INTERNATIONAL PATHOLOGY CONFERENCE OF THE „VICTOR BABEȘ” INSTITUTE BUCHAREST

7 – 8 November 2024

The event will be held in-person, at the „Victor Babeș” National Institute of Pathology

Splaiul Independenței 99-101, Bucharest, Romania

SCIENTIFIC PROGRAMME

Thursday 7, November 2024	
	Ioan Moraru amphitheater
09:00-09:45	**Registration**
09:45-10:00	***Conference opening***
10:00-12:00	***Session 1: Radiobiological research*** **Chair: Gina Manda**
	Introduction **Gina Manda**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Radiosensitization of tumor cells by modulating the transcription factor NRF2 **Gina Manda**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	The PORPHYDERM project – advancing the development of porphyrinic compounds for photodynamic therapy **Gina Manda**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Harnessing the abscopal effect of PDT by generating a whole cell vaccine **Laurentiu Anghelache**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Radiobiological effects of protons or gamma rays and UVB radiation on human cells **Angeliki Gkikoudi**, National Technical University of Athens, Greece
	The BIOSPHERE project – stress genes in normal cells co-exposed to energetic protons and UVB **Gina Manda**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Overview of the BIOSPHERE project – Impact of increased cosmic rays, UV radiation and fragility of ozone shield on the biosphere and our health **Alexandros Georgakilas**, National Technical University of Athens, Greece
	Discussions
12:00-13:00	**Lunch time**
13:00-15:00	***Session 2: Edited models in cell biology research*** **Chair: Elena Codrici**
	Biochemistry-Proteomics Lab in short: 2024 overview **Elena Codrici**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	CD36 editing of low malignancy breast cancer cells decreases cell proliferation and cell motility **Ana-Maria Enciu**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Protocol for patient-derived glioblastoma organoids generation: current applications and future directions **Elena Codrici**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	***In vitro* characterisation of phytosomes as carriers for bioactive compounds obtained from Hippophae rhamnoides berries** **Cristiana Tănase**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Piloting whole genome sequencing and analysis in Romania **Mircea Cretu Stancu**, Genomics Research and Development Institute, Bucharest, Romania
	Preventive potential of bioactive phytochemicals as modulators of cellular proliferation, oxidative stress and epigenetic alterations **Sevinci Pop**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Discussions
15:00-15:15	**Coffee break**

15:15-17:15	***Session 3: The pathophysiology of neurodegeneration and neuromuscular diseases*** **Chair: Laura Ceafalan**
	The effect of bacterial products on α-synuclein expression - an in vitro study modelling Parkinson's disease initiation **Octavian Ioghen**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Sarcopenia in Parkinson's disease **Bogdan Ovidiu Popescu**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Role of physical activity in Parkinson's disease **Emilia Manole**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Sarcopenia in neurodegenerative diseases: defining the molecular basis of intertissue cross-talk on the brain-muscle axis **Laura Ceafalan**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Neurogenic changes in muscle - patterns and challenges **Alexandra Bastian**, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
	Discussions
Victor Babeș amphitheater	
16:00-18:00	***Session 4: Histopathology*** **Chairs: Gabriel Becheanu, Andrei Niculae**
	Role of prognostic factors in personalized treatment of penile cancer patients **Luiza Dorofte,** Department of Laboratory Medicine, Örebro University Hospital, Faculty of Medicine and Health, Örebro University, Örebro, Sweden
	Molecular Features of Spitz Melanocytic Lesions **Adelina-Maria Cohn**, LNS - Laboratoire National de Santé, Luxembourg
	Macroscopic Assessment of Surgical Specimens for Colorectal Cancer **Valentin Enache**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Hamartomatous polyposis syndromes: morphopathological and clinical correlations **Gabriel Becheanu**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Key molecules in the initiation and progression of colorectal cancer **Andrei Marian Niculae**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Discussions

Friday 8, November 2024	
Ioan Moraru amphitheater	
09:00-11:15	***Session 5: Short communication - Biomedical research*** **Chairs: Carolina Constantin, Gheorghita Isvoranu**
	Alteration of immune parameters in experimental psoriasis **Mihaela Surcel**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Immunological investigation of functional immunodeficiencies with respiratory manifestations **Adriana Munteanu**, "Victor Babeș" National Institute of Pathology, Bucharest, Romania
	Are K+ currents required for the activation of adventitial fibroblasts? **Florentina Pluteanu**, Faculty of Biology, University of Bucharest, Romania
	Experimental study of hepatitis C virus E2 glycoprotein immunogenicity produced in the red microalga **Crina Stăvaru**, "Cantacuzino" Medical Military National Research and Development Institute, Bucharest, Romania
	Targeting unconventional protein secretion as therapeutic strategy in inflammatory disorders **Marioara Chiritoiu-Butnaru**, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

	Oxidation of methionine specifically boosts T cell responsiveness against a tyrosinase specific melanoma antigen **Gabriela Chiritoiu**, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania
	Targeting TGF-β signaling to reinforce the therapeutic potential of cytokine-activated Natural Killer cells as immunotherapy in cancer **Gheorghita Isvoranu**, “Victor Babeș” National Institute of Pathology, Bucharest, Romania
	Discussions
11:15-11:30	**Coffee break**
11:30-13:30	***Session 6:* Genomics of rare disorders** **Chair: Aurora Arghir, Sorina Papuc**
	Deleterious *ZNRF3* germline variants as a novel cause of neurodevelopmental disorders with mirror brain phenotypes due to distinct domain-specific effects on Wnt/β-catenin signaling **Prof. Dr. Anita Rauch**, Institute of Medical Genetics, University of Zurich, Zurich, Switzerland; Pediatric University Hospital Zurich, Zurich, Switzerland
	Clinical findings in rare disorders associated with autistic behavior **Magdalena Budisteanu**, Clinical Hospital of Psychiatry “Prof.Dr. Al. Obregia”, Bucharest, Romania
	Rare genomic variants in a cohort of children with neurodevelopmental disorders and autism **Sorina Papuc,** “Victor Babeș” National Institute of Pathology, Bucharest, Romania
	Updates in molecular monitoring of mutant nucleophosmin 1 **Valeriu Cismasiu,** “Victor Babeș” National Institute of Pathology, Bucharest, Romania
	Discussions
13:30-14:30	**Lunch time**
14:30-15:45	***Session 7: Poster Session*** **Chair: Ana-Maria Enciu and Emanuel Fertig**
	Mitochondrial dysfunction characterise peripheral artery disease in diabetic mouse. Preliminary data **Diana-Valentina Uță**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
	Regulation of alarmins in the skeletal muscle affected by the diabetic foot syndrome **Viorel-Iulian Suica**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
	Short-term S100A9 blockage after myocardial infarction favourably modulates energy metabolism in ischemic left ventricle **Raluca Maria Boteanu**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
	Assessment of primary human dermal fibroblasts for engineering skin equivalents - Preliminary data **Raluca Ţuţuianu**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
	Estrogen deficiency-induced signs of insulin resistance in ovariectomized APPNL-F and APPNL-G-F knock-in mice **Tudor-Fabian Troncea-Sandu**, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
	Discussions
15:45-16:00	**Coffee break**
16:00-18:00	***Session 8: Nephropathology*** **Chairs: Mihaela Gherghiceanu, Gener Ismail**
	Kidney biopsy processing for immunofluorescence and electron microscopy. **Mihaela Gherghiceanu**. “Victor Babeș” National Institute of Pathology, Bucharest, Romania

	Trends of biopsy-confirmed renal diseases in Romania: a single center study spanning 28 years **Iuliana Ciocănea-Teodorescu**, “Victor Babeș” National Institute of Pathology, Bucharest, Romania
	Redefining the pathological spectrum of COL4-related disorders. **Ștefan Lujinschi**, Fundeni Clinical Institute Bucharest, Romania
	Urinary soluble CD163 - potential biomarker for underlying histologic activity in proliferative glomerulonephritis. **Bogdan Obrișca**, Fundeni Clinical Institute Bucharest, Romania
	Microvascular inflammation: in and out of antibody-mediated rejection. **Bogdan Sorohan**, Fundeni Clinical Institute Bucharest, Romania
	The unsuspected heterogeneity of graft inflammatory infiltrate during kidney allograft rejection. **George Terinte-Balcan**. Hôpital Universitaire Necker, Paris, France
	Discussions
18:00-18:20	***Closing remarks***

SCIENTIFIC & ORGANIZING COMMITTEE

President: Mihail Eugen Hinescu
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Session 1: Radiobiological research

Radiosensitization of tumor cells by modulating the transcription factor NRF2

Elena Mihaela Dragnea[1], Maria Dobre[1], Ionela Victoria Neagoe[1], Ana-Maria Enciu[1], Cristian Postolache[2], and **Gina Manda**[1]

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Radiotherapy (RT) remains a standard in tumor treatment, but its application is limited not only by the intrinsic radioresistance of certain tumors, but also by the radiation-induced radioresistance that greatly decreases RT efficacy. Radioresistance derives partly from the tumor addiction to the transcription factor NRF2 that maintains cellular homeostasis by activating a plethora of cytoprotective genes in response to physical and xenobiotic stress. Moreover, as RT is based on an aggressive oxidative burst, NRF2 activation is expected to occur following RT, hence shielding tumor cells against the cytotoxic action of successive RT sessions.

The aim of the study was to define the NRF2 activation pattern after exposure of human colon carcinoma cells to γ rays.

Human colon carcinoma HCT116 cells line were exposed to 8 and 25 Gy γ rays, and were further cultivated for investing the dynamic of stress responses. The expression pattern of 84 stress genes was evaluated by qRT-PCR, addressing cell death, DNA damage & repair, endoplasmic reticulum stress, oxidative stress, hypoxia response, and inflammation. Transcriptomic data were placed in the context of cellular viability, proliferation, and adhesion. The natural product Brusatol was used for down-regulating the stress response by inhibiting the translation of many short-lived proteins, including NRF2. An animal study was performed on nude mice in which colon carcinoma cells, non-treated or *ex vivo* exposed to 25 Gy γ radiation, were subcutaneously inoculated. After six days mice were treated daily by intraperitoneal injection with 2 mg/kg b.w. brusatol for 4 consecutive days. This treatment scheme was repeated twice, with a pause of 3 days.

We emphasized the expression pattern of NRF2 target genes in human colon carcinoma cells exposed to γ rays, indicating that a persistent oxidative stress occurs, and that NRF2 appears to limit cell death. NRF2 activation was accompanied by hypoxia and DNA damage responses as well as by cytoprotective inflammation. We emphasize the over-expression of the cell-cycle arrest CDKN1A gene, a target of both the radiation-sensitive p53 tumor suppressor and of the NRF2 transcription factor, that feeds forward the non-canonical NRF2 activation.

