation

EFFECTS OF SALINOMYCIN ON LYMPHOMA CELLS VIA DISRUPTION OF MITOCHONDRIAL RESPIRATION AND STIMULATION OF CD20 EXPRESSION

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Introduction.
One of the effective treatment strategies in B-cell malignancies, including non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL), is based on the combination of chemotherapy with anti-CD20 monoclonal antibodies, such as rituximab. However, many patients develop resistance to such regimens. The inefficient response in such cases is frequently related to the diminished level of CD20 antigen on the surface of tumor cells. We have recently discovered that Salinomycin (SAL), a drug capable to eliminate tumor cells through restraining mitochondrial respiration, greatly increases the level of CD20 on the surface of lymphoma cells, making them more sensitive to rituximab treatment. We also found that SAL treatment induces changes in metabolic pathways that influence mitochondria function. To determine the mechanism of CD20 upregulation by SAL, we focused on elucidating whether the stimulation of CD20 expression is promoted by changes in mitochondria metabolism.

Materials and Methods.
For this purpose, we synthesized SAL derivatives that are unable to upregulate CD20 antigen (inactive derivatives). We examined the effects of SAL and its derivatives on lymphoma by measuring mitochondrial membrane potential (MMP), reactive oxygen species (ROS) production, and cellular respiration. We used mitochondrial potential-dependent dyes, such as JC-1 and TMRM (Tetramethylrhodamine, Methyl Ester, Perchlorate). The production of superoxide by mitochondria was detected by MitoSOX red reagent. Mitochondrial respiration and glycolysis were analyzed by monitoring the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using a Seahorse extracellular flux analyzer.

Results and conclusion.
Interestingly, we found that only salinomycin, but not its derivatives unable to upregulate CD20, increased the production of ROS, decreased the MMP, and decreased basal and maximal mitochondrial respiration. In summary, the results suggest that some signaling pathways connect mitochondrial function with the regulation of CD20 expression. However, the exact mechanism of the upregulation of CD20, involving mitochondrial activity, remains to be elucidated.

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Abstract 013 – poster presentation

EFFECT OF ZINC AND POLYPHENOLS CO-SUPPLEMENTATION ON THE DEVELOPMENT OF NEOPLASMS AND THE LEVEL OF MODIFIED NUCLEOSIDES IN RATS WITH BREST CANCER

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The aim of the study was to evaluate the effect of selected polyphenolic compounds: epicatechin, apigenin and naringenin, administered separately or in combination with zinc (Zn), on the growth and development of the neoplastic process induced by 7,12-dimethylbenz[a]anthracene (DMBA) in rats. The impact of supplementation with the above-mentioned compounds on the content of modified derivatives: 1-methyladenosine, N6-methyl-2'-deoxyadenosine, O-methylguanosine, 7-methylguanine, 3-methyladenine, 1-methylguanine, 2-amino-6,8-dihydroxypurine, 8-hydroxy-2'-deoxyguanosine in the urine of rats with mammary cancer was also assessed.

Female Spraque-Dawley rats divided into 7 groups were used in the study: animals without supplementation and supplemented with apigenin, epicatechin and naringenin separately or in combination with zinc (Zn). To induce the mammary cancer, rats were treated with DMBA. Modified derivatives were determined by a validated high performance liquid chromatography coupled to mass spectrometry method.

Based on the obtained results it can be said that supplementation of the animals with naringenin inhibits the development and progression of neoplastic process in rats treated with 7,12-dimethylbenzanthracene. Neoplastic tumors were found in only 2 of 8 rats (incidence: 25%) and were considered by at most grade 1 malignancy. The first palpable tumors in the group of animals receiving naringenin appeared at week two-three weeks later when comparing to other groups. The combination of zinc with flavonoids (apigenin, epicatechin and naringenin) seems to stimulate the process of carcinogenesis. The level of N6-methyl-2'-deoxyadenosine and 3-methyladenine in urine of rats were statistically significantly higher in the groups supplemented with

apigenin, epicatechin and naringenin administered in combination with Zn than in the groups receiving only polyphenolic compounds.

