Institutul Național de Cercetare-Dezvoltare
în Domeniul Patologiei și Științelor Biomedicale
„VICTOR BABEȘ”
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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

INTERNATIONAL PATHOLOGY CONFERENCE

OF THE „VICTOR BABEȘ” INSTITUTE

BUCHAREST

BOOK OF ABSTRACTS

2 – 4 November 2023

BUCHAREST, ROMANIA

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Contents

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SCIENTIFIC PROGRAMME

Thursday, November 2nd 2023

9:30 **Registration**

11:00-11:15 **Coffee break**

11:15-13:15 **Session 1: `Victor Babeș` National Institute of Pathology - research news**

Session Chair – Prof. Mihail Eugen Hinescu

Molecular approaches in tumoral 3D models gene-edited by the CRISPR/Cas9 method in the development of solutions for personalized medicine in cancer. Elena Codrici
Impact of high fat diet on phenotype and function of Natural Killer cells. Gheorghita Isvoranu
Inflammation-redox crosstalk in Alzheimer's diseases. Elena Milanesi
Assessment of EGFR-RAS-RAF pathway mutation status in healthy skin, benign nevi, and cutaneous melanomas – evaluation using droplet digital PCR. Monica Neagu
Rare genetic variants in brain heterotopia - results of an ERA NET E-RARE collaboration. Aurora Arghir

13:15- 14:15 **Lunch time**

14:15-15:15 **Molecular Aspects of Mesothelioma**

Keynote Lecture - Victor Babeș Honorary Scientist Award

Lucian R. Chirieac

Brigham And Women's Hospital

Professor of Pathology, Harvard Medical School, Boston, MA, USA

15:15-15:30 **Coffee break**

15:30-15:45 **AxioScan, latest technology**

Sommer KIEREN, Carl Zeiss Microscopy GmbH. (S)

15:45-17:45 **Session 2: Short communication - Young Pathologists**

Session Chair – Adelina Cohn

Prognostic Histopathological Factors in Thick Cutaneous Melanomas. Dana Țăpoi, Emergency Clinical Hospital Bucharest, Romania

Diagnostic and reporting challenges in papillary thyroid carcinoma developing in thyroglossal duct cyst. **Antonia-Carmen Georgescu**, Nephrology Clinical Hospital `Dr. Carol Davila`, Bucharest, Romania

Tumor immune microenvironment in lung carcinoma. **Florina Almarii,** Fundeni Clinical Institute, Bucharest, Romania

The clinicopathological spectrum of triple-negative breast cancer. Tiberiu Augustin Georgescu, `Alessandrescu- Rusescu` National Institute for Mother and Child Health, Bucharest, Romania

Peripheral neuroblastic tumors diagnosis and prognostic factors in the new WHO blue book on pediatric tumors. **Oana Neagu**, `Grigore Alexandrescu` Emergency Clinical Hospital for Children, Bucharest, Romania

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Thursday, November 2nd 2023

A practical approach in challenging `spitzoid` melanocytic lesions based on IHC panels. **Adelina Baltan**, Poundbury Cancer Institute for Personalized Medicine, Dorchester, UK

18:00 **Opening ceremony – Victor Babeș Library**

Friday, November 3rd 2023

	Victor Babeș amphitheater	Ioan Moraru amphitheater
09:00-11:00	**Session 3: Updates in Immunology** **Session Chair – Monica Neagu** **Combining immunotherapy with radiology, new pathways of research supported by particles provided by the high-power laser system at ELI-NP**. **Klaus Michael Spohr**, School of Computing, Engineering and Physical Sciences, University of the West of Scotland; Extreme Light Infrastructure & Horia Hulubei National Institute for RD in Physics and Nuclear Engineering, Romania **Enabling further breakthroughs in immunotherapy via interdisciplinary collaborations to enhance therapeutic monitoring capabilities.** **Marius Jurca**, Extreme Light Infrastructure – Nuclear Physics, `Horia Hulubei` National RD Institute for Physics and Nuclear Engineering; Engineering and Applications of Lasers and Accelerators Doctoral School, University Politehnica of Bucharest, Bucharest, Romania; ALSITEC srl, Haguenau, France **CAR-T and CAR-NK cells in cancer immunotherapy.** **Florina Bojin**, `Victor Babeș` University of Medicine and Pharmacy Timișoara; Center for Gene and Cellular Therapies in Cancer – OncoGen, `Pius Brînzeu` Clinical County Emergency Hospital Timișoara, Romania **Characterization of donor-specific alloreactive CD4+ and CD8+ cellular immune T cell responses in the lung allograft and blood in lung transplant recipients**. **Iulia Dana Popescu**, Pittsburgh University, Pittsburgh. **From high field to Earth field. Nuclear Magnetic Resonance spectroscopy methods to study the microglia and glioblastoma cells.** **Ioana Fidel**, Extreme Light Infrastructure – Nuclear Physics (ELI-NP), `Horia Hulubei` National R&D Institute for Physics and Nuclear Engineering; Interdisciplinary School of Doctoral Studies, University of Bucharest, Romania **Personalized vaccines in cancer – present and future directions.** **Virgil Păunescu**, `Victor Babeș` University of Medicine and Pharmacy Timișoara; Center for Gene and	**Session 4: Genomics and Proteomics - two facets of multi-omics in health care and research** **Session Chairs - Cristiana Tanase, Aurora Arghir** **Genomics and Metagenomics involved in Neurodevelopmental disorders: Fragile X syndrome and Autism Spectrum Disorders,** **Yolanda de Diego Otero,** Biomedical Research Institute of Málaga-IBIMA, Malaga Regional University Hospital, Spain **Personalized tumor models reveal mechanisms of glioblastoma immunosuppressive microenvironment and therapy resistance.** **Barbara Breznik,** MPharm, Cancer Biology Group Leader National Institute of Biology Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia **Microphysiological Systems as Disease Models** **Devrim Pesen Okvur,** Izmir Institute of Technology www.iyte.edu.tr Initio Cell BV www.initiocell.com. Turkey. **The Impact of Radiation on Cancer - Associated Fibroblasts in Head-and-Neck Cancer.** **Marleen Ansems,** Radiotherapy & OncoImmunology Laboratory, Department of Radiation Oncology, Radboud University Nijmegen,The Netherlands **COST CA21135 - IMMUNO-model - WG1: In vitro and ex vivo cancer immunotherapy models – establishing basic protocols for immunotherapy response evaluation.** **Devrim Pesen Okvur**, Izmir Institute of Technology, Turkey

Friday, November 3rd 2023

	Victor Babeș amphitheater	Ioan Moraru amphitheater
	Cellular Therapies in Cancer – OncoGen, `Pius Brînzeu` Clinical County Emergency Hospital Timișoara, Romania **COST CA21135 - IMMUNO-model - WG3: Solid tumors - models to study immunotherapy efficacy and toxicity in solid tumors, and validate biomarkers to monitor these effects**. Marleen Ansems, Radiotherapy & OncoImmunology Laboratory, Department of Radiation Oncology, Radboud University Nijmegen The Netherlands	
11:00-11:15	**Coffee break**	
11:15-12:45	**Session 5: Short communication - Biomedical research** **Session Chair – Dr. Gheorghița Isvoranu** **Plasma microRNAs as predictors of post acute myocardial infarction ventricular remodeling. Ioan Ovidiu Sîrbu,** `Victor Babeș` University of Medicine and Pharmacy, Timișoara, Romania **NK cell memory in unconventional models. Orhan Rashid.** Sir Henry Wellcome Fellow School of Infection and Immunity, University of Glasgow, UK **Drug repurposing screening for anti-inflammatory molecules. Marioara Chirițoiu-Butnaru,** Institute of Biochemistry of the Romanian Academy Bucharest, Romania **Experimental studies of immunization against hepatitis B and C. Crina-Georgeta Stăvaru,** `Cantacuzino` Medical Military National Research and Development Institute Bucharest, Romania **Methionine oxidation selectively enhances T cell reactivity against a melanoma antigen. Gabriela Chirițoiu,** Institute of Biochemistry of the Romanian Academy Bucharest, Romania **Chrysin-Based Supramolecular Cyclodextrin-Calixarene Drug Delivery System: A Novel Approach for Attenuating Cardiac Fibrosis in Diabetes**. **Anca Hermenean,** `Vasile Goldiș` Western University of Arad, Romania	**Session 6: Cardiovascular pathology** **Session Chair – Elisa Anamaria Liehn** **Reducing diabetic macrovascular complications due to peripheral arterial disease. Gustavo Crespo-Avilan,** Duke National University of Medicine, Singapore **ND-13, a small DJ-1 derived peptide as a novel treatment for mitoprotection in acute myocardial infarction**. **Sauri Hernandez-Resendiz**. National Heart Centre Singapore **The role of Phosphatidylserine oral supplementation in vascular regeneration. Dan Valentin Pistritu,** University of Medicine and Pharmacy `Carol Davila` Bucharest, Romania **3D Tissue Reconstruction from 2D histology slides for the study of the extracellular matrix. Victor Ungureanu,** University of Medicine and Pharmacy `Carol Davila` Bucharest, Romania
12:45-13:15	**Automated Solutions for Advancing Protein and Cell Biology Discovery. Sylvia De Bruin, Imaging specialist Molecular Devices. (S)**	**Pathologist centric case and image management system. Ákos Ágyi, PhD, Product Manager 3Dhistech, Budapest, Hungary (S)**