In vitro treatment of cells with nanomolar concentrations of brusatol drastically decreased cell adhesion and/or proliferation, counteracting rapidly the cytoprotection conferred by the radiation-induced stress response in tumor cells. Surprisingly, when brusatol was administered to tumor-bearing mice, human HCT116 tumors grew significantly faster, but this did not happen when *ex vivo* irradiated tumor cells were transplanted in mice.

Concluding, the timing when brusatol is administered post-irradiation is critical *in vivo*, brusatol exerting pro-tumoral effects depending on the context.

Acknowledgements. Work was supported by the Ministry of Research, Innovation and Digitization through the Nucleu project PN 23.16.02.01.

Additional keywords: colon carcinoma, oxidative stress, brusatol

The PORPHYDERM project – advancing the development of porphyrinic compounds for photodynamic therapy

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The increased incidence of skin cancer demands intensive research to discover new strategies for prevention and therapy. A relatively new method of early therapy in skin pathology is photodynamic therapy which can eliminate (pre)malignant tumors by triggering an oxidative burst because of the targeted activation of a photosensitizer by light. Photodynamic therapy has minimal adverse effects, and, unlike radio- or chemotherapy, can be repeated safely to achieve increased effectiveness.

The PORPHYDERM project "Customized photodynamic protocol with innovative porphyrins and redox modulators in premalignant cutaneous disorders - preclinical demonstration" (2022-2024) has finally generated two innovative, stable, and biocompatible porphyrin photosensitizers with good uptake by skin cells, having the ability to kill cells when activated by 635 nm light. An advantage of these compounds is that they do not require metabolization for exerting a photodynamic effect as compared with the most used commercial photosensitizer in skin disorders.

Selected photosensitizers were incorporated into pharmacologically-accepted gels for topical application, and 2 national patent requests were submitted at OSIM. A protocol was developed in animal model, combining photodynamic therapy for reducing (pre)malignant tumors, with redox modulation for healing potential photodynamic therapy-induced lesions of the surrounding healthy skin. In addition, a panel of methods was developed for non-invasive measurement of critical skin parameters, such as transdermal water loss, skin hydration, melanin, erythema, and skin vascularisation.

In addition to technological progress for the benefit of patients, dermatologists and the health system, the project supported the excellence research activity of team members, especially young researchers who had the opportunity to develop their scientific careers and to access international collaborations that foster future development of the medical solution proposed in the project.

Acknowledgement. Work was supported by UEFISCDI through the 637PED/2022 project.

Additional key words: targeted therapy, oxidative burst, (pre)malignant tumors, hydrogels

Harnessing the abscopal effect of PDT by generating a whole cell vaccine

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Photodynamic therapy (PDT) is a clinically approved treatment for various pathologies like localized tumors, skin disorders and infections. It is based on the cytotoxic power of the oxidative stress burst mediated by singlet oxygen.

Although it is a targeted therapy, distant effects were observed resembling the abscopal effects of radiotherapy, that are mediated by the immune system following the immunogenic cell death (ICD) of tumor cells. PDT-inflicted damage to cancer cells induces the release of cytokines/chemokines and damage associated molecular patterns that potentiate the systemic immune response. However, ICD might be inhibited by cytoprotective molecules, like heme oxygenase 1 (HO-1) that is overexpressed in several types of cancers and antitumor therapies, including PDT.

The aim of the study was to investigate the ability of an innovative porphyrinic photosensitizer, P2.2, to induce by *in vitro* PDT an antimelanoma immune response in B6 mice.

Whole cell vaccines were generated using mouse melanoma B16F10 cells (VacPDT) and HO-1 knock-down B16F10 cells (siVacPDT). Gene silencing was achieved by siRNA magnetofection. Both the investigated melanoma cells types were treated *in vitro* with a milder PDT regimen (10 J/cm^2 fluence and 10 mW/cm^2 irradiance). Vaccines were administered subcutaneously to B6 mice, in two doses, at 5 days interval, using polyinosinic:polycytidylic acid as adjuvant. Two weeks after the last administration, mice were intradermically transplanted with a low number ($8x10^4$) of B16F10 cells for modelling an incipient tumor or residual disease. Lymph nodes were harvested two weeks after tumor cells transplantation, and immune cells were isolated. The immune response triggered by the vaccines was evaluated by flow cytometry for analysing $CD3^+$ T lymphocytes subsets from lymph nodes: cytotoxic T cells ($CD8^+$) and helper T cells ($CD4^+$) in combination with $CD69^+$ as a marker for early activation, and $CD103^+$ for tissue resident memory cells ($CD8^+$, $CD8^+$ $CD69^+$, $CD8^+$ $CD69^+CD103^+$; $CD4^+$, $CD4^+CD69^+$, $CD4^+CD69^+$ $CD103^+$).

Some of the mice in all groups developed tumors. We observed a lower tumor growth rate in mice receiving vaccine, with a more prominent effect of siVacPDT. While VacPDT significantly increased the percentage of $CD8^+$ subpopulations ($CD8^+CD69^+$, $CD8^+CD103^+$ and $CD8^+CD69^+CD103^+$), siVacPDT increased the percentage of total $CD4^+$ T lymphocytes.

Concluding, the antimelanoma vaccines generated *in vitro* by PDT have shown activation of antitumor adaptive immune responses in B6 mice. These preliminary results also indicate that HO-1 silencing represented an advantage in shaping the immune response in melanoma-bearing mice. Further investigations are needed to increase the immunogenicity of the new PDT-generated vaccine.

Acknowledgement. Work was supported by the Ministry of Research Innovation and Digitization through Nucleu Project PN 23.16.02.01.

Key words: immunogenic, knock-down, HO-1, melanoma

Radiobiological effects of protons or gamma rays and UVB radiation on human cells

Angeliki Gkikoudi[1,2], Spyridon N. Vasilopoulos[1], Gina Manda[3], Amer Al-Qaaod[4], Ulrich Giesen[4], Faton Krasniqi[4], Georgia I. Terzoudi[2] and Alexandros G. Georgakilas[1]

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Secondary cosmic and solar radiation constitutes a significant environmental factor influencing human health on Earth in the scenario of complete or partial ozone depletion. While cosmic radiation originates mainly from outer space, solar radiation emanates from the Sun, both contributing to the complex radiation environment experienced by living organisms on our planet. Although studies have traditionally examined the effects of cosmic and solar radiation individually, emerging research suggests that their combined (mixed beams) exposure may induce synergistic effects, altering cellular responses and potentially impacting human health in ways yet to be fully understood. The WP4 of the BIOSPHERE project aims to delve into the intricate interplay between cosmic radiation and solar radiation at the cellular level, focusing on their synergistic effects on human cell biology. Through a multidisciplinary approach, we explored key aspects of synergistic interactions, including DNA damage and genomic instability. To assess the impact of the combined exposure, normal human cell lines (skin fibroblasts, keratinocytes, etc.) were exposed to gamma rays and protons (simulating muon radiation) followed by UVB. Cellular, molecular, and cytogenetic biomarkers of radiation exposure were utilized, such as DNA damage response proteins (γH2AX and 53BP1). Preliminary results have revealed elevated levels of persistent unrepaired DNA Damage in co-exposed samples compared to samples exposed to ionizing radiation only, using the γH2AX foci biomarker. Towards this direction, the experimental approaches used in the present study aim to comprehensively investigate the effects of the combination of solar UV and cosmic radiation to evaluate their radiobiological consequences on human cells. Our findings may eventually contribute to the advancement of our understanding of the complex radiation environment on Earth and its potential implications on human health for radiation protection purposes.

The BIOSPHERE project – stress genes in normal cells co-exposed to energetic protons and UVB

Gina Manda[1], Maria Dobre[1], Elena Mihaela Dragnea[1], Ionela-Victoria Neagoe[1], Ulrich Giessen[2], Amer Al-Qaaod[2], Faton Krasniqi[2], and Alexandros Georgakilas[3]

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The increasing atmospheric ionisation caused by extra-terrestrial radiation (cosmic rays and solar UV radiation) reaching the Earth due to partial ozone layer depletion is expected to impact human health, but little is known on how combined radiation fields would act at cellular level.

In this context, a joint biological study was developed for understanding the stress response in human normal keratinocytes and fibroblasts co-exposed to energetic protons of 0.5 Gy (15-16 MeV) and UVB radiation (50 J/m^2). The viability of cells at 24-48 h after exposure to protons and UVB was assessed by the MTS reduction tests in conjunction with the lactate dehydrogenase release assay. By pathway-focused RT-PCR, the expression changes of 84 stress genes were investigated in human HaCaT keratinocytes and Hs27 fibroblasts, as compared to unexposed control cells, using as reference genes ACTB, B2M, GAPDH, HPRT1 and RPLP0 for normalization. Gene expression changes were reported as fold change in exposed *vs* control cells.

Irradiation parameters were chosen for mimicking a mild exposure to protons and UVB not affecting cellular viability significantly. A synergistic effect of the co-exposure was shown in the number of metabolically-active cells that were decreased compared to cells exposed to single radiation. A partially overlapping pattern of DNA damage genes with modified expression was registered in cells exposed either to protons or UVB. Meanwhile, a broader panel of stress genes with expression alterations was evidenced in co-exposed cells that appear to be more stressed, although individual stress genes do not have a higher over-expression than cells exposed to protons or UVB alone. Moreover, the data showed that attached cells, presumably living cells, are partly committed to cell death. Besides the DNA damage response, other repair mechanisms were equally highlighted in the investigated irradiation settings, involving inflammation, antioxidant and hypoxia responses.

Concluding, a mild exposure to energetic protons and UVB is stressing skin cells more than individual radiations, inducing a prolonged stress response related to DNA and protein damage, oxidative stress, hypoxia and inflammation.

Acknowledgement. The project 21GRD02 BIOSPHERE has received funding from the European Partnership on Metrology, co-financed from the European Union's Horizon Europe Research and Innovation Programme and by the Participating States.

Additional key words: keratinocytes, fibroblasts, genotoxicity, oxidative stress, hypoxia, inflammation

Overview of the BIOSPHERE project – Impact of increased cosmic rays, UV radiation and fragility of ozone shield on the biosphere and our health

Alexandros Georgakilas

DNA Damage Laboratory, Physics Department, School of Applied Mathematical and Physical Sciences, National Technical University of Athens (NTUA)

One of the most significant ecological challenges facing EU member states and beyond is the impact on human and ecological health of the increasing atmospheric ionisation caused by extra-terrestrial radiation (cosmic rays and solar UV radiation) boosted by anthropogenic emissions. In this context, the BIOSPHERE project aims to develop the tools, methodologies, and measurement infrastructure for evaluating the mutual impact of the above-mentioned radiations, bringing together 22 European institutions with complementary expertise in metrology, meteorology, particle physics, chemistry, and biomedical sciences.