In conclusion, supplementation of rats with selected flavonoids administered separately or in combination with Zn has impact on the development of neoplasms and the level of modified nucleosides in urine of rats with breast cancer. For sure further studies are more than welcome in this field. The presented results call into question if the diet supplementation with more than one anti-cancer agent at the same time is likely to reduce the expected results of inhibiting the risk of carcinogenesis.

Abstract 014 – poster presentation

HELIGMOSOMOIDES POLYGYRUS **ANTIGENS ALTER THE MACROPHAGE ACTIVATION AND INCREASE THE PROLIFERATION OF BREAST CANCER CELLS *IN VITRO***

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Introduction:
Heligmosomoides polygyrus is an intestinal nematode of rodents. The antigens of this parasite have strong immunomodulatory properties. It has been shown that these antigens alter the phenotype of macrophages and the proliferation of some cancer cells.

Materials and Methods:
We explored the cytotoxic effect of somatic and excretory-secretory *H. polygyrus* antigens in concertation 10ug/ml on macrophages with the use of MTT test. The change of gene expression in macrophages stimulated by these antigens for 24 and 48 hours was evaluated using RT qPCR. We explored the gene expression encoding Arg1, iNOS, YM1, IL-4, IL-10, IL-6, TNF-alfa, CD206 and CCL2. Moreover, we measured the level of expression of surface markers CD124, CD206, CD64, CD11b, F4/80, TLR2 and TLR4 in macrophages stimulated by these antigens for 48 hours of culture. Then, we co-cultured macrophages stimulated by *H. polygyrus* antigens and breast cancer cells line EMT6, together for 48 hours.

Results:
The results we obtained prove that *H. polygyrus* antigens do not affect the viability of macrophages in 24 or 48-hour cultures. However, these antigens have an impact on the profile of the genes and surface receptors of macrophages. They increase the expression of both pro-inflammatory and anti-inflammatory molecules. What is more, *H. polygyrus* excretory-secretory antigens have a greater effect on gene and receptor expression than somatic *H. polygyrus* antigens of the same concentration. Moreover, *H. polygyrus* somatic and excretory-secretory antigens increase the proliferation of breast cancer cells in co-culture with macrophages and in single culture *in vitro*.

Conclusion:
H. polygyrus antigens contain molecules of pro-inflammatory and anti-inflammatory activity. What is more, some of these molecules can increase the proliferation of breast cancer cells *in vitro.*

Abstract 015 – poster presentation

RETINAL PIGMENTED EPITHELIUM GENERATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS AS A NEW DIRECTION FOR CELL-BASED TREATMENTS FOR AGE-RELATED MACULAR DEGENERATION

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Introduction:
Age-related macular degeneration (AMD) is the main cause of central vision loss in patients older than 55 years old globally, which is presented in two forms: neovascular (wet) and non-neovascular (dry). Existing treatments for wet AMD only partially improve outcomes and mostly maintain the condition. There are presently no effective treatments available to reverse dry AMD. Stem cell-based therapies have shown promising results for retinal degenerative diseases. The possibility to produce embryonic stem cell (ESC) called patient-specific induced pluripotent stem cells (iPSCs) provided a new horizon for AMD therapy.

Materials and Methods:
The review was written using information sources in the Pub Med. database, where articles of high scientific importance were selected. Keywords when writing include: AMD, stem cells, hiPSC, iPSC, hESC.

Results:
The most important challenge facing cell therapists in treating AMD is choosing the source of cells and methods to generate retinal pigmented epithelium (RPE) cells. However, since both hESCs and hiPSCs can differentiate toward RPE cells, controlling the potency of hPSCs differentiation into RPE cells is one of the important goals of many research teams. Several initiated studies distinguished straightforward differentiation of RPE from iPSCs. Researchers have differentiated iPSCs into RPE directly by adding chemical molecules affecting signaling pathways that are recognized to be critical in the development and specification of RPE.