Friday, November 3rd 2023

	Victor Babeș amphitheater	Ioan Moraru amphitheater
13:15-14:00	**Lunch time**	
14:00-15:00	**Keynote Lecture: Epigenetic regulation of therapy response in cancers** **Prof. Tugba Bagci Onder, PhD** Professor in Medical Biology, Brain Cancer Research and Therapy Laboratory, Koç University School of Medicine, Istanbul, Turkey	
15:00-16:30	**Session 7: Nephropathology** **Session Chairs – Mihaela Gherghiceanu** **What's new in antibody-mediated rejection of the kidney graft after the 2022 Banff meeting? Bogdan Sorohan**, Fundeni Clinical Institute Bucharest, Romania **Precise composition and localization of the inflammatory burden of 125 kidney transplant rejections: determinants and prognosis impact**. **George Terinte-Balcan**, Hôpital Universitaire Necker, Paris, France **Old and new in IgA nephropathy. Mihaela Gherghiceanu,** `Victor Babeș` National Institute of Pathology, Bucharest, Romania	**Session 8: Neurosciences** **Session Chair – Bogdan O. Popescu** **Modulation of TRPM8 function by the prostacyclin receptor: involvement of Gq/11 proteins**. **Alexandru Babeș,** University of Bucharest, Romania **Modeling human de novo heterozygosity using mosaic gene dosage differences in mice. Tudor Badea**, Transilvania University of Brasov, Romania **Vascular and glial changes in the brain of patients with SARS-CoV-2 infection. Daniel Pirici,** University of Medicine and Pharmacy of Craiova, Romania
16:30-16:45	Coffee break	
16:45-18:15	**Session 9: Next generation pathology** **Session Chair – Octavian Bucur** **Digital Pathology for cervical cancer screening in low and middle income countries. Rajan Dewar,** Vice-Chair, New York Medical College, New York, USA **Digital Pathology in automating hematology workflow - AI in peripheral smear and bone marrow cell classification**. **Sayed Shahabuddin Hoseini,** Westchester Medical Center. New York, USA **and Rajan Dewar**, New York Medical College, New York, USA **Classification of Early Breast Neoplastic Lesions using Deep Learning and Expansion Pathology**. **Victor Ungureanu**, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania	**Session 10: Muscle pathology** **Session Chair – Laura Cristina Ceafalan** **Intellectual disability in Duchenne muscular dystrophy; Considerations on a clinical case. Magda Budișteanu,** Clinical Hospital of Psychiatry `Prof.Dr. Al. Obregia` Bucharest, Romania **Pathological aspects and classification criteria of inflammatory myopathies. Alexandra Bastian,** `Carol Davila` University of Medicine and Pharmacy Bucharest, Romania **Idiopathic inflammatory myopathies - particular aspects in children. Niculina Butoianu** - Clinical Hospital of Psychiatry `Prof.Dr. Al. Obregia`, Pediatric Neurology Bucharest, Romania **The involvement of PARKIN in the skeletal muscle phenotype in Parkinson's disease. Emilia Manole,** `Victor Babeș` National Institute of Pathology, Bucharest, Romania

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Friday, November 3rd 2023		
	Victor Babeș amphitheater	**Ioan Moraru amphitheater**
		Nicotinamide Mononucleotide (NMN) effects in Type 2 Diabetes muscle: A Deep Proteomics Study. George Cătălin Marinescu, Independent Research Association Bucharest, Romania

Saturday 4, November 2023	
09:00-11:00	**Course – Electron microscopy for study and diagnosis of viruses** **Mihaela Gherghiceanu, Emanuel Fertig,** `Victor Babeș` National Institute of Pathology, Bucharest, Romania
11:00-11:15	**Coffee break**
11:15-12:30	**Session 11: Short communication - young researchers** **Best Presentation Award - Motic optical microscope** **Session Chairs – Ana-Maria Enciu & Emanuel Fertig** **In vitro reconstitution of human RQT dependent ribosome dissociation reaction. Leona Chițoiu,** Beckmann Lab, Gene Center, Ludwig-Maximilians-Universität, München, Germany ***In vitro* generation of functional melanoma models by CRISPR-Cas9 gene editing technology coupled with lentiviral transduction. Elena Georgiana Dobre,** `Victor Babeș` National Institute of Pathology, Bucharest, Romania **Impact of gut microbial molecules on the onset of Parkinson's disease – an *in vitro* study. Octavian Ioghen,** `Victor Babeș` National Institute of Pathology, Bucharest, Romania
12:30 - 13:00	**Session 12: Short communication - students** **Session Chairs – Ana-Maria Enciu & Emanuel Fertig** **Anti-tumor potential of novel synthesized thiosemicarbazide derivatives. Antonia Maria Stroe,** University of Medicine and Pharmacy `Carol Davila`, Faculty of Pharmacy, Bucharest, Romania **Biological activities of two plant extracts rich in isoflavones -In vitro investigation on breast adenocarcinoma cell line. Ana Maria Cirjan,** University of Medicine and Pharmacy `Carol Davila`, Faculty of Pharmacy, Bucharest, Romania
13:00 - 14:00	**Lunch time**
14:00-15:30	**Communication (K)now** **Session Chairs – Prof. Dan Anton Vasiliu, unatc.ro** **Andreea-Diana Jicman. Îmbunătățirea abilităților de relaționare și comunicare prin joc teatral la copiii și tinerii defavorizati / Improvement of the relational and communication skills through theatrical play in disadvantaged children and youth**

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Saturday 4, November 2023

Ana Maria Victoria Vicovan. Facilitarea jocurilor teatrale pentru copiii spitalizați: de la izolare la exprimare / Facilitating theatrical games for hospitalized children: from isolation to expression
Adelina Dobrea. Dezvoltarea competențelor de comunicare la tinerii refugiați. Jocul teatral - limbaj universal de comunicare / Developing communication skills in young refugees. Theatrical play - universal language of communication
Andreea-Diana Jicman, PhD, Georgiana Adelina Dobrea, Ioana-Mădălina Lixăndroaia, Ana Maria Victoria Vicovan. Intervenție prin teatru pentru alfabetizarea emoțională și optimizarea abilităților de comunicare la tinerii aflați în situații de risc / Theater intervention for emotional literacy and optimization of communication skills in young people at risk

15:30-15:45 **Closing remarks**

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DAY 1 - THURSDAY, NOVEMBER 2

Keynote Lecture - Molecular Aspects of Mesothelioma
Session 2: Short communication - Young Pathologists

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Keynote Lecture - Molecular Aspects of Mesothelioma

Molecular Aspects of Mesothelioma

Lucian R. Chirieac
Brigham And Women's Hospital
Professor of Pathology, Harvard Medical School, Boston, MA, USA

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Session 2: Short communication - Young Pathologists

PROGNOSTIC HISTOPATHOLOGICAL FACTORS IN THICK CUTANEOUS MELANOMAS

Țăpoi D.A. [1,2], Gheorghișan-Gălățeanu A.A. [2], Dumitru A.V. [1,2], Costache M. [1,2]
[1] Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[2] University Emergency Hospital, Bucharest, Romania

Introduction

Thick cutaneous melanomas (Breslow depth > 4 mm) are locally advanced tumors (pT4) and have a poor prognosis. This study aims to evaluate clinical and histopathological features that can predict the prognosis of thick melanomas.

Material and methods

We performed a study on 94 thick primary cutaneous melanomas, diagnosed between 2012-2018. The following parameters were assessed: age, gender, tumor location, histological subtype, Breslow depth, Clark level, mitotic index, the presence/absence of ulceration, necrosis, regression, microsatellites, neurotropism, lymphovascular invasion and resection margins to determine their association with disease progression and survival.

Results

On univariate analysis, progression-free survival (PFS) was significantly associated with female gender, Breslow depth, superficial spreading melanoma (SSM) subtype, mitotic index, necrosis, microsatellites, and perineural invasion. Overall survival (OS) was significantly associated with female gender, Breslow depth, SSM subtype, necrosis, microsatellites, and perineural invasion. On multivariate Cox proportional hazards regression, Breslow depth, necrosis, microsatellites, and perineural invasion were associated with PFS. Breslow depth, necrosis, microsatellites, and perineural invasion were associated with OS. Kaplan Meier survival analysis showed that OS significantly decreased as Breslow depth increased.

Conclusions

The only independent adverse prognostic factors are increasing Breslow depth, necrosis, microsatellites, and perineural invasion for both PFS and OS. Our study is among the very few to demonstrate that necrosis and perineural invasion are independent prognostic factors for PFS and OS. Additionally, we have demonstrated that even in pT4 melanomas, increasing Breslow depth remains an important prognostic factor for OS.

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DIAGNOSTIC AND REPORTING CHALLENGES IN PAPILLARY THYROID CARCINOMA DEVELOPING IN THRYROGLOSSAL DUCT CYST

Antonia-Carmen Georgescu

Spitalul Clinic de Nefrologie ¨Dr. Carol Davila¨, Bucuresti, Romania

Background

Papillary thyroid carcinoma developing in the background of a thryroglossal duct cyst represents a rare occurrence, which has an incidence of up to 2% of all thyroglossal duct cysts. Early recognition of this entity is important, as these patients have an indication for total thyroidectomy and postoperative radioactive iodine therapy. Further on we present a case of a patient with thryoglossal duct cyst with papillary thyroid carcinoma (PTC), with emphasis on the reporting and diagnostic issues of such a rare entity.

Material and methods

The purpose of this study is to present the case of a 35 year old female who underwent excision of the thyroglossal duct cyst for cosmetic purposes, without having a thorough preoperative endocrinologic consult. The resected thyroglossal cyst measured 20/20/17 mm, which featured on the cut surface a solid-papillary area that ranged up to 11/10 mm. The lesion was fully embedded in paraffin blocks which were then examined in Hematoxilin-Eosin stain.

Results

Histopathologic examination revealed a cystic nodule which was partially lined by a squamous epithelium and which featured a neoplastic proliferation of papillary thyroid carcinoma that extended through the capsule to the adjacent striated muscle. Multiple lymphatic tumour emboli were also observed in the surrounding tissue. Although the 8th edition of the AJCC and the reporting datasets from ICCR for thyroid carcinoma exclude PTC developing in the background of a thyroglossal duct cysts from the staging criteria, experts advise to extrapolate the TNM staging for thyroid carcinoma for these rare tumours*. Thus, the presented case would be categorised as a Pt3b, pNx, LV1.

Conclusion

Papillary thyroid carcinoma developing in the background of a thyroglossal duct cyst represents a rare entity that is currently not included in the reporting datasets from ICCR and AJCC, and thus, one might face difficulties in the process of reporting them.

*Thompson LDR, Herrera HB, Lau SK. Thyroglossal Duct Cyst Carcinomas: A Clinicopathologic Series of 22 Cases with Staging Recommendations. Head Neck Pathol. 2017 Jun;11(2):175-185. doi: 10.1007/s12105-016-0757-y. Epub 2016 Oct 4. PMID: 27704385; PMCID: PMC5429280.

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TUMOR IMMUNE MICROENVIRONMENT IN LUNG CARCINOMA

Florina Almarii [1,2], Maria Sajin [1,3], Monica Hortopan [4], Vlad Herlea [1,2]

[1] Carol Davila University of Medicine and Pharmacy, Bucharest
[2] Fundeni Clinical InsEtute, Bucharest
[3] University Emergency Hospital, Bucharest
[4] Sanador Hospital, Bucharest

Background

Tumor-infiltrating immune cells (TIICs) play a pivotal role in the tumor microenvironment, influencing tumor progression, patient prognosis, and therapeutic responses. The present study aimed to evaluate the profile of TIICs in primary pulmonary malignant tumors and correlate them with clinical and morphological parameters.

Methods

A total of 50 cases of lung carcinomas were retrospectively analyzed. Histopathological examination was conducted to assess morphological parameters, while immunohistochemical staining was employed to identify and quantify various TIICs.