This project will allow for the first time simultaneous and side-by-side measurement of the flux rate of secondary cosmic rays, terrestrial solar UV spectrum and total atmospheric ozone, complemented by meteorological data, and will support ground-based measurements with satellite measurements (PROBA-V and GOES), and advanced numerical models and tools (Copernicus Atmosphere Monitoring Service, and the Space Environment Information System).

The mutual effects of cosmic rays and biologically active solar UV radiation on human health is largely unexplored, but has become highly important in the context of the gradual ozone layer depletion, potential leading to increased harmful terrestrial levels of UVB and extra-terrestrial particles. Therefore, the biological impact of mixed radiation fields of cosmic rays and solar UV radiation is under investigation in Work Package 4 which addresses the damages inflicted in human "normal" cells, that may be responsible for their long-term dysfunction. The biological study is performed on skin and immune cells that are at higher risk to be affected by environmental challenges.

The experimental approach in WP4 is based on simulating environmental exposures under experimental conditions (energetic protons and UVB radiation) to establish correlations between radiation type and flux, on the one hand, and the changes of various cellular parameters such as viability/cell death, DNA damage and genomic instability, as well as stress responses, on the other hand. Through advanced bioinformatics and systems biology methodologies, the project will ultimately generate a network of biological events triggered by combined radiation fields, highlighting potential therapeutic targets for counteracting the deleterious effects of this type of environmental exposure. Health and environment regulatory bodies will benefit from experiment-based data for future informed decisions on radioprotection. Moreover, a Good Practice Guide for comprehensive radiobiological studies of human normal cell lines exposed to single and combined fields of secondary cosmic rays and UV radiation will be made publicly available in the benefit of the radiobiology community.

Acknowledgement. The project 21GRD02 BIOSPHERE has received funding from the European Partnership on Metrology, co-financed from the European Union's Horizon Europe Research and Innovation Programme and by the Participating States.

Additional key words: DNA damage response, cellular stress, keratinocytes, fibroblasts, monocytes, gene network

Session 2: Edited models in cell biology research

CD36 editing of low malignancy breast cancer cells decreases cell proliferation and cell motility

Maria Dudău[1], Elena Codrici[1,2], Ionela-Daniela Popescu[1], Alina Erbescu[1], Aura Arghir[1], Sorina Papuc[1], Cristiana Tanase[1,3] , **Ana - Maria Enciu**[1,2]

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Background. It has been repeatedly shown that malignant cells rely on fatty acids (FA) metabolism for increased malignancy and metastasis, including in breast cancer. Lowering the disponibility of FA as cellular fuel, could prove a useful target to lower tumor cell aggressiveness.

Aim. We aimed to generate edited cell lines for CD36, a fatty acid translocator, to further study the impact of this protein in cell behaviour.

Material and methods. MCF-7 cell line (ER/PR positive, HER2 negative) was edited using a CRISPR-Cas9 plasmidic system, followed by selection with puromycin and single cell cloning. Gene-editing was confirmed by Sanger sequencing. The cell population was tested with real-time cell adhesion and proliferation assays, by two different methods, as well as spheroid formation.

Results. Edited cells were characterized by higher adherence, slower proliferation rate and tendency to form duct-like structures, even in the absence of extracellular matrix.

Conclusion. CD36 gene editing decreases the proliferation rate of MCF-7 cells and their ability to form 3D spheroids.

Acknowledgment*: This work was supported by the Core Program within the National Research, Development and Innovation Plan, 2022–2027, with the support of MCID, project no. 10N/01.01.2023, PN 23.16.02.03*

Protocol for patient-derived glioblastoma organoids generation: current applications and future directions

Elena Codrici[1,2], Ionela-Daniela Popescu[1], Maria Dudău[1], Emanuel Tudor Fertig[1,2], Maria Savoaia[2], P.Mugur Rădoi[2,3], Cristiana Tanase[1,4] and Ana - Maria Enciu[1,2],

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Background: Glioblastoma is an aggressive form of brain tumour, with no resolution, despite recent advancement in understanding the molecular mechanisms. In order to find novel therapy targets, more suitable models are to be implemented and standardized.

Aim: to develop and implement a protocol for patient-derived glioblastoma spheroids/organoids, best fit for plasmid-based transfection.

Material and methods. Glioblastoma biopsies were harvested in high-glucose DMEM medium, kept at 4-8°C for up to 4 hours then processed by mechanical trituration and enzymatic digestion in Accutase (2 min at 37°C). The suspension was filtered through a 40-100µ mesh and the resulting filtrate incubated in ultralow adhesion plates, Matrigel Organoid or dextran-based hydrogels. Organoids were nucleofected with GFP plasmid to assess efficiency and penetrability of transfection.

Results. Of the tested methods, ultralow adhesion incubation yielded tumour spheroids with the highest efficiency, of macroscopic size, compatible with downstream imagistic detection and transfection. However, the non-adherence spheroids did not revealed the infiltrative nature of the glioblastoma, which was achieved in various hydrogels, such as Matrigel Organoid and precast gradient hydrogel plates (Merck). Incubating spheroids in hydrogels restricted their size and slowed growth. Analysis of the supernatants revealed a persistent release of immune molecules into the TME, including CCL2, a chemoattractant linked to poor prognosis in GBM; however, the release from dextran-based hydrogels was lower compared to other conditions.

Conclusion. For morphology assessment, biomarker detection and fast and high-throughput analysis, ultralow attachment plates are the most convenient method.

Acknowledgment: *This work was supported by the Core Program within the National Research, Development and Innovation Plan, 2022–2027, with the support of MCID, project no. 10N/01.01.2023, PN 23.16.02.03.*

In vitro characterisation of phytosomes as carriers for bioactive compounds obtained from Hippophae rhamnoides berries

Cristiana Tanase[1,2], Simona Lițescu[3], Elena Codrici[1,4], Ionela-Daniela Popescu[1], Maria Dudău[1], Emanuel Tudor Fertig[1,4], Lauentiu Anghelache[1], Sevinci Pop[1], Ana - Maria Enciu[1,4]

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Background. Phytocarriers are innovative drug delivery systems utilizing biocompatible and biodegradable materials to improve the efficacy, stability, and bioavailability of natural products. Various nanocarrier types, primarily organic (e.g., liposomes, polymeric nanoparticles), enhance the delivery of bioactive compounds like sea-buckthorn extract, which is rich in essential fatty acids and antioxidants.

Aim. The aim is to produce and characterize liposomes loaded with sea-buckthorn extract (phytosomes) of Romanian origin, for potential further development as cosmetic delivery agents or food supplements.

Material and methods. We analyzed the biocompatibility of both sodium cholate (NaC) and sodium deoxycholate (NaDC) liposomes and phytosomes on two cell lines: normal human fibroblasts Hs27 (ATCC CRL-1634™) and SC normal human monocytes (ATCC 9855). Biocompatibility of liposomes and phytosomes was assessed by end-point cytotoxicity (LDH release) and cell viability assays (MTS) as well as by real-time cellular proliferation and inflammation assay by multiplexing. The end-point cell proliferation assay was complemented by the real-time assays for cells adhesion and proliferation, where incubation time was increased, and data collection at intermediate times allowed calculation of doubling times of treated cells. Both liposomes and phytosomes were morphologically characterized using NS-TEM and cryo-TEM.

Results. NaC liposomes and phytosomes are uptaken by cells, making them useful vectors for active compounds delivery. Cell proliferation studies showed that NaC liposomes and their corresponding sea-buckthorn loaded phytosomes are well tolerated and do not induce cytotoxicity on either fibroblasts or monocytes. When assessed in real-time, the sea buckthorn extract phytosomes loaded in NaC and NaDC diluted 100 fold induced an anti-proliferative effect, when treated for longer periods of time. In terms of antiinflammatory properties, a dose-dependant response was noted for inflammatory cytokine release. Correlated with the lack of toxicity of the 1/100 diluted nanocarriers, we provided evidence for the anti-inflammatory effect of NaC phytosomes.

Conclusion. This study addressed the technical issue of delivery of sea buckthorn extract using liposomes, for increased intracellular delivery with potential therapeutic benefits. Tested NaC- diluted liposomes and phytosomes are uptaken by cells and induce an anti-inflammatory response in the presence of LPS. In conclusion, a cell compatible, therapeutically effective phytosome for sea-buckthorn extract delivery was obtained and can be further tested in *in vivo* models.

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Piloting whole genome sequencing and analysis in Romania

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In the last year, the Genomic Research and Development Institute has piloted all the necessary components that will enable it to scale to population-level sequencing, primarily whole-genome and whole-exome, whether for research or diagnostic purposes. We show here key components that were piloted, with an emphasis on the bioinformatics aspects. We illustrate the compute infrastructure needed for large scale projects and how we piloted our cluster, and further detail on a few whole-genome sequencing pilots that ICDG initiated, what data we produced, how we are analyzing it and how it will help us to scale up in an efficient manner. This will have a significant impact on the development of the Romanian reference genome.

Keywords: bioinformatic analysis, reference genome, big data

Preventive potential of bioactive phytochemicals as modulators of cellular proliferation, oxidative stress and epigenetic alterations

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The demand for plant-based dietary supplements has increased worldwide. Therefore, the scientific data is needed to prove their claimed preventive potential as well as to explore their toxicological profile. Our aim was to assess the biological effects of two plant extracts rich in phytochemicals, especially isoflavones, on in vitro breast cancer models. Using a wide range of concentrations (0.1 to 3.33 mg/mL) we analyzed how the extracts modulate cellular processes such as oxidative stress and proliferation on highly invasive ER- and non-invasive ER+ breast adenocarcinoma cells; and on non-tumorigenic ER- normal breast cells. Cytotoxicity and RTCA assays showed that both extracts exerted a biphasic dose effect on ER+ adenocarcinoma and normal ER- cell proliferation and oxidative stress. We demonstrated that a dose-dependent monotonic cytotoxicity occurred on highly invasive adenocarcinoma ER-cells, and the induced apoptosis was based on the pro-oxidant activity of the extracts. ROS generation by high dose of ethanolic extract was observed in all cells, followed by mitochondrial dysfunction. Oxidative stress parameters such as MDA and GSH levels and SOD activity were affected. Moreover, the extracts have the capacity to modulate epigenetic events such as DNA methylation on highly malignant breast cancer cells. Our study demonstrates that T. pratense extracts can induce differential biological activities on breast cells, depending on the dose and the state of cell malignancy and estrogen receptors status.

Session 3: The pathophysiology of neurodegeneration and neuromuscular diseases

Sarcopenia in neurodegenerative diseases: defining the molecular basis of intertissue cross-talk on the brain-muscle axis

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Sarcopenia affects 40% of patients in advanced stages of Parkinson's Disease (PD) and is associated with disease severity and poor prognosis. Sarcopenia contributes to the progression of cognitive decline - the relationship between muscle loss and neurological deficit has not yet been demonstrated.