Conclusion:
Following a series of preclinical investigations, PSC-derived RPE transplants for AMD patients are now a novel available therapy option. Phase I/II clinical trials have provided sufficient data to support the safety of proposed treatment methods. Since only RPE cells with Bruch's membrane need to be replaced, it is therefore likely that we will have a stem cell-based therapy for acute wet AMD sooner rather than later. We are still far from being able to cure late dry AMD due to the continuous loss of RPE inducing a subsequent loss of photoreceptors above the affected retina. To increase the effectiveness of RPE fabrication and their integration into the retina as well as to enhance the retina microenvironment for long-term integration and survival of transplanted cells, additional research must concentrate on subretinal delivery of the transplanted cell.

Abstract 016 – poster presentation

FINGERPRINT OF CIRCULATING MICRORNAS IDENTIFY ACUTE ISCHEMIC STROKE PATIENTS

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Introduction:
Stroke is the second-most common cause of death worldwide. Aim of the present study is to identify circulating miRNAs that are modulated in patients with stroke by to select specific miRNAs to be used as disease biomarkers to improve both diagnosis and prognosis.

Methods:
RNA was extracted from plasma samples and quality was assessed using a fluorometric electrophoretic assay. MiRNA profiling was performed using the Affymetrix platform using GeneChip 4.0. and they were validated by RT-qPCR using the Taqman Advanced Protocol.

Results:
Statistical analysis of the microarray data identified 404 differentially expressed probes. We found that targets of up regulated top miRNAs were most significantly associated with following Bioplanet pathways including: BDNF signaling pathway, Interleukin-2 signaling pathway, Pathways in cancer, FSH regulation of apoptosis, Axon guidance and TGFbeta regulation of extracellular matrix. ANKRD52, AGO1 were targeted by all types of differentially expressed miRNAs. Most susceptible to regulation by up-regulated miRNAs were ANKRD12 and HIF1A. Most susceptible to regulation by down-regulated miRNAs were GNAI2 and GRIN1.
Validation analysis showed that, the expression of miR-18a-5p was higher in ischemic stroke patients both at day-1 and day-7 compared to the control group ($p=0.001$, $p=0.009$, respectively). Similarly, miR-199a-5p was significantly higher in the ischemic stroke patient acute phase, and kept upregulated at day-7 as well ($p=0.001$, $p=0.009$, respectively). Similarly, miR-199a-5p was significantly higher in the ischemic stroke patient acute phase, and kept upregulated at day-7 as well ($p < 0.001$, $p=0.002$, respectively). The expression of miR-4467 was significantly lower in the ischemic stroke patients at day-1 compared to controls ($p<0.001$); the expression levels significantly increased at day-7 compared to day-1 ($p=0.004$). MiR-3135b expression was significantly downregulated in the ischemic stroke patients both at day-1 and day-7 compared to the control group ($p<0.001$, $p<0.001$, respectively).
Diagnostic values of baseline miRNAs were evaluated with ROC curve analysis. Baseline expression of all studied miRNAs showed diagnostic value for ischemic stroke in the acute stage. The area under the ROC curve for miR-18a-5p was 0.76 (95% CI, 0.62-0.91) $p=0.001$, for miR-199a-5p, it was 0.89 (95% CI, 0.80-0.97) $p<0.0001$.

Conclusions:
Our results identified several circulating miRNAs that are down- or up-regulated in stroke patients. Among those with the most relevant differential expression, several miRNAs were identified that are known to play a role in the pathophysiology of neurovascular diseases, paving the way to a new class of smart pathophysiology-based biomarkers in stroke.

Funding:
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Abstract 017 – poster presentation

DIAGNOSTIC UTILITY OF CIRCULATING MICRORNAS AND THE ROLE OF BDNF SIGNALING PATHWAY IDENTIFY PATIENTS WITH STROKE-EMBOLIC STROKE OF UNDETERMINED SOURCE

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Introduction:
Stroke is the second-most common cause of death worldwide. The aim of the present study is to identify and select specific miRNAs to be used as disease biomarkers to improve both prognosis and prediction.