Results

Distinct patterns of immune cell infiltration were observed across different histological subtypes of lung carcinoma. Notable associations were found between the density and type of TIICs and specific clinical parameters, suggesting potential prognostic implications. Further, certain TIIC profiles correlated with specific morphological tumor features, emphasizing the intricate interplay between tumor cells and the immune microenvironment.

Conclusion

The pathological evaluation of TIICs in primary pulmonary malignant tumors provides valuable insights into tumor biology and patient prognosis. This study underscores the importance of a comprehensive assessment of the tumor immune microenvironment in lung carcinomas, with potential implications for therapeutic strategies.

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THE CLINICOPATHOLOGICAL SPECTRUM OF TRIPLE-NEGATIVE BREAST CANCER

Tiberiu-Augustin Georgescu[1,2]

[1] Discipline of Pathology, Carol Davila University of Medicine and Pharmacy, Bucharest

[2] Department of Pathology, National Institute for Mother and Child Health “Alessandrescu-Rusescu” Bucharest, Romania

Introduction

Triple negative breast cancer (TNBC) is defined by the absence of hormonal and HER2 receptors within tumor cells. This subgroup is extremely heterogeneous in regards to their molecular, morphological and clinical features. Most TNBCs tend to be high-grade tumors, which grow rapidly and metastasize frequently. However, there is a small subset of low-grade TNBCs which are very different from their high-grade counterparts in how they look under the microscope, how they behave in the body and also how they respond to treatment.

Material and methods

The purpose of this presentation is to highlight the wide spectrum of triple-negative breast cancer, focusing on the low-grade subset, which includes salivary gland-like tumors, such as: adenoid cystic carcinoma, secretory carcinoma, acinic cell carcinoma and mucoepidermoid carcinoma, low-grade metaplastic carcinomas, like: low-grade adenosquamous carcinoma and fibromatosis-like carcinoma and the newly described entity, tall cell carcinoma with reversed polarity.

Results

In order to highlight the clinical and histopathological particularities of this subset of tumors, the presenter reports the case of a 58-year-old female diagnosed on core needle biopsy with a particular subtype of low-grade TNBC, which underwent a very peculiar clinical outcome, eventually developing large metastatic deposits in the ipsilateral axillary lymph nodes.

Conclusion

Acknowledgement that triple-negative breast cancer is an operational term and that triple-negative disease is extremelyheterogeneous and includes both high-grade and low-grade tumours determined by particular genetic alterations, is mandatory for the successful implementation of precision medicine.

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PERIPHERAL NEUROBLASTIC TUMOURS- DIAGNOSIS AND PROGNOSTIC FACTORS IN THE NEW WHO BLUE BOOK ON PEDIATRIC TUMOURS

Oana Neagu[1,2], Anca Simona Constantin[1], Dana Terzea[2]

[1] Children’s Emergency Hospital “Grigore Alexandrescu” Bucharest

[2] Onco Team Diagnostic Bucharest

Peripheral neuroblastic tumours originate in the sympathoadrenal lineage of neural crest–derived tissues and account for approximately 11% of all paediatric cancers. The International Neuroblastoma Pathology Classification divides them into two prognostic categories based on histology and age. However, there are several genetic anomalies that can impact disease evolution. The WHO blue book on Paediatric Tumours highlights these mutations and recommends ancillary tests for an accurate and complete diagnosis. We selected three representative cases to illustrate these entities with a focus on diagnostic criteria, staging and molecular testing needed for a complete diagnosis. One case is an undifferentiated neuroblastoma in a 10-year-old female with a loss of ATRX in tumour cells. The particularity was the mediastinal location, a rapid growth and respiratory distress at an advanced age for this tumour category. Nonetheless, ATRX mutation is rare in neuroblastomas and is related to an unfavourable prognosis. The second case is from an 8-year-old boy who presented with a forearm mass first diagnosed as a haematoma. On imaging, the tumour was well delineated, with axillary adenopathy. Histologically and immunohistochemically, the tumour cells were confirmed as neuroblasts. The PET-CT scan did not show any primary site connected to the sympathetic chain ganglia. The third case is a 2-year-old boy who presented with hip pain and a large tumour in the proximal femur. The biopsy showed a metastasis from an undifferentiated neuroblastoma, later identified as a primary location in the adrenal gland. Nmyc amplification was detected by FISH in this case, which correlates with rapid and aggressive progression. In conclusion, the diverse manifestations and genetic variations observed in peripheral neuroblastic tumours underscore the complexity of their diagnosis and management. Understanding the intricacies of these tumours, including their histological, molecular, and clinical features, is crucial for tailoring effective and targeted therapy.

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A PRACTICAL APPROACH IN CHALLENGING ‘SPITZOID’ MELANOCYTIC LESIONS BASED ON IHC PANELS

Adelina Baltan, Simona Costache, Teresa Thomas, Corrado D’Arrigo, Saleem Taibjee
Poundbury Cancer Institute for Personalised Medicine, Dorchester, UK

Introduction

Driven in part by molecular profiling, the classification of melanocytic tumours has seen a rapid and recent evolution. New diagnostic categories have been defined, especially in the areas of Spitz, blue and deep penetrating tumours. Our diagnostic approach to melanocytic lesions needs to be reconsidered. The identification of these categories without molecular genetics tests can be challenging. Molecular testing may not be available or may result in lengthy delays. Immunohistochemistry (IHC), on the other hand, is widely available, has short turnaround time (TAT) and has been used successfully in oncology to identify some of these genetic changes.

Methods

We developed IHC panels guided by the morphological differential diagnosis. The markers used include: $BRAF^{V600E}$, ALK, ROS1, pan-TRK, BAP1, β-catenin, $p16^{INK4a}$, PRAME alongside more traditional markers such as PHH3, Ki-67, Melan-A and S-100. We started this work in the summer 2022 and we have used this approach on 70 cases of challenging cutaneous melanocytic lesions with spitzoid morphology.

Results

Using variations of this large panel (‘Spitz panel’), an identifiable alteration could be demonstrated by IHC in around 65% of the cases (46 cases out of 70). This helped distinguishing between conventional melanoma pathway, Spitz tumour, WNT-inactivated/deep penetrating tumour and BAP1 inactivated tumour. Spitz lineage has high prevalence of single translocation in one of three proto-oncogenes (ROS1, ALK and NTRK). Conversely, mutation in BRAF, particularly BRAFV600E, are rare in Spitz tumour and more prevalent in melanoma, particularly those related to intermittent sun exposure. Dysregulation of the β-catenin pathways (that results in nuclear expression of this marker) is not seen in Spitz while it is prevalent in deep penetrating tumours; this may also be useful in distinguishing DP tumours from melanoma with deep penetrating growth pattern in selected cases.

Conclusion

Our data shows that the ‘Spitz panel’ adds value in the diagnosis of challenging melanocytic tumours and allows for valuable input in the management of these lesions. Because these are IHC-based studies, the TAT is very short. This is particularly important for busy dermatopathology services and in view of long TAT with next-generation sequencing.

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DAY 2 - FRIDAY, NOVEMBER 3

Session 3: Updates in Immunology
Session 4: Genomics and Proteomics - two facets of multi-omics in health care and research
Session 5: Short communication - Biomedical research
Session 6: Cardiovascular pathology
Keynote Lecture: Epigenetic regulation of therapy response in cancers
Session 7: Nephropathology
Session 8: Neurosciences
Session 9: Next generation pathology
Session 10: Muscle pathology

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Session 3: Updates in Immunology

COMBINING IMMUNOTHERAPY WITH RADIOLOGY, NEW PATHWAYS OF RESEARCH SUPPORTED BY PARTICLE RADIATION PROVIDED BY THE HIGH-POWER LASER SYSTEM AT ELI-NP

Klaus Michael Spohr[1,2] for the ELI-NP collaboration

[1] Extreme Light Infrastructure – Nuclear Physics (ELI-NP), “Horia Hulubei” National R&D Institute for Physics and Nuclear Engineering, 30 Reactorului Str., RO 0771255, Măgurele-Bucharest, Romania

[2] School of Computing, Engineering, and Physical Sciences, University of the West of Scotland, Paisley PA1 2BE, Scotland

The Royal Marsden in London and the Memorial Sloan Kettering in New York can be acknowledged as the cradle of immuno- and radiotherapy dating back over 130 years. Since then, a gamut of cancer therapies has been developed in both fields using inherently different strategies with tremendous success but also suffering limitations interwoven with the different nature of their approach. Often, immunotherapies, in which progress has been outstanding in the last decades, are curtailed by the chemical escapism of the mutating cancer cells exhibit to fool the body’s own defense mechanisms. On the contrary, radiotherapy is inert to chemical matters in first approximation but does not distinguish between cancerous and healthy cells. Having the unique opportunities of a series of particle beams given the new high-power laser systems (HPLS) at ELI-NP at the IFIN-HH in Bucharest Magurele, a novel strategy to close the gap between immuno- and radiotherapy is envisaged. We aim to establish T-cell-supported radiologic therapies in which particular gene-manipulated T-cells are used as selective ultra-precise delivery agents for significant payloads of nanoparticles for treatments. For some of the envisaged therapies, the radiological effect relevant to the therapy can be triggered at any stage during the procedure by, e.g., neutron activation. Herein, the particle and radiation beams by the 10 Petawatt HPLS flagship installation at ELI-NP will play a significant role.

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ENABLING FURTHER BREAKTHROUGHS IN IMMUNOTHERAPY VIA INTERDISCIPLINARY COLLABORATIONS TO ENHANCE THERAPEUTIC MONITORING CAPABILITIES

Jurca M. C. [1,2,3], Spohr K. M. [2] for the ELI-NP collaboration

[1]Extreme Light Infrastructure – Nuclear Physics (ELI-NP), “Horia Hulubei” National R&D Institute for Physics and Nuclear Engineering, 30 Reactorului Str., RO 0771255, Măgurele- Bucharest, Romania

[2]Engineering and Applications of Lasers and Accelerators Doctoral School (SDIALA), University POLITEHNICA of Bucharest, Bucharest, Romania

[3]ALSITEC sarl, 4, Avenue de Wissembourg, 67500 Haguenau, France

Cancer is alive and tries to survive by eluding hostilities from its environment’. Charles Graeber summarizes that escapism in his book “The Breakthrough” by stating that “it (cancer) dances away”. Immunotherapy tries to harvest the body’s inert ability to fight cancer with its own means. The fight against malignant cells might be undertaken by surgery, chemotherapy, radiotherapy, or a combination of those treatments. Those treatments often act as catalysts for the healing process, but they all induce collateral damages associated with the nature of the applied therapy. ELI-NP and its research partners are dedicated to instigating applicable and customizable therapy approaches capable of matching cancer mutations. Using the unique abilities of laser-induced particle and radiation beams, we identified a series of approaches to enhance and monitor therapeutic measures in simultaneously with a precise observation of the overall patient’s health. The presentation proposes several potential solutions for improving the efficiency of intermediate immunotherapy steps using future laser-driven beams at ELI-NP.