The study aims to establish a group of circulating muscle biomarkers, myokines and altered epigenetic factors in advanced neurodegenerative diseases accompanied by muscle loss, with a role in evaluating the prognosis of the disease and as possible therapeutic targets.

For this purpose, we optimised experimental proof-of-concept in vitro and in vivo models to prove inter-tissue communication on the muscle-brain axis through soluble molecules.

The in vitro study used co-cultures between muscle and neuronal cells with and without PD-induced changes. The Luminex protein multiplexing technique was used to determine the myokine profile and MicroRNA A+B v3.0 PCR card blocks were used to test 768 miRNAs for the miRNA profile.

The expression profile of modified myokines in muscle cells after co-cultivation demonstrated a tendency to increase the expression of LIF and IL6 and a decrease in the expression of FSTL-1, FABP-3 and Osteonectin in the case of cells maintained in co-culture with neuronal cells. These results require further validation by WB.

To analyze the impact of the presence of dopaminergic neurons on miRNA expression in muscle cells, as well as their alteration of the progression of NDs (especially PD), the relative expression level (dCt) was evaluated in myocytes cocultured with neuronal cells, either untreated or treated with rotenone as a PB model. We identified 14 miRNAs whose expression is increased in myocytes co-cultured with PB neurons and 10 miRNAs showing the opposite evolution. No information was found in the literature showing a functional link between these microRNAs and muscle fibre homeostasis in the context of neuromuscular pathologies. We intend to investigate the correlations further.

For the in vivo approach we are in the process of validating the PD phenotype in a transgenic mouse strain, B6; C3-Tg(Prnp-SNCA*A53T)83Vle/J, expressing human A53T variant alpha-synuclein, by using behavioural testing and Western blot to determine the level of alpha-synuclein in the brain. The aim is to determine a panel of circulating myokines correlated with muscle wasting and the severity of the phenotype, as prognostic factors, as well as the impact of moderate exercise on the onset and progression of these changes.

Sarcopenia in Parkinson’s disease

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Parkinson’s disease (PD) is the second most prevalent neurodegenerative disorder. In PD there are a variety of signs and symptoms that can occur, both motor and non-motor. Bradykinesia, rigidity and rest tremor are so called cardinal motor signs, defining the core parkinsonian motor syndrome. However, depression, anxiety, hallucinations, cognitive decay, orthostatic hypotension, REM behavioural disorder, neuropathy and many others are frequently present in the clinical picture of advanced PD patients. Sarcopenia (reduced skeletal muscle strength and mass) it is common in PD, according to various recent clinical studies and it might be associated with disease severity. However, no robust scientific data explain this correlation and therefore we try to investigate a possible direct communication through different signalling factors between brain and striated muscles, which might be altered in PD.

Role of Physical Activity in Parkinson's Disease

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More and more recent studies show the positive effects of non-pharmacological approaches in the management of Parkinson's disease (PD) symptoms, especially the effects of exercise on both motor (balance and mobility) and non-motor symptoms (depression, constipation, thinking skills).

New research has shown that exercise may have a protective effect on the brain and help slowing the disease progression. Despite the marked loss of dopaminergic neurons in mice models of PD, exercise training showed a reversal of behavioral deficits related to regular movement, balance and gait performance. It seems that the striatum plays an important role in providing input to the basal ganglia circuit and it is implicated in the PD pathological process.

Aerobic training could upregulate the expression of striatal dopamine D2 receptors and also significantly could boost the rate of mitochondrial biogenesis and the quantity of mitochondrial subunits. Thus, the mitochondrion is another target of physical exercise. The low intensity exercise improved the mitochondrial function both in skeletal muscle and brain in rats model of PD, inducing a neuroprotective effect against the loss of dopaminergic neurons.

Another target is represented by exerkines (including myokines), bioactive substances that are synthesized and released by many types of tissues during exercise and which have been implicated in neuroprotection. It was shown that exerkines protect neuronal cells in vitro and in vivo, in rodent PD models.

Myokines also are implicated in the skeletal muscle atrophy in PD and researching the change in their levels during physical activity could improve muscle therapy in PD patients with sarcopenia.

Coclusion: Studying a field underestimated until recently in neurodegenerative diseases can bring new data and a new vision regarding non-pharmacological therapy in these pathologies, focusing here on exercise.

Key words - aerobic activity, dopamine D2 receptors, mitochondria, exerkines

The effect of bacterial products on α-synuclein expression - an in vitro study modelling Parkinson's disease initiation

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Parkinson's Disease (PD) is a prevalent and escalating neurodegenerative disorder with significant societal implications. Despite being considered a proteinopathy in which the aggregation of α-synuclein is the main pathological change, the intricacies of PD initiation remain elusive. Recent evidence suggests a potential link between gut microbiota and PD initiation, emphasizing the need to explore the effects of microbiota-derived molecules on neuronal cells.

In this study, we exposed dopaminergic-differentiated SH-SY5Y cells to microbial molecules such as lipopolysaccharide (LPS), rhamnolipid, curli CsgA, phenol soluble modulin α-1 (PSMα1) and assessed cellular viability, cytotoxicity, growth curves and α-synuclein levels by performing MTS, LDH, real-time impedance readings, qRT-PCR and Western Blot assays respectively.

Statistical analysis revealed that rhamnolipid exhibited concentration-dependent effects, reducing viability and inducing cytotoxicity at higher concentrations, increasing α-synuclein mRNA and protein levels with negative effects on cell morphology and adhesion. LPS exposure also increased α-synuclein levels. Curli CsgA and PSMα-1 showed minimal or no changes.

Our findings suggest that microbiota-derived molecules, particularly rhamnolipid and LPS, impact dopaminergic neurons by increasing α-synuclein levels. This study highlights the potential involvement of gut microbiota in initiating the upregulation of α-synuclein that may further initiate PD, indicating the complex interplay between microbiota and neuronal cells.

Key Words: Parkinson's disease, microbiota, alpha-synuclein overexpression, microbiota-derived molecules, rhamnolipid, lipopolysaccharide, curli CsgA, phenol soluble modulin α-1

Neurogenic changes in muscle - patterns and challenges

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Background and objectives: Muscle denervation occurs when intercellular interaction between anterior horn cells, axons and motor end-plates with the contractile muscle proteins is damaged. Differentiating between myopathic and neurogenic weakness, a starting point in the diagnostic evaluation, is not always easy for both neurologist and pathologist.

We present, from a pathology standpoint, essential morphological features that can orient towards the neurogenic origin of various disorders, main elements of differential diagnosis, focusing on the basic neurogenic patterns, but also on the similarities and overlaps between different morphological pictures in neuromuscular pathology.

Matherial and method: We reassessed 100 consecutive representative muscle biopsies diagnosed in the Pathology Department of Colentina Clinical Hospital with different neurogenic disorders, to describe and exemplify various aspects and challenges encountered in their pathological evaluation. All the slides were obtained from fresh biopsy specimens snap-frozen in isopentane precooled in liquid nitrogen. Cryosections of muscle were routinely stained with hematoxylin and eosin, modified Gomori trichrome, ATPase pH 4.35, 4.63 and 9.4, nicotinamide adenine dinucleotide, succinate and lactic dehydrogenases, COX, van Gieson, PAS and oil red O.

Results: The histopathology of neurogenic atrophy in varying stages comprise angulated denervated fibers in small or large groups, pyknotic nuclear clusters, fascicular atrophy, reinnervation with fiber type grouping on ATP-ases, target or targetoid fibers on histochemical stains, even necrosis. Long-standing neurogenic atrophy often shows features mimicking myopathic changes like increased internal nuclei, hypertrophy, disordered internal architecture. Neurogenic changes may also occur in „pure" myopathies with concurrent peripheral neuropathy highlighting mixed myopathological features. Identification of such aspects and certain patterns provide clues that may suggest the type of neurogenic insult in amyotrophic lateral sclerosis, spinal muscular atrophies, Kennedy disease, mitochondrial or inflammatory neuromyopathies and peripheral neuropathies.

Conclusion: Muscle biopsy is a useful investigative tool, in addition to clinical, electrophysiological and molecular evaluation, for positive and differential diagnosis in selected cases.

Keywords: muscle biopsy, muscle denervation, histopathology

Session 4: Histopathology

Role of prognostic factors in personalized treatment of penile cancer patients

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Penile squamous cell carcinoma is a rare type of tumor, particularly in developed countries. Approximately 50% of the affected men have a Human Papillomavirus (HPV) infection. The remaining patients exhibit chronic penile inflammatory conditions, such as lichen sclerosus. The diagnosis of penile cancer significantly affects the quality of life and sexual health of the patient. The most important prognostic factor linked to survival outcomes in these patients is the presence of inguinal nodal metastasis. Prophylactic inguinal lymph node surgery is not advised in all cases because of the significant morbidity. Given that many patients with penile cancer are still sexually active, opting for a conservative surgical approach can help maintain sexual function and enhance overall quality of life.

The objective of our research is to investigate whether the presence of HPV, lichen and certain characteristics of penile tumors can be used as prognostic indicators for metastatic disease. The identification of patients with pT1 tumors who may benefit from limited, organ-sparing surgery, as well as those at significant risk for lymph node metastasis, relies on identification of histopathological prognostic factors like tumor grade and subtype of squamous cell carcinoma.

We previously examined interobserver agreement in assessing penile cancers histological subtype and grade. Pathologists from three Swedish and Italian hospitals analyzed 207 cases. The findings of our study indicate poor-to-moderate agreement in the evaluation of both histological grade an subtype. Low interobserver concordance may lead to the under- and over-treatment of a significant number of patients with penile cancer, which raises questions about the efficacy of tumor histological subtype and tumor grade in guiding treatment decisions for patients with pT1 tumors. There is a necessity for a more reproducible risk-grading system for penile cancer patients.

In our recent study, we proposed a histological risk grading system based on more objective histological criteria. Compared to the European guidelines' existing risk grading, our proposed method exhibits enhanced sensitivity and classifies a significantly larger number of patients as low-risk, potentially eliminating the need for lymph node surgery in more cases. Patients categorized within the intermediate and high-risk groups for inguinal lymph node metastasis continue to be recognized, yet an increased number of patients with pT1 tumors may be considered for monitoring of lymph node status rather than undergoing additional surgical procedures.

Keywords: Penile cancer · Interobserver agreement · Histological grading · Inguinal lymph node metastasis · Organ-sparing surgery

Molecular features of Spitz melanocytic lesions

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Spitz melanocytic lesions are characterized by unique morphological features and specific genetic alterations[1]. This presentation aims to highlight the molecular findings of Spitz nevi and Spitz melanocytomas, in correlation with histological aspects and immunohistochemical profile.