Method:
In the exploratory cohort, we included 48 patients with an embolic stroke of undetermined source (ESUS). Among them, 24 of them had a single stroke, whereas 24 of them had a second stroke or TIA. In the validation cohort, we enrolled 60 patients with acute ischemic stroke and 32 healthy individuals in this study. Total RNA was extracted from plasma and quality was assessed with fluorometric electrophoresis. In total 48 microarray analysis was performed with the Affymetrix platform and microarray analysis results were validated by RT-qPCR. Statistical analysis was performed in TAC software and R using Signal information obtained from the TAC output. We performed FDR correction, logistic regression, Mann-Whitney test, and t-test and calculated AUC using ROCp R package. Coexpression analysis to identify genes authentically expressed was performed using Spearman correlation (cutoff=0.9, Rpval=0.05). qRT-PCR was done for validation analysis.

Results:
MiR-4786 (AUC=0.88; p=0.008), miR-1288 (AUC=0.93; p=0.027), miR-548ar-3p (AUC=0.85; p=0.009), Let-7e-5p (AUC= 0.52; p=0.005) and miR-125a-5p (AUC= 0.52; p=0.008) were upregulated, whereas miR-4676 (AUC= 0.91; p=0.003) was downregulated, in patients with multiple stroke compared to single stroke ESUS in microarray analysis. Besides, in our bioinformatic analysis, enrichment analysis showed IL-2 signaling pathway, lipid and lipoprotein metabolism, BDNF signaling pathway, MAPK signaling pathway, Intellectual Disability and Alzheimer's Disease are significantly related to ESUS- patients. Moreover, we validated these miRNAs between patients with acute ischemic stroke and healthy individuals by RT-qPCR. MiR-1288, miR-548ar were significantly lower ($p<0.0001$, $p<0.0001$), miR-4676 and miR-4786 were significantly higher in in patients with acute stroke ($p<0.0001$, $p<0.0001$), compared to healthy individuals.

Conclusions:
Our results identified several novel circulating miRNAs that are down- or upregulated in ESUS-stroke patients, as showing the predictive significance for the assessment of risk of the second stroke. Moreover, with an additional cohort we have also tested most significantly differentially expressed miRNAs in acute stroke patients compared to controls as a diagnostic biomarker. Our results showed that miRNAs can have potential as both diagnostic and prognostic utility in neurovascular diseases.

Funding:
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Abstract 018 – poster presentation

ALTERATION OF CIRCULATING ACE2-NETWORK RELATED MICRORNAS IN PATIENTS WITH COVID-19

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Introduction:
In this study we aimed to analyse the diagnostic and predictive utility of miRNAs, which were identified in our previous in silico analysis (miR-10b-5p, miR-124-3p, miR-200b-3p, miR-26b-5p, miR-302c-5p) in patients with COVID-19. We also aimed to unravel the functions of analyzed miRNAs, by using machine learning based tools, including SHAP for clinical data analysis, and bioinformatic tools for data mining, target predictions and enrichment analyses.

Methods:
Blood samples and clinical data of 79 COVID-19 patients were collected at three different time points, including the day of admission, 7-days and 21-days after admission, as well as one time-point of 32 healthy volunteers. The composite endpoint was hospitalisation length of stay (>21 days) and/or death in follow-up.