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CAR-T AND CAR-NK CELLS IN CANCER IMMUNOTHERAPY

Florina Bojin
`Victor Babeș` University of Medicine and Pharmacy Timișoara; Center for Gene and Cellular Therapies in Cancer – OncoGen, `Pius Brînzeu` Clinical County Emergency Hospital Timișoara, Romania

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

CHARACTERIZATION OF DONOR-SPECIFIC ALLOREACTIVE CD4$^+$ AND CD8$^+$ CELLULAR IMMUNE T CELL RESPONSES IN THE LUNG ALLOGRAFT AND BLOOD IN LUNG TRANSPLANT RECIPIENTS

Iulia Popescu

Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh

Lung transplantation remains the only therapeutic option for patients with end-stage lung diseases, however chronic lung allograft dysfunction (CLAD) significantly limits long-term survival in lung transplant recipients (LTRs). Episodes of acute cellular rejection (ACR) are common and the major risk factor for developing CLAD, however little is known about donor-specific cellular T cell responses, as these have not been previously characterized in LTRs. We used a novel *ex vivo* flow cytometric assay to assess donor-specific alloimmune responses from LTRs cells in lung allograft resident effector T cells (BAL-derived) and PBMC. Using a 6h *in vitro* re-stimulation protocol with either irradiated donor cells or donor lysate, we measured the frequencies of effector responses (IFN-γ, TNF-α, the cytotoxic marker CD107a, IL-17a, IL-13, IL-2 and the costimulation surface molecule, CD154) from CD4$^+$ and CD8$^+$ lung resident or blood compartment T cells. Overall the predominant alloreactive effector response was donor-specific CD154 surface expression in lung resident CD4$^+$ T cells compared to the PBMC compartment, with little to none CD154 expression on CD8$^+$ T cells. Expression of surface CD154 was highly co-expressed with allospecific CD4$^+$ T cells producing the Type-1 cytokines IFN-γ, TNF-α and CD107a indicating CD154 as a potential marker for effector multifunction, but not co-expressed on Type-2 or Type-17 cells. In fact, donor-specific IL-13 and IL-17 responses were detectable in some patients but at significantly lower frequencies compared to Type-1 effector responses. Comparison between the lung resident T cells and blood T cells revealed consistently increased donor-specific alloreactive frequencies in the lung allograft versus the periphery. Ongoing experiments are assessing the proliferative capacities of donor-specific alloreactive T cell populations in the blood compartment and RNA sequencing. Together, these data indicate donor-specific alloreactive multifunctional effector CD4$^+$ lung resident T cells are present in high frequencies in select LTRs with histologic evidence of allograft rejection.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

FROM HIGH FIELD TO EARTH FIELD. NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY METHODS

TO STUDY THE MICROGLIA AND GLIOBLASTOMA CELLS

I.I. Fidel[1,2], O. Teșileanu[1], M. Iovea[3], I.C. Chiricuță[4] and P.R. Vasos[1,2] - for the collaborating team

[1] Biophysics and Biomedical Application Group, Laser Gamma Experiment Department, Extreme Light Infrastructure – Nuclear Physics (ELI-NP), “Horia Hulubei” National R&D Institute for Physics and Nuclear Engineering, 30 Reactorului Str., RO 0771255, Măgurele, România

[2]Interdisciplinary School of Doctoral Studies, University of Bucharest, Regina Elisabeta Blv., 050663 Bucharest, România

[3]AccentPro2000 S.R.L (AP2K), 4 Mărășești Str., RO 077125, Măgurele-Ilfov, România

[4]Amethyst Radiotherapy, Șoseaua Odăii 42, Otopeni 075100, România

Objectives

The short time in which secondary radiation generated by a high-intensity laser beam impacts a gas or solid target (pulses of fs to ns)[1] results in a radiobiology setting, in the recombination of free-radicals in cells. Biochemical pathways are altered and mechanisms related to the FLASH effect are triggered.[2,3] At ELI-NP, the radiobiological experiments aim to establish biomarkers for cells, ex-vivo and in-vivo experiments. Metabolic changes induced in cells during irradiation are detected via magnetic resonance spectroscopy. Another goal of our studies is the detection of free-radicals formation via magnetic resonance relaxometry in Earth`s magnetic field.[4]

Methods

Microglia and glioblastoma cells were irradiated in suspension using the secondary radiation delivered outside the laser interaction chamber. Nuclear Magnetic Resonance Spectroscopy was used to detect the molecular biomarkers of radiation in cells. Earth`s field magnetic resonance relaxometry was used to quantify the free radicals in different biological samples.

Results

At ELI-NP, we developed a protocol and irradiation setup using secondary radiation stemming from the interaction chamber of a 100 TW laser operating in pulses of 1.5-2 J and 28 fs duration delivered onto a gas target. Variations in metabolite concentrations between control and irradiated samples were measured using high-field Nuclear Magnetic Resonance spectroscopy. We also present a new experimental method based on Earth-field magnetic resonance to detect free radical formation by magnetic resonance imaging on different timescales.

Conclusions

The first trial of high dose-rate radiation effects in cells using the infrastructure at ELI-NP is based on the proposed biomolecular analysis of radiation effects advances towards frontier biomedical experiments at ELI-NP.

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

PERSONALIZED VACCINES IN CANCER – PRESENT AND FUTURE DIRECTIONS

Virgil Păunescu

`Victor Babeș` University of Medicine and Pharmacy Timișoara; Center for Gene and Cellular Therapies in Cancer – OncoGen, `Pius Brînzeu` Clinical County Emergency Hospital Timișoara, Romania

Human papillomavirus types 16 and 18 are responsible for the majority of cervical cancers worldwide. Despite the existence of three prophylactic vaccines based on virus-like particles (VLP) of the major capsid protein (L1), these vaccines are ineffective in clearing existing infections. Individuals with such infections face an elevated risk of neoplastic transformation. To address this challenge, this study proposes an alternative synthetic long peptide (SLP)-based vaccine designed for individuals already infected, including those with precancerous lesions. This innovative vaccine aims to activate both CD8+ and CD4+ T cells, eliciting a robust and enduring immune response. The SLP construct incorporates HLA class I- and class II-restricted epitopes, identified from IEDB or predicted using NetMHCPan and NetMHCIIPan. Through in silico studies, none of the SLPs were found to be allergenic or toxic. Population coverage assessments revealed 98.18% coverage for class I epitopes and 99.81% coverage for class II peptides in the IEDB world population's allele set. Ab initio prediction of three-dimensional structures using Rosetta yielded high-quality models, verified through PROCHECK and QMEAN4. Molecular docking with toll-like receptor 2 identified potential intrinsic TLR2 agonist activity, while molecular dynamics studies of SLPs in water indicated stability with favorable thermodynamic properties.

Keywords: cervical cancer; epitopes; human papillomavirus; in silico; molecular docking; synthetic long peptides; therapeutic vaccine.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

COST CA21135 - IMMUNO-MODEL - WG3: SOLID TUMORS - MODELS TO STUDY IMMUNOTHERAPY EFFICACY AND TOXICITY IN SOLID TUMORS, AND VALIDATE BIOMARKERS TO MONITOR THESE EFFECTS

Marleen Ansems

Radiotherapy & OncoImmunology Laboratory, Department of Radiation Oncology, Radboud University Nijmegen The Netherlands

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Session 4: Genomics and Proteomics - two facets of multi-omics in health care and research

GENOMICS AND METAGENOMICS INVOLVED IN NEURODEVELOPMENT: FRAGILE X SYNDROME AND AUTISM SPECTRUM DISORDERS

Yolanda de Diego Otero MSc. PhD.
Molecular and Cellular Biologist. Geneticist. Neurodevelopmental Senior Researcher. Newborn Screening Center of Eastern Andalusia. Regional University Hospital of Malaga. Grupo A-01. Biomedical Research Institute of Málaga IBIMA-plataforma BIONAND.

Neurodevelopment is a biological process that leads to the development and maturation of the nervous system. Nowadays, neurodevelopmental disorders are still a medical challenge. Progressively, several improved and sophisticated diagnostic tools continue to uncover a very complex genomic structure that included various types of genetic mutations (chromosomal rearrangements, copy number variations, small indels, and nucleotide substitutions) at varying frequencies (common, rare, emerging). The genomic variants and the metagenome present in the microbiome also interact to increase the different presentations of the diseases. This network of interacting actors makes it difficult to establish strict genotype-phenotype correlations. Furthermore, individual lifestyle also contributes to disorder onset, symptom severity and induces extensive gene-environment interactions that play a key role in the neurodevelopmental disorders.

Fragile X syndrome and autism are two distinct neurodevelopmental disorders, but they share some similarities and are often associated with each other due to overlapping symptoms and genetic factors. FXS is the most common genetic cause of autism.

Fragile X syndrome (FXS). FXS is the most common known inherited cause of intellectual disability and occurs more frequently in males than females (www.omim.org/entry/300624). It is a genetic disorder caused by a dynamic mutation in the FMR1 gene, located on the X chromosome. The CGG repeat expansion is the principal mutation that leads to a deficiency or absence of the fragile X mental retardation protein (FMRP), which plays a crucial role in brain development and synaptic function. FXS is characterized by a range of developmental and intellectual disabilities, including: Cognitive impairments, often in the form of intellectual disability. Behavioral challenges, such as social anxiety, attention difficulties, and hyperactivity. Speech and language delays. Sensory sensitivities. Repetitive behaviors. Some individuals with FXS may have physical characteristics, like a long face, large ears, and a prominent jaw, hyperextensive joints, macroorchidism, epilepsy and strabismus.

Autism Spectrum Disorder (ASD). It is relatively common with estimates of its prevalence varying by region and diagnostic criteria (1 in 45-100). It affects individuals of all races, ethnicities, and socio-economic backgrounds, but it is more commonly diagnosed in males than females. The exact cause of autism is complex and likely involves a combination of genetic and environmental factors. Many genes are associated with an increased risk of developing autism. ASD is a spectrum disorder, which means it encompasses a wide range of symptoms and severity levels. Common characteristics include: Impaired social communication and interaction. Restricted and repetitive behaviors and interests. Sensory sensitivities. Difficulty with verbal and nonverbal communication.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Relationship between FXS & ASD. Approximately 30% of individuals with FXS also meet the criteria for ASD diagnosis. This overlap has led to the concept of the "autistic-like" features seen in many individuals with FXS. The FMR1 gene mutation associated with FXS has been linked to increased risk for autism spectrum disorders. When individuals with FXS meet the criteria for autism, they are often diagnosed with both FXS and ASD. It's important to note that while there is a significant overlap between the two conditions, not all individuals with FXS have autism, and not all individuals with autism have FXS.