The study presents a series of nine Spitz melanocytic lesions, including five Spitz nevi and four Spitz melanocytomas. No case met the criteria for Spitz melanoma.
Microscopic examination and immunohistochemical studies for ALK, ROS1 and pan-TRK were performed in all cases. The spitzoid nature of the lesions was confirmed by molecular biology studies.

Two Spitz nevi presented a ROS1 fusion. While one of the lesions had a plaque-like silhouette, the other showed a wedge-shaped architecture. In both cases, the melanocytes had a fusiform morphology with fibrillary cytoplasm. Focal presence of pseudo-rosettes was documented in one case. The ROS1 immunohistochemical stain showed a blush positivity in one case and a strong positivity in the other.

Morphological clues for NTRK1 fusion Spitz neoplasms, such as filigree-like rete ridges and lobulated nests[2], were identified in two Spitz nevi. The pan-TRK IHC stain was strongly positive. One of the cases showed an LMNA-NTRK1 fusion.

The fusions KIF5B-RET and CCDC6-RET were described in one Spitz nevus. Histologically, the lesion displayed a nested architecture with dyscohesive appearance and was composed of monotonous epithelioid melanocytes.

Spitz melanocytomas were diagnosed after careful examination of the morphological and immunohistochemical features. The criteria favoring a melanocytoma included: cytologic atypia, architectural distortion, cellular density, presence of multinucleated melanocytes, dermal mitoses, loss or decrease of p16 IHC expression, or focal PRAME1 positivity. All cases were sent for second opinion and confirmation of the diagnosis to an expert dermatopathologist.
In our study, two of the Spitz melanocytomas had MAP3K8 fusions. The ETV6-NTRK3 fusion and a BRAF fusion were identified in the other two cases.

In conclusion, some molecular events associated with Spitz lesions can be predicted by morphological features. Furthermore, genetic characterization of Spitz neoplasms may increase diagnostic accuracy.

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Macroscopic Assessment of Surgical Specimens for Colorectal Cancer

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Macroscopic Assessment of Surgical Specimens for Colorectal Cancer

The macroscopic examination of colorectal cancer specimens provides crucial information for staging, margin assessment, and determining the extent of disease, which are vital for treatment planning and prognosis. The assessment should be systematic, covering the appearance, size, and involvement of both the primary tumor and surrounding tissues. Begin by confirming the specimen type (e.g., right hemicolectomy, left hemicolectomy, or rectal resection) and orienting the anatomy. Note any specific anatomical landmarks, such as the mesenteric margin or peritoneal reflection, which assist in determining tumor location and margin involvement. Document the tumor's distance from resection margins (proximal, distal, and circumferential). Record the tumor's location within the bowel segment (e.g., right colon, sigmoid, or rectum). Measure the tumor size. Describe the gross appearance of the tumor. Assess the surgical margins, including the proximal, distal, and nonperitonelized (radial) margins. For rectal cancers, the circumferential resection margin (CRM) is especially critical, as involvement here is associated with higher recurrence rates. Record the distance between the tumor edge and each margin. Identify ideally, a minimum of 12 lymph nodes to provide an adequate assessment of nodal involvement. Note any other abnormalities within the specimen, such as polyps, diverticula, or inflammatory changes. Photograph the specimen for record-keeping and potential consultations. Inking the specimen at the resection margins, ensure clear identification of margin involvement during microscopic evaluation. This systematic macroscopic assessment provides a foundational understanding of the specimen's disease extent and aids in accurate histopathological analysis and staging.

Key words: margins, peritoneum, lymphnodes.

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Key molecules in the initiation and progression of colorectal cancer

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Colorectal cancer represents one of the cancers with the highest morbidity and mortality rates among cancer patients. The new discoveries regarding the genetic component of the colorectal carcinogenesis process and the involvement of metabolic processes or cellular processes give a new vision in the approach of this complex pathological entity.

The aim of the studies was to investigate some molecular mechanisms involved in the occurrence and progression of colorectal cancers and to identify new predictive biomarkers and possible therapeutic targets.

In the first study, the expression level of CD36, FASN, GPC4, BIRC5, SLC27A3 and SLC27A4 genes was comparatively analyzed in paired samples collected from 39 patients with colorectal cancer and 18 normal tissues collected from the colonic mucosa of some individuals without a diagnosis of colorectal adenocarcinoma. Also, seven microRNAs targeting the CD36 gene and most of the analyzed genes were evaluated in 25 patients and all analyzed controls. A significant impairment of the expression of all analyzed genes, except GPC4, was identified in tumor tissues compared to peritumoral ones. CD36 and SLC27A4 genes were underexpressed in tumor tissue, while FASN was overexpressed. SLC27A3, FASN and GPC4 were overexpressed and BIRC5 was underexpressed in peritumoral tissue.

Comparing the expression of seven microRNAs that target CD36 between tumor and peritumoral tissues we identified the underexpression of miR-16-5p, miR-26b-5p, miR-107 and miR-195-5p. By comparing the expression of microRNAs between tumor and normal tissues we identified the underexpression of miR-195-5p and the overexpression of miR-27a-3p. Both microRNAs were overexpressed in peritumoral tissue compared with normal tissue. miR-27a-3p was overexpressed in tumor tissues from 11 patients with lymph node metastases; in the same patients we identified a negative linear correlation between miR-27a-3p and CD36 expression and a positive linear correlation between miR-195-5p and FASN. miR-27a-3p was overexpressed in tumor tissues from 6 patients with perineural invasion.

In the second study we identified a general impairment of the expression of microRNAs of the let7 family in colorectal mucosa harvested from 25 patients with colorectal cancer compared to adjacent non-tumorous mucosa. In tumor tissues with perineural invasion let-7a-5p, let-7b-5p, let-7c-5p, let-7d-5p and let-7i-5p were overexpressed compared to tumor tissues without perineural invasion. In tumor tissues with KRAS mutations we found that miR-let-7e-5p was overexpressed.

In the third study, we identified interactions between let7 family microRNAs and genes involved in the apoptosis process and in the NF-κB signaling pathway. BCL2L1 and CASP8 genes were overexpressed in moderately differentiated tumor tissues compared to poorly differentiated tumor tissues.

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4.

Session 5: Short communication - Biomedical research

Alteration of immune parameters in experimental psoriasis

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Introduction. Psoriasis (Ps) is a is a lifelong inflammatory T-cell mediated disease, with cutaneous and articular manifestations with a significant negative impact on patients' quality of life. The aberrant interaction between keratinocytes, immune cells, and cytokines determines the chronic inflammatory state. Despite the numerous therapeutic options available, Ps remains an incurable disease and adjuvant/personalized therapies represent a challenge. In this study, the Imiquimod (IMQ)-based model of Ps was characterized and the changes induced by IgY raised against pathological human bacteria resistant to antibiotics were observed.

Material and methods. IMQ-based model was performed using 4 groups of C57BL/6 mice: Ps group - received a topical dose of IMQ cream (5% Aldara Cream, Sweden) for 6 consecutive days; Ps IgY group - received (starting with day 7) a gavage dose of IgY for 5 days; Remitted Ps group - received (starting with day 7) a gavage dose of PBS for 5 days and control group. The severity of the disease was assessed using in vivo measurements for erythema, desquamation and induration, PASI score, splenomegaly assessment and histopathological examination. Cellular (T, B, NK cells) and humoral (inflammatory cytokines) immune parameters were monitored by flow cytometry, respectively xMAP array.

Results. Measurements for erythema, desquamation, induration and PASI score revealed a progressive evolution during the IMQ-treatment. Splenomegaly assessment showed that both spleen weight and spleen weight/body weight ratio were significantly higher in Ps group as compared to controls. Histopathological evaluation of Ps group skin samples revealed hyperkeratosis, acanthosis and elongation of rete ridges. For Ps group, the main changes observed were decreased percentages of T-CD4+ and B cells ($p<0.05$), and increased values for T-CD8a+, NK cells and serum levels of pro-inflammatory cytokines ($p<0.05$). These values were normalized post-IgY therapy.

Conclusions. IMQ-based murine model of psoriasiform dermatitis was clinically, histopathologically, immunologically and proteomically characterized, and all evaluated parameters showed that this model exhibited human Ps' specific features. Improved clinical evolution along with the restoration of immune parameters were obtained after IgY therapy. As IgY preparation can be raised against individualized microbiome, using this compound can open the personalized medicine domain in Ps.

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Keywords: imiquimod; inflammation; IgY

Immunological investigation of functional immunodeficiencies with respiratory manifestations

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Introduction: Recurrent respiratory infections (RRI) represent a high percentage of general childhood pathology, in conditions of an immune system without major apparent defects. Because they may be associated with an altered cellular immune response, the aim of the study was to quantify T and B lymphocyte subpopulations from peripheral circulation in order to identify possible immunological changes with impact on the pathogenesis of RRI.

Material and methods: The casuistry includes two main groups of children (1-18 years), thus: i) **RRI groups: 1-5 years** (n=85), **6-10 years** (n=38) and **11-18 years** (n=16) – children with at least 6 episodes of RI/year; ii) **control groups** (n=10, 12, 9) for every age group – clinically healthy children. Blood samples were collected for dosing serum immunoglobulins (IgG, IgA, IgM) by nephelometry and for extended lymphocyte immunophenotyping by flow cytometry (BD FACSCanto II): total T-lymphocytes ($CD3^+$), T-helper ($CD4^+$), T-suppressor/cytotoxic ($CD8^+$), double-negative T cells ($CD4^-CD8^-CD1d^-$), regulatory T cells ($CD4^+CD25^+CD127^-$), NKT cells ($CD3^+CD16/56^+CD1d^+$), NK cells ($CD16/56^+$) and total B cells ($CD19^+CD20^+$) with mature/naive B cells ($CD27^-IgD^+$), memory B cells ($CD27^+$) and plasmocytes ($CD10^-CD27^+CD38^{bright}$).

Results: The most important changes were increased T-$CD4^+$ (p=0.0036) (1-5 years group), decreased T-$CD8^+$ (p=0.0002) (1-5 and 6-10 years groups) and increased T-$CD4^+$/T-$CD8^+$ ratio (p=0.0025) (1-5 years group). For 1-5 years group, regulatory T cells showed increased percentages (p=0.0036) and 80% of cases showed significant decreases in total B cells (p=0.002). A significant decrease in mature/naive B (p=0.04) (1-5 and 6-10 years groups) and increase in memory B (p=0.04) (1-5 years group) were observed. No differences were observed for hummoral parameters.

Conclusion: Investigation of cellular immunological parameters from peripheral blood may complete the clinical diagnosis, especially in cases where humoral parameters are within normal limits. Considering that RRI can cause disorders in children's development, detection of causes and prophylaxis of these infections are major elements for improving living conditions of affected child population.

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Keywords: recurrent respiratory infections, memory B cells, double-negative T cells

Are K+ currents required for the activation of adventitial fibroblasts?