Results:
Delta low miR-200b-3p expression (after 7-days of admission) presents predictive utility in assessment of the hospital length of stay and/or death in ROC curve analysis (AUC:0.730, p=0.002). Delta low miR-200b-3p expression, together with diabetes mellitus (DM) are independent predictors of increased hospital length of stay and/or death (OR= 5.775; CI= 1.572- 21.214; p= 0.008, OR= 4,888; CI= 1.001- 23.858; p= 0.050, respectively). The expression levels of miR-26b-5p and miR-10b-5p in COVID-19 patients were found lower at the baseline, 7 and 21-days after admission compared to the healthy controls ($p<0.0001$ for all time points). SHAP analysis indicated levels of miR 200b-3p (day 7th), miR-302c-5p (day 7th), CRP (day 7th), neutrophils (day 0), and D-Dimer (day 0) as the most promising predictors of long hospitalisation for the COVID-19 patients. Pathway enrichment analysis showed that among top shared pathways between targets of analyzed miRNAs were Interleukin-2 signaling pathway, and Pathways in cancer. miR-200b3p showed regulation of COVID-19-related targets associated with T cell protein tyrosine phosphatase and HIF-1 transcriptional activity in hypoxia, key pathways in COVID-19.

Conclusions:
In this study we validated and characterized miRNAs which could serve as novel, predictive biomarkers of the COVID-19 long term hospitalisation, and can be used for early stratification of patients and prediction of severity of infection development in an individual. Bioinformatics analysis pointed out the role of those miRNAs in multiple CVDs phenotypes associated with COVID-19 disease. Identifying novel biomarkers by means of machine learning based tools for feature identification and bioinformatics tools may improve clinical data interpretation and prediction of the outcome in patients with COVID-19.

Abstract 019 – poster presentation

DETECTION OF ARGINASE CONTAINING SMALL EXTRACELLULAR VESICLES IN BIOLOGICAL FLUIDS OF ENDOMETRIOSIS PATIENTS AS A POTENTIAL IMMUNOSUPPRESSIVE FACTOR

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Introduction:
Endometriosis is a chronic gynaecological disorder characterized by the growth of the endometrium outside its cavity. Reports suggest that it may be related to the impaired immune response. Our preliminary results indicate an increased level of two isoforms of arginase (ARG) enzyme, arginase-1 (ARG1) and arginase-2 (ARG2) as well as increased ARG activity in the peripheral blood of patients. Arginases are well-known regulators of amino acid metabolism, with a strong immunosuppressive effect. We assume that an immunosuppressive mechanism mediated by (ARG)-carrying extracellular vesicles (ARG+EVs), may be responsible for the observed immune dysfunction in endometriosis, resulting in the disease progression. The aim of the project is to identify ARG+EVs in the serum and peritoneal fluid (PF) of endometriosis patients and to decipher their impact on the dysfunction of the immune system in endometriosis.

Materials and Methods:
Small EVs were isolated from serum and PF of endometriosis and control patients using SEC and were verified by Western blotting, nanoparticle tracking analysis (NTA) and imaging flow cytometry (IFCM). The presence of arginases in EVs was determined by Western blotting, ELISA and IFCM. The functionality of ARG+ EVs was investigated using a multidonor mixed lymphocyte reaction (mdMLR) assay.

Results:
We detected small EVs in serum and PF samples from endometriosis patients and controls. According to NTA measurements, there was a trend towards higher total number of particles in endometriosis patients in comparison to controls both in serum and PF. We confirmed the presence of ARG1 and ARG2 in single EV samples from serum and PF of endometriosis patients along with the detection of some classical markers of small EVs. Based on single EV analyses by imaging flow cytometry, we showed a decrease in ARG1 expression in serumderived EV samples after the laparoscopic surgery. According to ELISA results, the concentration of vesicular ARG2 was several times higher than the ARG1 concentration, that was in the range of several ng per ml of serum or PF. In addition, EVs showed a minor immunomodulatory effect in the mdMLR, with a decline in activated CD4 and CD8 cells (CD25+CD54+), which was reversed by the addition of an ARG inhibitor.

Conclusion:
Our findings provide the first evidence for the presence of the immunosuppressive enzyme - ARG in the cargo of small EVs isolated from serum and PF of endometriosis patients. We believe that ARG+EVs may impact endometriosis progression, in terms of immune dysfunction, as well as provide a potential diagnostic/prognostic biomarker or therapeutic target.

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