In summary, fragile X syndrome and autism are distinct but related neurodevelopmental disorders. They share some common features, including social communication difficulties and sensory sensitivities, and there is a genetic connection between the two conditions due to the FMR1 gene mutation. However, they also have their own unique characteristics and diagnostic criteria. Early intervention and tailored therapies can help individuals with these conditions lead fulfilling lives and reach their full potential.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

PERSONALIZED TUMOR MODELS REVEAL MECHANISMS OF GLIOBLASTOMA IMMUNOSUPPRESSIVE MICROENVIRONMENT AND THERAPY RESISTANCE

Barbara Breznik, Metka Novak

Department of Genetic Toxicology and Cancer Biology, National Institute of Biology

Brain tumor glioblastoma remains one of the most aggressive malignancies with poor prognosis due to its heterogeneity and lack of understanding of treatment resistance. We have developed in vitro glioblastoma models such as organoids and glioblastoma spheroid immune cell cocultures that mimic the tumor microenvironment of patients. These models allow us to explore the pathobiology of glioblastoma under clinically relevant conditions, and initial results shed light on potential mechanisms underlying glioblastoma therapy resistance and immunosuppression. Using established glioblastoma models, the effects of standard and immunotherapies are examined in the context of the dynamic and complex tumor microenvironment. Our results highlight the importance of 3D in vitro tumor models for more accurate immunotherapy research.

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MICROPHYSIOLOGICAL SYSTEMS AS DISEASE MODELS

Devrim Pesen Okvur
Izmir Institute of Technology www.iyte.edu.tr; Initio Cell BV www.initiocell.com, Turkey

Drug discovery has a failure rate of 90%. It takes billions of dollars and tens of years to bring a drug to the market. The weakest link is the preclinical stage where 2D cell culture and animal models are used. In addition, there are scientific and ethical concerns with animal testing; person-to-person variation is unaccounted for. Microphysiological Systems (MPS) offer successful preclinical stages where time to market can be reduced 40% and R&D expense can be reduced 32%. MPS support 3R+ and are suitable for precision medicine. We have been developing and using MPS for cancer research. Our results show that distance dependent cell-to-cell interactions, invasion, chemotaxis, extravasation, homing choices, dose response and drug combinations can be examined in 3D with single cell resolution in real time using MPS. Complementing MPS with patient samples can enable translation to clinic and personalized medicine.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

RADIATION OF HEAD AND NECK SQUAMOUS CELL CARCINOMA DERIVED CAFS AFFECTS THEIR PHENOTYPE

Marleen Ansems

Radiotherapy & OncoImmunology Laboratory, Department of Radiation Oncology, Radboud University Nijmegen,The Netherlands

Purpose: Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most common cancer worldwide, resulting in more than 450.000 deaths a year. Half of the patients with advanced HNSCC die within five years. Most treatments aim to kill cancer cells, but this is often insufficient to cure advanced HNSCC. Besides cancer cells, also cancer-associated fibroblasts (CAFs) make up a large part of the tumour. Their presence, however, is often ignored. Currently, the majority of HNSCC patients will be treated with radiotherapy. Despite the abundance of CAFs, the effect of radiotherapy on their function in HNSCC is largely unknown. To increase the success rate of treatment strategies in HNSCC, more in-depth knowledge regarding CAFs and the effect of radiotherapy on their role is essential.

Experimental procedures: CAFs were isolated from HNSCC patients and exposed to ionizing radiation ex vivo. The effect of different doses of radiation on CAFs was determined by microscopic analyses of cell growth, cell size, DNA damage and quantification of senescence.

Summary of the data: Our data shows that human HNSCC patient-derived CAFs are widely affected by radiotherapy; they dose-dependently decrease cell growth, increase their cell size and have permanent DNA damage. As these effects are often associated with senescence, we established -and confirmed- radiation-induced senescence.

Conclusions: Our data show that radiotherapy modulates the phenotype of HNSCC patient-derived CAFs. Radiation of CAFs affects their morphology and induces senescence. Current research focuses on the effect of radiotherapy on the function of CAFs in vivo and their effect to modulate the anti-tumour immune response.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

COST CA21135 - IMMUNO-MODEL - WG1: IN VITRO AND EX VIVO CANCER IMMUNOTHERAPY MODELS – ESTABLISHING BASIC PROTOCOLS FOR IMMUNOTHERAPY RESPONSE EVALUATION

Devrim Pesen Okvur
Izmir Institute of Technology, Turkey

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Session 5: Short communication - Biomedical research

NK CELL MEMORY IN UNCONVENTIONAL MODELS

Orhan Rasid
Sir Henry Wellcome Fellow
School of Infection & Immunity, University of Glasgow

Natural killer cells are unique players in innate immunity due to their inflammatory and cytotoxic potential. Following viral infection, NK cells display immune memory properties, defined by heightened responses to re-stimulation, an expansion of specific NK cell sup-populations and a protective role against re-infection. However, if similar innate memory develops in other models remained more elusive. We studied NK cell memory in systemic inflammation, respiratory bacterial infection and are exploring new avenues in helminth infection. Defining NK cell memory in new models provides insight into new attributes of NK cell biology and establishes these cells as targets for prophylaxis and immunotherapy.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

DRUG REPURPOSING SCREENING FOR ANTI-INFLAMMATORY MOLECULES

Gabriela Chiritoiu[1], Simona Ghenea[1], Cristian V.A. Munteanu[1], Cosmin Trif[1], Stefana M. Petrescu[1], Gheorghita Isvoranu[2]*, **Marioara Chiriţoiu-Butnaru[1]***

[1] Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

[2] „Victor Babeș” National Institute of Pathology, Bucharest, Romania

**authors with equal contribution*

Cytokines are key mediators of inflammation and amongst these interleukin (IL)-1β is the most potent pro-inflammatory cytokine. It is produced in high amounts as response to tissue damage or infections by cells of the immune system. In the acute phase, secretion of IL-1β is beneficial facilitating pathogen engulfment and damaged tissue removal, however sustaining an inflammatory response and IL-1β secretion, despite at low level, leads to chronic inflammatory syndrome associated with several disorders, such as atherosclerosis, diabetes, rheumatoid arthritis and nonetheless, neuroinflammation.

Cells of the immune system, primarily macrophages and monocytes, undergo a complex process to produce and secrete IL-1β as response to external stimuli. The cytokine is synthesized as inactive form (proIL-1β) which is cleaved by activated caspase-1 to its mature form (mIL-1β) and rapidly secreted to the extracellular space without entering the classical endoplasmic reticulum-Golgi pathway.

Engineering an IL-1β reporter cell line is challenging due to the complex process of producing the biologically active form of the protein. Moreover, any exogenous overexpression system would induce a constitutive inflammatory response which does not maintain the physiological conditions. Therefore, to overcome these drawbacks, we generated a reporter macrophage cell line by tagging IL-1β at its genetic locus using CRISPR/Cas9 knock-in technology, which recapitulates the physiological response to classical inflammatory stimuli and can be used for quantitative assessment of IL-1β secretion and identify drugs/small molecules to control its secretion associated with inflammatory syndromes.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

EXPERIMENTAL STUDIES OF IMMUNIZATION AGAINST HEPATITIS B AND C

Crina Stăvaru[1], Iuliana Caraș[1], Irina Ionescu[1], Adriana Costache[1], Cătalin Țucureanu[1], Ana-Maria Pantazică[2], Lia Cucos[2], André van Eerde[3], Mihaela-Olivia Dobrică[2], Hege Steen[3], Cătalin Lazar[2], Inger Heldal[3], Sissel Haugslien[3], Ion Popescu[2], Vlad Tofan[1], Adrian Onu[1], Jihong Liu Clarke[3] ,Norica Branza-Nichita[2]

[1] "Cantacuzino" Medical Military National Research and Development Institute, Bucharest, Romania.
[2] Institute of Biochemistry of the Romanian Academy, Bucharest, Romania.
[3] Norwegian Institute of Bioeconomy Research, Ås, Norway

Objectives

Hepatitis B and C viruses chronically affect approximately 3.5% of the global population, making this pathogens one of the leading global health problems and vaccination is the primary and most effective tool in combating these infectious diseases. This study investigated the immunogenicity induced HBV/HCV chimeric antigens in mice.

Methods

Balb/c female mice, 6-8 weeks aged were immunized in different protocols with chimeric antigens HBV and HCV produced in mamalian cells and plants. The HBV the S/preS$^{116–42}$ protein was expressed in HEK293T cells and in *Nicotiana benthamiana (N.bent.)* standard (WT) and in generated CRISPR/Cas9-edited plant line (FX-KO), with genetic knockouts of two 1,2-xylosyltransferase and four α-1,3-fucosyltransferase genes, respectively. For HCV the wild-type (wt) E1E2 polypeptide and an E2 N-glycosylation mutant (E1E2ΔN6) were expressed in lettuce using Agrobacterium-mediated transient expression. The antigen specific antibodies neutralization activities were detected by ELISA and IFNγ/IL-5 ELISPOT for specific cellular immune response investigation.

Results

The both HBV antigens elicited a significant amount of IgG with higher values obtained for the FX-KO N. bent-derivedS/preS1$^{16-42}$. Same profiles resulted, for neutralization tests and for IFNg/IL-5 producing cells detection. The humoral immune response induced by the HCV proteins was modulated by the type of administration and showed the increased immunogenic property of E1E2ΔN6.

Conclusions

The study showed the feasibility of producing complex viral antigens in plants and the importance of plant glycosylation in relation to the immunogenicity of subunitary proteins.

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

METHIONINE OXIDATION SELECTIVELY ENHANCES T CELL REACTIVITY AGAINST A MELANOMA ANTIGEN

Gabriela N Chiriţoiu[1], Cristian V A Munteanu[2], Teodor A Şulea[2], Laurenţiu Spiridon[2], Andrei-Jose Petrescu[2], Camilla Jandus[3,4], Pedro Romero[5], Ştefana M Petrescu[1]

[1] Department of Molecular Cell Biology, Institute of Biochemistry, Splaiul Independenței 296, 060031 Bucharest, Romania.
[2] Department of Bioinformatics and Structural Biochemistry, Institute of Biochemistry, Splaiul Independenței 296, 060031 Bucharest, Romania.
[3] Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland.
[4] Ludwig Institute for Cancer Research, Lausanne Branch, Epalinges, Switzerland.
[5] Departement of Oncology, UNIL-CHUV, University of Lausanne, Epalinges, Switzerland.