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Background and Objectives

Adventitial fibroblasts can be activated by various plasma factors delivered to them via vasa vasorum. Upon activation, fibroblasts will proliferate, migrate and increase the secretion of extracellular matrix proteins leading to fibrosis, neointima formation. Levels of the nerve growth factor, NGF, or catecholamines are increased in inflammation, or hypertension, respectively. Little is known about the activation or the changes in electrical activity of the activated fibroblasts in response to these factors. We hypothesize that fibroblasts activation by NGF or by a β-adrenergic receptor agonist, isoprenaline (Iso), is accompanied by alterations of ion channels activities. Our objectives were to evaluate the voltage dependent potassium (K+) channels in fibroblasts stimulated with NGF or Iso and to correlate their activity with the proliferation and migration of these cells.

Methods

Adventitial fibroblasts were isolated from normal adult rat aorta. Fibroblasts were synchronized in G0 phase prior to stimulation for 24 – 72h with 100ng/mL NGF or 1μM Iso. Different levels of fibroblasts' activation were mimicked by the level of serum in the cultured medium. Patch-clamp, MTT and wound healing assays were used to assess the electrical phenotype, the proliferation and migration of fibroblasts.

Results

Patch-clamp experiments indicated alterations in several different potassium currents. In fibroblasts activated by serum, both stimuli induced an increase of the sustained outward K+ current IDR. The transient outward K+ current (Ito) was significantly decreased by Iso and unaffected by NGF. Hyperpolarization activated K current (Ih), a hallmark of myofibroblast state, was increased by NGF and decreased by Iso. These results correlate with an increase in migration and proliferation rate by NGF, while Iso failed to further increase fibroblasts' activity.

Conclusions

These results demonstrate for the first time that K+-currents such as Ito and Ih are involved in the proliferation and migration of the adventitial fibroblasts.

Experimental study of hepatitis C virus E2 glycoprotein immunogenicity produced in the red microalga

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Aim. The present study focused on testing the immunogenicity of subunit vaccine against the hepatitis C virus (HCV) construct (sE2$^{\Delta HVR1}$) expressed in the red alga *Porphyridium purpureum*. The study was approved by the national designated authority, ANSVSA.

Methods. BALB/c female mice, age 6–8 weeks, were 3 times immunized parenteral, oral or mixed first parenteral and then boost by feeding. The intramuscular administration consists of 25 µg/dose of sE2$^{\Delta HVR1}$ purified from Expi293 cell media, oil-in-water nano-emulsion adjuvanted, followed by two oral administrations of either wild-type or sE2$^{\Delta HVR1}$ *P. purpureum* biomass, at 15 mg/dose. In parenteral administration, groups mice were both primed and boosted by intramuscular injection with either Expi293- or *P. purpureum*-derived sE2$^{\Delta HVR}$.

HCV-specific IgG, IgG1, IgG2a subclasses and IgA were detected by ELISA and by neutralization of HCV-E1E2 pseudo-particles (pp) infection the neutralizating antibodies.

Results. The parenteral immunization with the sE2$^{\Delta HVR1}$ Expi293 triggered the strongest immune response, with high anti-HCV antibody titers (IgG, IgG1, IgG2a) being already evidenced after the first boost, at day 27 and also immune response was also observed in the animal group boost by feeding with sE2$^{\Delta HVR1}$. IgA levels remained undetectable at all time points. The presence of neutralizing antibodies against the homologous HCV isolate were identified by using a luciferase-based HCVpp neutralization assay.

Conclusion. The HCV antigen produced in red algae elicits a strong immune response in mice and showed the fact that microalgae are cost-effective promising production platforms for the production of recombinant proteins.

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Targeting unconventional protein secretion as therapeutic strategy in inflammatory disorders

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The innate immune response is crucial for recognizing and responding to pathogens and harmful substances. Central to this response is the formation of inflammasomes, multi-protein complexes that activate signaling pathways leading to inflammation. While inflammasomes are vital for controlling infections, their dysregulation can result in chronic inflammatory diseases such as atherosclerosis, septic shock, neuroinflammation, post-stroke cardiac dysfunction, and cancer. A key player in inflammatory processes is interleukin (IL)-1β, initially produced as an inactive precursor (proIL-1β). Upon inflammasome activation, caspase-1 cleaves proIL-1β to generate its active form (mIL-1β), which is then secreted into the extracellular space. IL-1β along many other pro-inflammatory cytokines/proteins are exported by an endoplasmic reticulum (ER)/Golgi-independent pathway, generally described as unconventional protein secretion (UPS). One common factor required for the secretion of unconventionally secreted cargoes, both in mammals as well as lower Eukaryotes, are the peripheral Golgi proteins GRASP55/65 which were discovered as Golgi stacking proteins and later studies shown these proteins play key role in unconventional protein secretion both in physiological and pathological conditions

Designing a reporter cell line for IL-1β presents significant challenges, particularly because classical overexpression systems can trigger a persistent inflammatory response, deviating from the physiological context. To address this, we aimed to develop a CRISPR/Cas9 knock-in IL-1β reporter cell line that accurately reflects the physiological response to inflammatory stimuli, enabling us to quantitatively assess IL-1β secretion. We validated this cell line for its ability to secrete a tagged form of IL-1β in response to inflammatory stimuli. With this model established, we screened an FDA-approved drug library to identify existing drugs that could be repurposed as anti-inflammatory agents by inhibiting IL-1β release from innate immune cells. From our screening, we identified three promising candidates that effectively blocked IL-1β secretion. We confirmed their efficacy in primary macrophages and selected the most effective drug for further validation in an animal model of sepsis.

Given our previous findings that deletion of GRASP55 impairs IL-1β secretion, we conducted additional experiments using primary macrophages from GRASP55$^{-/-}$ mice. This allowed us to determine whether the identified drugs act downstream of GRASP55 or if they operate via an independent export pathway. This distinction is crucial for understanding the mechanism of action of these drugs and their potential as therapeutic agents for managing inflammatory conditions.

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Keywords: interleukin-1β, CRISPR/Cas9, drug repurposing

Oxidation of methionine specifically boosts T cell responsiveness against a tyrosinase specific melanoma antigen

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Malignant melanoma is one of the most aggressive cancers, characterized by a rising incidence and poor patient outcomes. A promising therapeutic alternative that avoids the toxicity often associated with chemotherapy is peptide-based vaccination immunotherapy. This approach leverages the ability of cytotoxic CD8+ T lymphocytes to specifically target and kill tumor cells presenting the appropriate HLA-peptide complexes on their surface. However, despite its potential, the clinical efficacy of peptide-based vaccination has been limited. One strategy to enhance effectiveness is to design antigens with improved immunogenicity by modifying the amino acid sequences of peptides. Unfortunately, there is still a lack of understanding regarding how modifications to the side chains of peptide amino acids affect immunological recognition.

In this study, we examined the antigenicity of the melanoma immunodominant peptide from tyrosinase, YMDQTMSQV, after oxidizing its methionine residues. Oxidation of the YMD peptide resulted in several distinct oxidized species, which were purified to homogeneity using HPLC. When assessing the immunological potential of these oxidized forms, we found that at least five CD8+ T cell clones derived from the peripheral blood mononuclear cells (PBMCs) of melanoma patients could recognize them. Moreover, the antigenicity of the oxidized species was significantly greater compared to that of the non-oxidized (native) peptide. Additionally, we found that a small fraction of the oxidation is a result of natural intracellular oxidation, as assessed by mass spectrometry. Our findings suggest that these oxidized forms of the epitope could be promising candidates for immunotherapy, potentially leading to enhanced clinical responses in melanoma patients, and that high-affinity CD8+ T cells could be naturally primed during melanoma progression through immunosurveillance.

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Targeting TGF-β signaling to reinforce the therapeutic potential of cytokine-activated Natural Killer cells as immunotherapy in cancer

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The tumor microenvironment has an important function in the progression of cancer by releasing immunosuppressive factors, such as transforming growth factor β (TGF-β). These factors inhibit the activity and viability of cytotoxic lymphocytes like Natural Killer (NK) cells, allowing evasion of immune cell-mediated killing. The use of inhibitors targeting immunosuppressive TGF-β signaling may be a promising strategy for boosting the antitumor immune response of NK cells.

In this study, we evaluated the effects of a small molecule inhibitor of the TGF-β receptor type I/II kinases, LY2109761, on NK cells' phenotype and functionality following exposure to pathologic levels of TGF-β *in vitro*.

NK cells isolated from C57BL/6 mice spleens were cultured overnight with different cytokine combinations (IL-12/15/18 or IL-12/15/21), TGF-β and LY2109761. The activation conditions were evaluated based on the production of interferon (IFN)γ, granzyme B and perforin, and on the expression of a wide range of surface markers, such as chemokine receptors, nutrient transporters, programmed death receptor ligands, activating and inhibitory receptors. Melanoma and lymphoma cell lines were used to show how TGF-β impacts NK cell cytotoxicity.

NK cells activated e*x vivo* with cytokines had superior cytotoxicity against tumor cells as compared to unstimulated NK cells. Exposing the activated NK cells to pathologic levels of TGF-β led to decrease in cytotoxicity. Flow cytometry and functional assays revealed that TGF-β exposure decreased the following characteristics of activated NK cells: killing of melanoma (B16F10) and lymphoma (YAC-1) cells; expression of chemokine receptors (CCR6, CX3CR1, CXCR4 and CXCR3); nutrient transporters (CD71 and CD98); expression of NK cell activating receptors (NKp46, NKG2D, DNAM-1, CD25 and CD122); and production of IFN-γ, granzyme B and perforin. These changes were reversed by adding LY2109761, an inhibitor of TGF-β receptor.

In conclusion, TGF-β altered the phenotype and functionality of NK cells activated with cytokine, and LY2109761 eliminated the immunosuppressive effect of TGF-β in our assay setups. Our findings emphasize the relationship between TGF-β signaling and NK cell dysfunction, providing insights for potential therapeutic interventions to enhance immune responses against cancer.

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Keywords: NK cells, TGF-β, cancer immunotherapy

Session 6: Genomics of rare disorders

DELETERIOUS *ZNRF3* GERMLINE VARIANTS AS A NOVEL CAUSE OF NEURODEVELOPMENTAL DISORDERS WITH MIRROR BRAIN PHENOTYPES DUE TO DISTINCT DOMAIN-SPECIFIC EFFECTS ON WNT/B-CATENIN SIGNALING

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ZNRF3 (Zinc and RING finger 3) is a negative-feedback regulator of Wnt/β-catenin signaling, which plays an important role in human brain development. Although somatic mutations are frequently observed in cancer, germline variants in ZNRF3 have not been established as causative for neurodevelopmental disorders (NDDs). We identified 12 affected individuals with ZNRF3 variants and various phenotypes via GeneMatcher and evaluated genotype-phenotype correlation.