Antigenic peptide presentation and recognition by cells of the immune system and identification of methods to modulate this process represents a major interest for cancer therapy. A particular case of immune recognition based on antigenic peptides is the case of melanoma, where self-antigens can be presented by melanoma cells to the cell surface to elicit $CD8^+$T recognition and activation. Up to date, research in the field has focused on the amount of peptide required for immune recognition, and much less on the peptide aminoacid side-chains modifications to improve it.
We investigated the effect of methionine oxidation on the antigenicity of the melanoma immunodominant peptide 369-YMDGTMSQV-377 (YMD). Our results indicate that the antigenicity of the sulfoxide form is higher when compared to the YMD peptide as shown by $CD8^+$T cell activation assays and cell surface identification by LC-MS/MS analysis. Moreover, this is supported by free energy computations performed on HLA-A∗02:01/YMD/TCR complex showing that this is lowered upon oxidation, paired with a steep increase in order at atomic level.
These results demonstrate that methionine oxidation in the antigenic peptides may generate altered peptide ligands with increased antigenicity, and that this oxidation may occur *in vivo*, opening up the possibility that high-affinity $CD8^+$T cells might be naturally primed in the course of melanoma progression, as a result of immunosurveillance.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

CHRYSIN-BASED SUPRAMOLECULAR CYCLODEXTRIN-CALIXARENE DRUG DELIVERY SYSTEM: A NOVEL APPROACH FOR ATTENUATING CARDIAC FIBROSIS IN DIABETES

Maria Consiglia Trotta[1], Hildegard Herman[2], Alina Ciceu[2], Bianca Mladin[2], Marcel Rosu[2], Caterina C. Lepre[3, 2], Marina Russo[4, 5], Ildikó Bácskay[6, 7], Ferenc Fenyvesi[6], Raffaele Marfella[8], Cornel Balta[2], Michele D'Amico[1], **Anca Hermenean[2, 9*]**

[1]Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Italy
[2]”Aurel Ardelean” Institute of Life Sciences, Vasile Goldis Western University of Arad, Romania
[3]PhD Course in Translational Medicine, University of Campania “Luigi Vanvitelli”, Italy
[4]PhD Course in National Interest in Public Administration and Innovation for Disability and Social Inclusion, University of Campania “Luigi Vanvitelli”, Italy
[5]School of Pharmacology and Clinical Toxicology, University of Campania “Luigi Vanvitelli”, Italy
[6]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, Hungary
[7]Institute of Healthcare Industry, University of Debrecen, Hungary
[8]Department of Advanced Medical and Surgical Sciences, University of Campania “Luigi Vanvitelli”, Italy
[9]Department of Histology, Faculty of Medicine, Vasile Goldis Western University of Arad, Romania

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PLASMA MICRORNAS AS EARLY PREDICTORS OF POST-ACUTE MYOCARDIAL INFARCTION VENTRICULAR REMODELING

Liana Maries[1], Alexandra Ioana Moatar[1,2], Aimee Rodica Chis[1,3], Catalin Marian[1,3], Constantin Tudor Luca[4,5], Dan Gaita[4,5] and **Ioan-Ovidiu Sirbu[1,3]**

[1] Biochemistry Department, “Victor Babes” University of Medicine and Pharmacy, 300041 Timisoara, Romania
[2] Doctoral School, “Victor Babes” University of Medicine and Pharmacy, 300041 Timisoara, Romania
[3] Center for Complex Network Science, “Victor Babes” University of Medicine and Pharmacy, 300041 Timisoara, Romania
[4] Cardiology Department, “Victor Babes” University of Medicine and Pharmacy, 300041 Timisoara, Romania
[5] Research Center of the Institute of Cardiovascular Diseases Timisoara, 13A Gheorghe Adam Street, 300310 Timisoara, Romania

Aim

Left ventricle remodeling (LVR) is a complication of acute myocardial infarction (aMI) that leads to heart failure (HF) through impairment of both systolic and diastolic function. Timely prediction of post-aMI LVR represents a significant challenge despite decades of clinical research. Multiple circulant aMI-associated microRNAs have been advanced as LVR predictors; however, the results are contradictory. We aim to evaluate the LVR-predictive ability of four best-known aMI-associated plasma microRNAs: miR-101, miR-150, miR-21, and miR-22.

Methods

We used RT-qPCR to evaluate the normalized expression levels of the four microRNAs in plasma samples collected on day 0 of aMI patients' admission to the hospital. The microRNA expression data were analyzed in correlation with the clinical and paraclinical features of the patients at hospital admission (day zero) and at one-year follow-up.

Results

Our data confirm that miR-101, miR-150, miR-21, and miR-22 are excellent aMI discriminators and show that Diabetes Mellitus, hemoglobin level, and the number of erythrocytes influence their expression level. However, only miR-22 can discriminate between LVR and non-LVR patients and significantly improves the predictive power in a multiple logistic regression model based on troponin, CK-MB, baseline ejection fraction, and end-diastolic volume.

Conclusion

Our data show that early (day of admission) plasma miR-22 might be a useful LVR predictor biomarker.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Session 6: Cardiovascular pathology

REDUCING DIABETIC MACROVASCULAR COMPLICATIONS DUE TO PERIPHERAL ARTERIAL DISEASE

Gustavo E. Crespo-Avilan[1,2,3]; Elisa A. Liehn[1]; Sauri Hernandez-Resendiz[1,2]; Loo Sze Jie[1,2] ; Derek J. Hausenloy[1,2,4]

[1]National Heart Research Institute Singapore, Singapore
[2]Cardiovascular and Metabolic Disorders Program, Duke-National University of Singapore
[3]Institute of Biochemistry, Medical School, JLU, Giessen, Germany
[4]Hatter Cardiovascular Institute, University College London, UK

Peripheral Arterial Disease (PAD) is a common comorbidity in individuals with diabetes, contributing significantly to macrovascular complications, such as cardiovascular disease and stroke. Despite advances in the management of diabetes, PAD remains a critical health concern. This work aims to shed light on a novel approach for mitigating diabetic macrovascular complications through the targeting of Interleukin-11 (IL-11).

The cytokine IL-11 has gained attention in recent years for its role in inflammation, vascular endothelial dysfunction, and atherosclerosis, all of which are central to the pathogenesis of macrovascular complications in diabetic patients. Our study investigates the impact of specifically targeting IL-11 in the context of diabetic macrovascular complications. We hypothesize that IL-11 inhibition can offer a promising avenue for reducing the burden of PD in diabetic individuals.

In our preclinical study in a murine diabetic animal model, we administered an anti- IL-11 cytokine to diabetic animal models with induced PAD, using the wire-in jury model. The results of this study demonstrate a significant reduction in atheromatous plaque formation and possibly lead to an overall improvement in the macrovascular health of diabetic subjects.

Furthermore, we will discuss the potential translational implications of our findings, emphasizing the significance of IL-11 cytokine targeting as a therapeutic strategy for reducing diabetic macrovascular complications. This innovative approach offers new hope for more effective treatment and prevention of PAD-related cardiovascular events in the diabetic population.

In conclusion, our research underscores the potential of targeting IL-11 cytokine to reduce diabetic macrovascular complications associated with PAD. By elucidating the mechanisms by which IL-11 contributes to vascular pathology and presenting promising preclinical data, this work encourages further exploration of IL-11 inhibitors as a therapeutic intervention in the management of diabetic macrovascular complications.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

ND-13, A SMALL DJ-1 INSPIRED PEPTIDE, TO INDUCE MITOPROTECTION IN ACUTE MYOCARDIAL INFARCTION

Sauri Hernandez-Resendiz [1,5], Aishwarya Prakash [1,5], Loo Sze Jie [1,5] ,Yu Fan [1,2], Cong Shuo [1,2], Siavash Beikoghli Kalkhoran[3] , Gustavo E Crespo-Avilan [1,5] , Chrishan Ramachandra [1,5] , Derek J Hausenloy [1,2,5]

[1]Cardiovascular and Metabolic Disorders Programme, Duke-NUS Medical School, Singapore
[2]Yong Loo Lin School of Medicine, National University Singapore, Singapore
[3]The Hatter Cardiovascular Institute, Institute of Cardiovascular Science, University College London, UK,
[5]National Heart Research Institute Singapore, National Heart Centre Singapore, Singapore

Background

DJ-1 protein has shown cytoprotective effects in cardiomyocytes damaged by ischemia-reperfusion injury (IRI). In this study we are investigating the potential of ND-13, a small 13 amino acid peptide derived from a segment of the DJ-1 protein. This peptide is made up of 13 amino acids derived from DJ-1. We hypothesized that ND-13 targets the balance and functioning of mitochondria, which in turn improves their ability to produce energy and maintain the mitochondrial dynamics. By regulating these key components of mitochondrial homeostasis, ND-13 may provide a novel therapeutic strategy to protect the mitochondria in cardiomyocytes following IRI.

Methods

In vitro, ex vivo and in vivo murine models of IRI were used to elucidate the cardioprotective effects of ND-13. Infarct size, cardiac function, mitochondrial function, oxidative stress, and survival signaling pathways were assessed.

Results

We have demonstrated that ND-13 protects cardiomyocytes against IRI by modulating oxidative phosphorylation (OXPHOS) at the onset of reperfusion. Robust cell protection was reproduced in in vitro, ex vivo and in vivo models of IRI, highlighting the efficacy and efficiency of this small peptide in reducing cell death acutely. Basal, maximal, and spare respiratory capacity was significantly increased in CMs treated with ND-13, indicating an optimized functioning of the electron transport chain (ETC) complexes, oxygen consumption and glucose oxidation. We speculate that ND-13 may either directly bind to the ETC complexes to maximize their functionality and/or may have a direct effect in preserving the mitochondrial network by regulating the fission/fusion process.

Discussion and Conclusions

ND-13 confers acute cardioprotection to CMs adversely both functionally and metabolically by SIRI. The cell protection appears to stem from two primary mechanisms: 1) by increasing mitochondrial oxygen consumption and ATP production and 2) mitigating the excessive mitochondrial fragmentation associated with acute myocardial ischemia-reperfusion injury.

THE ROLE OF PHOSPHATIDYLSERINE ORAL SUPPLEMENTATION IN VASCULAR REGENERATION

Dan-Valentin Pistritu [1,2], Yichen Xu[3], Chung Chee Hooi[4], Paula Martínez Anghel[1,5], Victor Ungureanu[1,2], Anisia-Cristiana Vasiliniuc[1,2], Anda Vasiliu[1,2], Antoanela Tanca[1,2], Roshni Singaraja[4], Octavian Bucur[1,6], Elisa Anamaria Liehn[1,3,7]

[1] Victor Babes' National Institute of Pathology, 99-101 Splaiul Independentei, 050096 Bucharest, Romania.
[2] Faculty of Human Medicine, Carol Davila University of Medicine and Pharmacy, 37 Dionisie Lupu Street, 020021 Bucharest, Romania.
[3] Institute for Molecular Medicine, University of Southern Denmark, 25 J.B Winsløws Vej, 5230 Odense, Denmark.
[4] National University of Singapore, 14 Medical Dr, Singapore 117599, Singapore
[5] Business Academy Aarhus, 30 Sønderhøj, 8260 Viby J, Denmark.
[6] Viron Molecular Medicine Institute, 201 Washington Street, Boston, MA 02108, USA.
[7] National Heart Center Singapore, 5 Hospital Dr., Singapore 169609, Singapore.

Aim

Cardiovascular diseases continue to represent the main cause of death, despite of the latest progress in the interventional therapies. Phosphatidylserine proved to have positive and valuable effects in all kinds of pathology, including protection of myocardial tissue and cardiac regeneration, while presenting negligible side effects. However, if phosphatidylserine can be used to accelerate the vascular regeneration after mechanical injury is still unknown.

Methods

8 weeks old LDLR-/- mice were fed fat-rich diet for 1 week and underwent de-endothelialization of the left carotid artery using a 14” stiff wire. The fat diet was continued for 2 more weeks, until the carotid arteries were explanted for analysis. Before explanting, echocardiography analysis was performed using Vevo3100 high-frequency ultrasound equipment. Orcein staining was used for identification of the vascular laminas for morphometric analysis of the plaque size and morphology. Immunohistology analysis was performed using anti-SMA and anti-Mac2 antibody to recognize the smooth muscle cells and macrophages, respectively.

Results

The preliminary results showed that phosphatidylserine oral supplementation has no particular benefit regarding the reduction of restenosis size or changing the cellular content after mechanical injury of the arterial wall. However, echocardiography showed a decrease in the stiffness of the vascular wall compared with the untreated carotid arteries after mechanical injury.

Conclusions

Our findings point out that phosphatidylserine supplementation can be beneficial in certain extent for patients undergoing vascular interventions, such as stent or graft implantation, reducing the stiffness of the vascular wall. This could have a significant impact on the improvement of the outcome after vascular interventions, particularly because of the negligible side effects. How the decrease in the vascular stiffness influences the future vascular healing process is still unknown and needs further investigations.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

3D TISSUE RECONSTRUCTION FROM 2D HISTOLOGY SLIDES FOR THE STUDY OF THE EXTRACELLULAR MATRIX

Victor Ungureanu
University of Medicine and Pharmacy `Carol Davila` Bucharest, Romania

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Keynote Lecture: Epigenetic regulation of therapy response in cancers

EPIGENETIC REGULATION OF THERAPY RESPONSE IN CANCERS

Tugba Bagci-Onder, PhD

Koç University School of Medicine

A mechanism of therapy resistance is transcriptional dysregulation of cell death and survival-related genes and changes in the epigenome are thought to play a critical role in acquired therapy resistance. Epigenetic modifications occur on chromatin and consist mainly of DNA methylation, histone acetylation and methylation, governed by the activity of several chromatin-modifying proteins and recognized by the activity of reader proteins. To decipher the relationship between therapy response and epigenetics, we first generate therapy-resistant cell lines by exposing tumor cells to different treatment modalities, such as chemotherapy with DNA alkylating/damaging agents or microtubule (de)polymerizing agents, or radiotherapy. We then profile the differences between therapy-sensitive and therapy-resistant populations of tumor cells. Our findings demonstrate that while acquisition of resistance for DNA damaging agents involve the activation of defense mechanisms through augmented DNA repair, most other chemo-resistance mechanisms involve the induction of multi-drug resistance transporters. We then undertake loss-of-function screens and interrogate the effects of chromatin modifying proteins in treatment response through genetic and chemical approaches. In this talk, we will cover examples to our current approaches and highlight recently identified molecular mechanisms governing epigenetic adaptation of cancer cells, while they transition to a therapy-resistant state.

Session 7: Nephropathology

WHAT'S NEW IN ANTIBODY-MEDIATED REJECTION OF THE KIDNEY GRAFT AFTER 2022 BANFF MEETING?

Bogdan Marian Sorohan [1,2]

[1] “Carol Davila” University of Medicine and Pharmacy, Department of Nephrology, Bucharest, Romania

[2] Fundeni Clinical Institute, Department of Kidney Transplantation, Bucharest, Romania

Antibody-mediated rejection (ABMR) remains a major cause of kidney graft failure. Since its first definition in the international Banff classification system, the diagnosis of ABMR is based on three criteria, which partially overlap: histological evidence of acute or chronic tissue injury, evidence of a recent/current interaction between antibody and endothelium and the serologic evidence of donor-specific antibodies (DSA). These criteria have been continuously changed over the years. Some of the most important changes between 2013 and 2022 were the inclusion of the C4d-negative ABMR, the introduction and subsequent removal of suspect ABMR entity and the validation, as well as the implementation of transcript genes associated with ABMR, leading to a change in the number of rejection diagnoses. However, one of the drawbacks remained poor prognostic ability, so at the XVI Banff Meeting for allograft pathology in 2022 the expert group focused more on this aspect. Thus, following this meeting, the following changes were made regarding ABMR: implementation of microvascular inflammatory lesions as a stand-alone entity, in the absence of C4d and DSA, reintroduction of the diagnosis of "Probable ABMR", removal of acute tubular injury and intimal fibrosis of new onset as key histological criteria for ABMR, as well as details on non-HLA antibodies and tissue gene transcripts. All these changes were made to improve the prognosis. Although the diagnosis of rejection points to a non-invasive area where biomarkers, digital pathology and artificial intelligence have their say, there is still much to be clarified from the histological side.

Keywords: antibody, rejection, C4d, microvascular inflammation, acute, chronic, Banff

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

PRECISE COMPOSITION AND LOCALIZATION OF THE INFLAMMATORY BURDEN OF 125 KIDNEY TRANSPLANT REJECTIONS: DETERMINANTS AND PROGNOSIS IMPACT

George Terinte-Balcan[1], Angela Mobio[1,3], Maëva Eloudzeri[3], Lise Morin[3], Jean Paul Duong Van Huyen[1], Dany Anglicheau[2,3], Marion Rabant[1,3]

[1] Laboratoire d'anatomopathologie, Hôpital Necker Enfants Malades, Paris
[2] Transplantation rénale adulte, Hôpital Necker Enfants Malades, Paris
[3] Inserm U1151, Paris, France

Background

The exact composition of the inflammatory burden during kidney transplant rejection has not been studied on large cohorts. We have recently shown an important heterogeneity in the cellular composition from one patient to another for the same type of rejection. We aim to study the determinants and impact of the cellular composition of infiltrates in a large cohort of 125 first episode rejection biopsies.

Methods

We used multiplex immunofluorescence on a cohort of 125 rejections (ABMR n=69; TCMR n=18; Borderline n=23; mixed n=15) to phenotype T and B lymphocytes, M1 (CD68+CD206-) and M2 (CD68+CD206+) macrophages and NK cells on the same biopsy slide with automated localization (intra- or extravascular) and quantification.

Results

The total cellular density was maximal during mixed rejection (1242/mm2), significatively superior to ABMR (604/mm2) or BL (564/mm2). During TCMR, the mean cellular density was 892/mm2. In all rejection types, most cells were located within the interstitium but there were more cells in the microcirculation during ABMR. In all types of rejection, the 2 main cell types were macrophages (59.2%), mostly M2, and T lymphocytes (33.2%), with few NK cells and B lymphocytes. However, ABMR was significantly enriched in macrophages, mainly M2, compared to TCMR (64.2% vs 50.8%; p=0.03). In the microcirculation, T lymphocytes were the main population in all types of rejections (55.2%).

We confirmed the vast heterogeneity of the cellular composition within the same type of rejection. We found a global decrease in macrophage density and increase in T lymphocytes density with time post transplantation. Using a Cox model, we found that the most impactful population was macrophages. The intravascular density of M2 macrophages was associated with a better graft survival.

Conclusions

Multiplex IF applied on a large cohort of 125 rejection confirmed the predominance of macrophages, mainly M2, and T lymphocytes during all types of rejection, with a majority of cells located within the interstitium, and a large heterogeneity from one patient to another. Macrophages density is associated with prognosis with a positive correlation between M2 macrophages and graft survival.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

OLD AND NEW IN IgA NEPHROPATHY

Mihaela Gherghiceanu
`Victor Babeș` National Institute of Pathology, Bucharest, Romania

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Session 8: Neurosciences

MODULATION OF TRPM8 FUNCTION BY THE PROSTACYCLIN RECEPTOR: INVOLVEMENT OF $G_{q/11}$ PROTEINS

Cosmin Trif[1], Alexandra-Maria Banica[1], Alexandra Manolache[2], Debora Huţanu[2], Teodora Stratulat[1,2], George Oprita[2], Tudor Selescu[2], Stefana Petrescu[1], **Alexandru Babes**[2] & Sorin Tunaru[1]

[1] Cell Signalling Research Group, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania
[2] Department of Anatomy, Physiology, and Biophysics, Faculty of Biology, University of Bucharest, Romania

Objectives

The Transient Receptor Potential Melastatin subtype 8 (TRPM8) receptor-channel is involved in innocuous cold sensing and has a potent anti-inflammatory action. Its activation by lower temperature or chemical agonists such as menthol and icilin induces analgesic effects, reversing hypersensitivity and reducing chronic pain. On the other hand, prostacyclin (PGI_2) enhances pain and inflammation by activating the prostacyclin receptor (IP-R). Due to the critical roles of TRPM8 and IP-R in the regulation of inflammatory pain, and considering their overlapping expression pattern, we analyzed the functional interaction between human TRPM8 and IP-R.

Methods

We employed transient expression of human TRPM8 and IP-R in HEK293T cells and performed intracellular calcium and cAMP measurements. Additionally, we cultured neurons from the dorsal root ganglia of mice and determined the increase in intracellular calcium triggered by TRPM8 agonist, icilin, in the presence of the IP-R agonist, cicaprost, IP-R antagonist, CAY10144 and the $G_{q/11}$ inhibitor YM254890.

Results

Our results demonstrate that the activation of IP-R by selective agonists, such as cicaprost, beraprost, and iloprost, inhibits TRPM8 independently of the G_s-cAMP pathway. The potent inhibition of TRPM8 by IP-R involves $G_{q/11}$ coupling of IP-R. These effects were also observed in neurons isolated from the dorsal root ganglia (DRGs) of mice.