Of the 12 individuals, eight harbored de novo missense variants and presented with NDD. The other four affected individuals carried truncating/large in-frame deletion variants with non-NDD phenotypes, including heart, adrenal, or nephrotic problems. After structural modelling, representative deleterious and control variants were assessed using in vitro transcriptional reporter assays with and without Wnt-ligand Wnt3a and/or Wnt-potentiator R-spondin (RSPO).

We found missense variants associated with macrocephalic NDD to cluster in the RING ligase domain. Structural modeling predicted disruption of the ubiquitin ligase function likely compromising Wnt receptor turnover. Accordingly, the transcriptional reporter assays showed enhanced Wnt/β-catenin signaling for these variants in a dominant negative manner. Contrarily, a patient with microcephalic NDD harbored a missense variant in the RSPO binding domain predicted to disrupt binding affinity to RSPO and showed attenuated Wnt/β-catenin signaling in the transcriptional reporter assays. In contrast to NDD-associated missense variants, the effects on Wnt/β-catenin signaling were comparable between the truncating variant and the empty vector, as well as benign variants and the wild-type.

In summary we provide evidence for mirror brain size phenotypes caused by distinct pathomechanisms in Wnt/β-catenin signaling through protein domain-specific deleterious ZNRF3 germline missense variants.

Clinical findings in rare disorders associated with autistic behavior

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Background: Autism spectrum disorders (ASDs) represent a heterogeneous group of neurodevelopmental conditions, often with a complex clinical picture, especially in cases with a genetic background. Our paper summarizes the clinical data of children with ASDs and rare disorders.

Methods: The clinical evaluation included neurological, psychiatric, and psychological evaluation, and specific investigations (MRI, EEG, ultrasounds etc.). Genetic tests, including array-based comparative genomic hybridization (array-CGH) and MLPA for fragile X, were performed for all ASD patients.

Results: Twenty-seven patients presented rare genetic syndromes, including both deletions and duplications. The most common clinical features included global developmental delay or intellectual disability, speech problems, and dysmorphic features. Epileptic seizures or EEG epileptiform discharges, and brain anomalies were also noted in some patients.

Conclusions: The clinical and genetic aspects of our cohort were very heterogeneous, revealing the fact that ASD is a condition with different etiopathogenic mechanisms, often as part of a complex phenotype. The new molecular technologies can contribute to a better understanding of the genetic causes of ASD, with important implications on management plan of the patients.

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Rare genomic variants in a cohort of children with neurodevelopmental disorders and autism

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Background: Autism spectrum disorders (ASDs) are complex neurodevelopmental conditions (NDC) associated with lifelong deficits in social interaction and communication, as well as many co-morbidities. ASDs have a well-described genetic background characterized by a wide spectrum of defects, from single nucleotide variants to large deletions and duplications.

Aim: Our paper summarizes the genomic findings in a Romanian cohort with ASDs, previously uncharacterized by genetic testing, presenting with autistic behavior and various phenotypic features.

Material and methods: 305 children diagnosed with ASD were enrolled from the patients referred to the hospital for various NDCs and neuropsychiatric problems, between September 2019 and February 2022. ASD diagnoses were based on the ICD-10. Array-CGH was performed on whole blood gDNA using 4x180K SurePrint G3 Human CGH microarray (Agilent Technologies).

Results: Array-CGH analysis detected 469 rare CNVs in the ASD group. Twenty-seven ASD individuals harbored rare clinically relevant genomic imbalances: one complex rearrangement (deletion/duplication), seven duplications, 17 deletions and two Y-chromosome aneuploidies. Rare CNVs overlapping known ASD-associated CNV loci included duplications of 1q21.1, 15q11.2q13.1, 17p11.2, as well as deletions of 3q29, 15q11.2. Several dosage sensitive genes robustly associated with ASD and NDC, for example *NRXN1, MBD5*, and *TRIP12* were detected in our ASD group.

Conclusions: Array-CGH detection yield, expressed as the number of ASD patients with solved genetic etiology in our cohort, was 9%. Our results highlight once more the genetic heterogeneity which underlies a wide array of pathogenic mechanisms in ASD.

Funding: The research leading to these results has received funding from the EEA Grant 2014-2021, under the project contract No 6/2019.

Updates in molecular monitoring of mutant nucleophosmin 1

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NPM1 mutations are the most common genetic alterations in adult AML, occurring in approximately one-third of cases. The mutant NPM1 is found in 50% to 60% of adult acute myeloid leukemia (AML) cases with a normal karyotype. The mutations in NPM1 lead to the loss of nucleolar localization signals and the acquisition of a strong nuclear export signal, which promotes cytoplasmic accumulation. However, recent studies have shown that NPM1c directly drives the expression of AML promoting genes from within the nucleus. Therefore, the oncogenic function of mutant NPM1 may involve dual mechanisms, acting in both the nucleus and the cytoplasm. Because of the unique features, NPM1-mutated AML is recognized as a distinct entity in the 2017 World Health Organization (WHO) classification of myeloid neoplasms as well as in the 2022 International Consensus Classification (ICC).

Monitoring of the NPM1 mutations in acute myeloid leukemia (AML) is crucial for several reasons, as emphasized in the measurable residual disease (MRD) guidelines published in 2018 by the European Leukemia Network (ELN) Working Party. Persistence of NPM1mut transcripts above a given threshold at the end of the treatment is associated with higher risk of disease relapse. NPM1-mutated MRD is the sole prognostic factor, independent of the cytogenetic and molecular context. Furthermore, NPM1-mutated MRD is a predictive factor for determining the indication of hematopoietic transplant. The ELN recommended method for MRD assessment in case of NPM1 is the quantitative PCR coupled with mRNA reverse transcription (RT-qPCR). However, a critical step is identification of the exact NPM1 mutation by sequencing of each patient sample. This information is required to set up RT-qPCR for measurable residual disease monitoring. We developed an alternative assay for detection of any NMP1 mutations with only one pair of primers. The method is simple to use and eliminates the need for sequencing.

Kye words: acute myeloid leukemia, measurable residual disease, NPM1

Session 7: Poster Session

MITOCHONDRIAL DYSFUNCTION CHARACTERISE PERIPHERAL ARTERY DISEASE IN DIABETIC MOUSE. PRELIMINARY DATA

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Introduction. Peripheral artery disease (PAD) occurs as a side effect of type 2 diabetes (T2D). PAD combined with lower limb skin wounds and neuropathy, results in diabetic foot syndrome (DFS). Mitochondria is a cell organelle that produce most of the chemical energy needed to power the cell's biochemical reactions. Hypothesis. In pathological circumstances, dysfunction in the chemical energy balance may be involved in the progression of the disease. Aim. To find out whether the mitochondrial proteins' change in murine lower-limb wounds during T2D with PAD and anti-TLR4 therapy settings.

Methodology. We used 4 groups of C57Bl/J6 mice with experimental diabetes induced by administrations of streptozotocin (5x40 mg/kg body): 1. a control group (D, n=3), 2. a group with induced plantar wound (DW, n=3), 3. a group with a lower left ischemic limb created by excision of a part of the femoral artery and wound (DIW, n=5), and 4. an ischemic mouse group with the previously described wound (DIWT, n=5) that received anti-TLR4 treatment. The hyperglycemic state was confirmed by the presence of ≥300 mg/dL glucose. After 14 days, the mice were euthanized, and plantar fascia was taken for proteome analysis using mass spectrometry.

Results. Bioinformatic analysis (protein FDR005 and Sequest score≥10) identified 2667 proteins having a statistically significant difference in abundance with a factor of ≥1.2 when compared to the D group. There are 185 mitochondrial proteins significantly modified, which correspond to the enrichment of citrate cycle, synthesis and degradation of ketone bodies and oxidative phosphorylation in KEGG Pathways. Further analysis is in progress to assess the mechanism of anti-TLR4 treatment during T2D with PAD.

Conclusions. The preliminary results demonstrate modifications of mitochondrial activity of the lower ischemic limb wound tissue in response to TLR4 inhibition treatment during experimental T2D with PAD.

Keywords: proteomics, lower-limb wounds, energy balance, TLR4 therap

REGULATION OF ALARMINS IN THE SKELETAL MUSCLE AFFECTED BY THE DIABETIC FOOT SYNDROME

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Introduction: The diabetic foot syndrome (DFS) is a complex, multifaceted inflammatory disease having as hallmark the diabetic foot ulcer. The patient's extensive limb muscle injury/death and impaired muscle regeneration negatively affects the patient's quality of life. Alarmins are key players in generating a physiological immune response, but their action has also been associated with exacerbation of inflammation and progression of many diseases.

Aim: To uncover potential therapeutic targets of DFS by analyzing the proteomic alarmin fingerprint in the lower limb skeletal muscle of an experimental DFS mouse model.

Materials and Methods: C57BL/6 mice with streptozotocin-induced diabetes were subjected to lower limb plantar ulceration and femoral artery ligation-induced ischemia (DIR, n=5). Another experimental group subjected to similar conditions received four (3 mg/kg) doses of i.p.-administered TLR4 inhibitor (DIRT1, n=5). A third group consisting of diabetic mice with plantar wounds (DR, n=3) was used as control. Gastrocnemius muscle samples were harvested from all mice and suitably processed for mass-spectrometry based proteomic analysis using the LTQ Orbitrap Velos Pro ETD.

Results: Pathway enrichment bioinformatic analysis demonstrated a statistical association of the DIRT1/DR differentially abundant proteins with processes such as citrate cycle, oxidative phosphorylation, thermogenesis and fatty acid degradation. We unambiguously identified 38 alarmins, comprising members of heat shock proteins, annexin, galectin, histone and S100 families. The HSP10, HSPB2, Annexin A1, S100-A10 were all upregulated by the treatment, while S100-A8 reverted the upregulation caused by the ischemic event.

Conclusions: The identified alarmin regulation pattern can aid in the development of therapeutical strategies for DFS-related myopathies.

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Keywords: alarmins, diabetic foot syndrome, proteomics, mass spectrometry

SHORT-TERM S100A9 BLOCKAGE AFTER MYOCARDIAL INFARCTION FAVOURABLY MODULATES ENERGY METABOLISM IN ISCHEMIC LEFT VENTRICLE

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Introduction: The infarcted heart is energetically compromised, a feature characterized by reduced adenosine triphosphate (ATP) concentrations with catastrophic consequences on cardiac function. Others and we have previously shown that short-term blockade of the protein S100A9 improves cardiac performance in mice after myocardial infarction (MI). In this study we questioned whether short-term S100A9 blockade after MI affect metabolic processes that generate ATP.

Methods: Nine C57BL/6 mice were subjected to MI and divided into 2 experimental groups: mice with MI and mice with MI treated with a specific S100A9 blocker (ABR-238901) during the first 48 hours post-MI (MI+ABR). MI was induced by permanent left coronary ligation. Mass spectrometry, pathway enrichment analysis, Western blot, and RT-PCR were performed on left ventricle tissues harvested seven days post-MI from all experimental mice.

Results: Compared to the MI group, in the MI+ABR mice the abundance of 600 proteins was significantly altered (abundance ratio ≥ 1.5 or ≤ 0.667; adjusted P-value ≤ 0.05). The pathway enrichment analysis revealed the association of several of these proteins with oxidative phosphorylation, mitochondrial fatty acid beta-oxidation, glycolysis and citrate cycle. We detected that some proteins (NDUFAB1, UQCRC1, HADHA, ACAA2, ALDOA, PKM1, DLD, DLAT, PDHX, ACO2, IDH3A, FH1) with key roles in early metabolic changes and ATP production exhibited increased abundances in the ischemic heart of mice that received the S100A9 blocker.

Conclusion: This study provides direct evidence that blocking S100A9 in the first 48 h post-MI significantly improves ATP production. The data expand the knowledge of the critical players involved in the recovery of energy metabolism post-MI.

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Keywords: myocardial infarction; S100A9 blockade; proteomic analysis

ASSESSMENT OF PRIMARY HUMAN DERMAL FIBROBLASTS FOR ENGINEERING SKIN EQUIVALENTS - Preliminary data

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Background. Dermal fibroblasts (Fb) represent a heterogeneous cell population which differ depending on the anatomical site of origin, i.e. the dermis area from which they are isolated (reticular or papillary). It has been shown that these cells secrete important growth factors such as EGF, GM-CSF, HGF, IGF-1, PDGF-BB, TGFβ2, and also VEGF, but the level of these secreted cytokines may differ between cell batches. As such, their capacity to induce epidermal differentiation in skin equivalents is widely variable.

Aim. To establish an evaluation method for the ability of various human dermal Fb batches to induce epidermal differentiation to be used for the development of skin equivalents.

Methodology. Fb were derived using the explant method from skin samples originating from human subjects undergoing esthetic surgery procedures. Early passage cells (up to 5) from 3 subjects were checked for mesenchymal stem cells markers (CD90, CD73, CD105) via flow cytometry. Furthermore, the expression of type I and III collagen, decorin, integrin α5β5, fibronectin and vimentin, was evaluated using immunocytochemistry. Fb were seeded on collagen porous scaffolds and cultivated for 4 weeks. Keratinocytes (HaCaT cell line) were added on top of the scaffolds, and after 3 days, the matrices were lifted to the air-liquid interface. Haematoxylin-eosin and Ayoub-Shklar staining were performed on paraffin sections. Epidermis differentiation markers were assessed on cryosections via immunofluorescence microscopy

Results. Flow cytometry data showed that Fb derived from all human subjects were positive for CD90, CD73, CD105 at similar levels. Also, all cell batches expressed type I collagen, vimentin and deposited fibronectin. For one of the three batches decorin was expressed intracellularly, while being absent in the other two. The same Fb batch was also able to induce the thickest epidermis in the skin equivalents. Also, it promoted the differentiation of the keratinocytes layer as evidenced by the presence of keratin 10 and involucrin as well as by Ayoub-Shklar staining.

Conclusion. The results indicate that, although the Fb derived from the skin of three human subjects expressed specific MSC markers, only the batch with an intracellularly expression of decorin had an appropriate capacity for engineering skin equivalents using a collagen porous matrix.

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Keywords: in vitro skin model, fibroblasts heterogeneity, differentiation

Estrogen deficiency-induced signs of insulin resistance in ovariectomized APPNL-F and APPNL-G-F knock-in mice

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Alzheimer's Disease (AD) is a neurodegenerative disorder that affects more than 55 million people worldwide, representing 60-70% of all cases of dementia. Women, especially after menopause, bear a larger burden of the epidemic, running a two-fold risk of developing AD. Acting as a neuroprotective agent, estrogen is a master regulator of bioenergetic systems in the brain. Furthermore, there is a growing consensus indicating that insulin influences cerebral bioenergetics too. Having an important role in proteostasis, insulin can influence clearance of amyloid β peptide and phosphorylation of tau, which are telltale signs of AD.

To investigate the relationship between estrogen deficiency and insulin resistance ovariectomized APPNL-G-F and APPNL-F knock-in mice were used. We also aimed to extend our knowledge regarding brain carbohydrate metabolism using SH-SY5Y cell cultures, especially fructose metabolism, as HCFS foods could be a contributing answer to why AD is a disease limited to humans.

APPNL-G-F 6 months old mice expressed a decrease in ESR2 mRNA production, suggesting a paradoxal increase in estrogen levels, APPNL-F presenting the opposite effect. Our hypothesis is that the brain presents a failsafe system of estrogen production for it to become ultimately overloaded in APPNL-F 18 months old mice. Moreover, changes in Glut4 and Igf1 mRNA expression overlapped with insulin resistance specific biomarkers. Preliminary similar results in SH-SY5Y cells cultured in high-fructose concentration media were obtained.

Moving forward, for more accurate results there is a need to create an aging timeline, comparing APPNL-F and APPNL-G-F mice separetly, and to assess the levels of more biomarkers and amyloid β peptide levels in the SH-SY5Y cell cultures. The lack of neurological symptoms associated with neurodegeneration in the mice calls for the use of a more biosimilar model, such as iPSC-derived hippocampal spheroids.

Keywords: Alzheimer's Disease, Menopause

Session 8: Nephropathology

Kidney biopsy processing for immunofluorescence and electron microscopy

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Kidney biopsy is essential to diagnose kidney diseases or to monitor kidney diseases progression. For accurate evaluation, the renal tissue needs to be examined with different techniques: immunofluorescence, light microscopy and electron microscopy. For maximum information, the tissue for each technique need to contain glomeruli and the biopsy cores should be divided properly to assure optimal material for diagnostic. Each technique requires different processing steps and special attention should be payed from the beginning, with fragment for IF placed in saline solution or transport media and different fixation for light and electron microscopy. The techniques required for kidney biopsy evaluation generate different kind of information with equal value for an accurate diagnosis.

Recently, new therapies have been introduced for specific lesions at the level of the glomerulus. Morphological changes in glomerulopathies must be properly documented so that patients can receive a targeted therapy. That is why the processing of the biopsy fragment must be done precisely from the beginning.

Trends of biopsy confirmed renal diseases in Romania: a single-center study spanning 28 years

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Background: The epidemiology of biopsy-confirmed renal diseases in Romania is difficult to establish due to the lack of a national renal biopsy registry. Nonetheless, several single center reports are available in the literature, from which a partial picture can be reconstructed.

Objectives: The primary aim of the study was to report on the trends of biopsy-confirmed renal diseases recorded in the database of the Victor Babeș National Institute of Pathology. A secondary goal of our analysis was to compare these data with previously published single center reports in Romania, as well as other regional or national European renal biopsy registries.

Methods: Patients whose renal biopsies were evaluated at the Victor Babeș National Institute of Pathology between 1996 and 2023 were included in the study. We analysed diagnosis frequency and age distribution trends over the 28 years spanned by the database.

Results: A total of 1954 adult native renal biopsies were included in the analysis. Male subjects accounted for 52% of the studied population. The median age at diagnosis over the entire study period was 46 years (IQR: 33-58), with an increasing trend over time ($p<0.01$). Overall, the most frequent diagnosis was IgA nephropathy (15%), followed by membranous nephropathy (12%). Diabetic kidney disease and lupus nephritis each accounted for approximately 7% of diagnoses.

Conclusion: The diagnosis frequencies and trends observed in our database are largely consistent with other European reports. Comparison and aggregation with reports from other Romanian centers proved difficult, due to differing subject inclusion criteria and diagnosis reporting practices. This highlights the need for a national biopsy registry, where data can be recorded according to harmonised practices. This would contribute to a better understanding of the epidemiology of renal diseases in Romania, and would constitute a valuable resource for further research in the field of renal diseases.

Keywords: adult native kidney biopsy, diagnosis frequency, nephropathology.

Microvascular inflammation: in and out of antibody-mediated rejection

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Microvascular inflammation (MVI) is a hallmark feature of activity in antibody-mediated injury according to Banff. It is also helpful in stratifying antibody-mediated rejection (ABMR) as active ABMR, chronic active ABMR and chronic ABMR. Because MVI is considered an independent risk factor for kidney graft loss, it has gained much attention in the transplant field.

MVI has been described as a histopathologic entity, besides the classical forms of ABMR with HLA-DSA and non-HLA-DSA, in ABMR without DSA, high HLA mismatch without rejection, T-cell mediated rejection (TCMR), borderline TCMR, transplant-related viral infections (CMV, BKVN), recurrent glomerulopathies or associated with ischemia reperfusion injury. Also, an important concept emphasized in the most recent studies is related to the involvement of NK cells through the missing-self process in the production of MVI, in patients with ABMR and no DSA. The dichotomization of ABMR and TCMR based on serological, molecular and histological elements, including MVI, seems to be contradicted by the heterogeneity of the inflammatory cell population and their overlap in the two types of rejection and missing-self mechanism explains it partially.

The interest in MVI and its role in kidney graft injury led the Banff Working Group 2022 to introduce the entity of "MVI, DSA-negative and C4d-negative". This entity is a pure descriptive phenotype with unclear cause which requires further research for pathophysiology understanding, prevalence, causes and the appropriate management approach.

Keywords: microvascular inflammation, ABMR, TCMR, DSA, Banff

The unsuspected heterogeneity of graft inflammatory infiltrate during kidney allograft rejection

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Kidney transplantation remains the treatment of choice when it comes to chronic kidney disease (CKD) (1). Given the fact that one of the biggest factors behind graft loss is rejection, understanding the exact pathophysiological mechanisms is crucial (2). For the moment, the diagnosis of rejection is based on the criteria formulated by the Banff classification with two major types currently recognized: antibody mediated rejection (ABMR) and T-cell mediated rejection (TCMR) (1). Although there is an increasing number of studies that show that the exact cellular composition is important in prognosis, the current Banff classification does not currently recommend taking this heterogeneity into account (1).

In the past few decades, not much progress has been made in the way we read kidney biopsies (3). However, in the last few years several new techniques have revolutionized the way we understand rejection. For example, multiplex immunofluorescence can bypass the constraints of immunohistochemistry by analyzing multiple markers of the same slide (4). Single cell transcriptomics (scRNA-seq) and spatial transcriptomics allow to pinpoint the exact cellular types with the advantage of accurately describing their molecular signature and even their location (in the case of spatial transcriptomics) (3). All these different methods have highlighted the remarkable heterogeneity of the cellular infiltrate and also have allowed us to better describe and understand the cell-to-cell interactions and immunological pathways that take place during rejection (5).

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