Conclusions

Our results demonstrate that an unusual signaling pathway of IP-R, namely the coupling to $G_{q/11}$ proteins, inhibits TRPM8 which may contribute to a better understanding of the role of TRPM8 and IP-R in the regulation of pain and inflammation.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

MODELING HUMAN DE NOVO HETEROZYGOSITY USING MOSAIC GENE DOSAGE DIFFERENCES IN MICE

Tudor C. Badea[1,2]

[1] Transilvania Neurogenetics Group, Institutul de Cercetare si Dezvoltare, Facultatea de Medicina, Universitatea Transilvania din Brasov

[2] Centrul National de Cercetare a Creierului, ICIA – Academia Romana

Objectives

Neuronal cell type development is a complex process involving both intrinsic transcriptional programs and external cues from the tissular environment. We seek to understand how neuronal cell types develop, focusing on Retinal Ganglion Cells, and using mouse genetics as a tool. In this work we explore the interaction between the transcription factor Brn3a/Pou4f1 and the neurotrophin receptor Ret.

Methods

We crossed a Ret^{CreERt} allele - harboring a tamoxifen-inducible Cre recombinase knocked-in at the Ret genetic locus - and a $Brn3a^{CKOAP}$ allele - in which the coding exons of Brn3a are replaced by the Alkaline Phosphatase reporter gene upon Cre recombination. We then induced sparse random recombination in Ret^{CreERt}; $Brn3a^{CKOAP}$ mice by injecting 4- OH - tamoxifen in pregnant females, postnatal pups, or adults, and characterized the dendritic arbors of RGCs in which one Brn3a allele was recombined ($Brn3a^{AP}$ RGCs). In addition, we used deep sequencing and immuno-histochemistry to diagnose the consequences of Brn3a or Ret ablation in RGCs.

Results

$Brn3a^{AP}$ RGCs exhibit cell type fate specification switches and dendritic arbor defects, only when recombination is induced in a sparse fashion early in RGC development (E15), but not in postnatal development or adult. Ret ablation does not significantly alter Brn3a, Brn3b and Brn3c distribution in RGCs, however Brn3a ablation results in cell autonomous and non-autonomous shifts in neurotrophic receptors distribution in RGCs.

Conclusions

Early embryonic gene dosage imbalances in combined Ret/Brn3a heterozygotes result in cell fate specification defects. These defects could explain the developmental deficits seen in children with de novo, heterozygous, loss of function mutations in Brn3a.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

VASCULAR AND GLIAL CHANGES IN THE BRAINS OF PATIENTS WITH SARS-COV- 2 INFECTION

Pirici D[1], Rosu GC[1], Mateescu VO[1], Simionescu A[2], Istrate-Ofiteru AM[1], Curcă GC[2,3], Pirici I[4], Mogoanta L[1], Mindrila I[4], Kumar-Singh S[5], Hostiuc S[2,3]

[1] Department of Histology, University of Medicine and Pharmacy of Craiova, Craiova, Romania
[2] 'Mina Minovici' National Institute for Legal Medicine, Bucharest, Romania
[3] Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[4] Department of Human Anatomy, University of Medicine and Pharmacy of Craiova, Craiova, Romania
[5] Laboratory of Cell Biology and Histology, Molecular Pathology Group, Faculty of Medical and Health Sciences, University of Antwerp, Antwerp, Belgium

Objectives

A multitude of changes associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have been described in the central nervous system (CNS), such as microglial activation, perivascular lymphocyte cuffing, hypoxic-ischemic changes, microthrombosis, stroke or hemorrhages. The aim of this study was to evaluate in detail the morphology of vascular basement membranes (VBM) and perivascular astrocytes in patients with acute respiratory syndrome (COVID-19 disease).

Methods

We have analyzed the microscopic morphology of the VBM and perivascular astrocytes on brain fragments taken from 14 patients with confirmed SARS-CoV-2 infection, and was compared with four control patients without CNS pathology, utilizing fluorescence immunohistochemistry for collagen IV, astrocytes (GFAP), vascular endothelium (CD31), inter-endothelial tight junctions (TJ1), as well as for the water channel aquaporin 4 (AQP4). The images taken from the cortical areas and from the white matter were processed by 2D and 3D deconvolution and to calculate vascular densities, diameters, degree of gliosis, colocalizations between collagen IV/GFAP and GFAP/AQP4, as well as the fractal dimension (FD) of astrocytes and VBM seen in tangential planes.

Results

FD analysis of astrocytes revealed lower branching complexities and decreased GFAP/collagen IV colocalization for patients with COVID-19. Interestingly, VBMs were more irregular (interpreted as higher FD values) compared to control tissue. Vessel diameters were increased in COVID-19 cases, especially for the white matter, TJ1 protein decreased colocalization with vascular endothelium, and AQP4 decreased co-expression in astrocytes.

Conclusions

Our data on VBM irregularity, loss of inter-endothelial tight junctions, reduction of astrocytic end-feets, and decreased AQP4 expression suggest subtle morphological changes of the blood-brain barrier in the brain of patients with COVID-19, which may be related to the inflammatory cascade and have hypoxic/ischemic consequences on long term.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Session 9: Next generation pathology

DIGITAL PATHOLOGY FOR CERVICAL CANCER SCREENING IN LOW AND MIDDLE INCOME COUNTRIES

Rajan Dewar
Vice-Chair, New York Medical College, New York, USA

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

DIGITAL PATHOLOGY IN AUTOMATING HEMATOLOGY WORKFLOW - AI IN PERIPHERAL SMEAR AND BONE MARROW CELL CLASSIFICATION

Rajan Dewar[1,*], Sayed Shahabuddin Hoseini[1];
[1]Department of Pathology, Westchester Medical Center, 100 Woods Rd, Valhalla, NY 10595, USA

The integration of artificial intelligence (AI) and machine learning applications is anticipated to revolutionize healthcare, particularly in the realm of diagnostic patient management. Nevertheless, a significant challenge in developing effective AI models lies in the need for domain knowledge, clinical expertise, and coding skills. Often, informatics and AI experts lack familiarity with the intricacies of the medical field, and many healthcare professionals are not proficient in coding. To overcome this obstacle, a new category of "no-code" AI platforms has emerged, empowering medical practitioners to construct AI models without requiring coding skills.

In this research, we investigate the utility of Teachable Machine™, a no-code AI platform, for the classification of white blood cells into the five most common WBC types. We employed training data from publicly accessible datasets and enhanced model accuracy by fine-tuning hyperparameters. The performance of the model was assessed using sensitivity, precision, and F1 score calculations, and independent datasets were used for testing. We explored various factors that influence the model's performance. Remarkably, the model achieved a 97% accuracy rate in classifying white blood cells, demonstrating high sensitivity and precision. Independent validation further underscored its potential for continued development.

In the second part of the talk, we will cover bone marrow differential count automation efforts using AI: Work volumes and staff shortages pose significant challenges to lab operations. Manual blood differentials (100-200 cells) and bone marrow differentials (currently n=500) remain a key contributing factor, because they are time-consuming and require morphological expertise on premises. Many hospitals and laboratory service providers find themselves forgoing morphology expertise during nights and weekends, instead sending samples out for review, creating operational burdens and the potential for delaying care.

This talk provides evidence-based validation of a full-field peripheral blood smear workflow solution in a laboratory service provider setting. In addition, it examines the workflow implications of a full-field bone marrow solution in a hospital network. The speakers will share their experiences with full-field digital cell morphology, AI-supported workflows, and remote reviews that eliminate the need for manual microscopy. They will also discuss the technology’s importance given the latest WHO/ICC classification guidelines.

This study stands as the pioneering effort to showcase the value of no-code algorithm-based AI platforms in the field of hematopathology, using authentic datasets for training. It paves the way for healthcare professionals to gain hands-on experience with AI and create practical AI models without the need for coding expertise.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

CLASSIFICATION OF EARLY BREAST NEOPLASTIC LESIONS USING DEEP LEARNING AND EXPANSION PATHOLOGY

Victor Ungureanu
Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Session 10: Muscle pathology

INTELLECTUAL DISABILITY IN DUCHENNE MUSCULAR DYSTROPHY. A CASE REPORT

Magdalena Budisteanu[1,2,3], Sorina Mihaela Papuc[1], Alina Erbescu[1], Gisela Gaina[1], Florentina Linca[2], Adelina Glangher[2], Doina Ioana[2], Aurora Arghir[1]

[1]Institutul National de Cercetare-Dezvoltare in Domeniul Stiintelor Biomedicale “Victor Babes”, Bucuresti, Romania,
[2] Spitalul Clinic de Psihiatrie “Prof. Dr. Alex. Obregia”, Bucuresti, Romania,
[3] Universitatea Titu Maiorescu, Facultatea de Medicina, Bucuresti, Romania

Objective

Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy during childhood. The genetic defect is represented by the mutations in *dystrophin* gene. Cognitive impairment has frequently been reported in patients with DMD. In this paper we report on a boy with DMD and severe developmental delay and autistic behavior.

Material and methods

The patient is a boy who was included in a research study for children with autism at the age of 5 years. He was first evaluated at the age of 2 years 6 months for cognitive delay (he had no words, he performed no orders) and autistic behavior (no eye contact, stereotypic movements). He was evaluated by clinical general exam, neurologic and psychologic evaluations, and by specific investigations (biological tests, EEG, brain MRI), and genetic tests (array CGH, PCR and MLPA for fragile X).

Results

The child was diagnosed with a severe developmental delay (QD 35) and was included in a complex therapy program, including physical, speech, behavioral and occupational therapies, without clinical improvement. No anomalies were identified on EEG and brain MRI, and genetic tests revealed no pathogenic results. Blood tests showed very high value of CK and LDH , and genetic test for DMD was recommended (MLPA), which was negative. At the age of 3 years 9 months the boy started to present motor problems with progressive aggravation. Panel gene sequencing for muscular diseases revealed a new variant on dystrophin gene, classified as likely pathogenic.

Discussion and conclusions

DMD is a severe neurological condition with progressive evolution. Cognitive impairment and autistic behavior have been reported in these patients, usually in a mild form. The severity of our case is unusual for DMD, so we can consider the possible association of another genetic anomaly, still unidentified.

Funding

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

PATHOLOGICAL ASPECTS AND CLASSIFICATION CRITERIA OF INFLAMMATORY MYOPATHIES

Bastian A. [1,2], Manole E.[1,3]

[1]Colentina University Hospital, Bucharest, Romania
[2]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[3]Victor Babes National Institute of Pathology

Alexandra Bastian e-mail: aleca.bastian@yahoo.com

Background and objectives

Inflammatory myopathies (IM) are rare heterogeneous conditions with challenging diagnosis and classification, currently comprising polymyositis (PM), dermatomyositis (DM) affecting adults and children, immune mediated necrotizing myopathy (NM), sporadic inclusion body myositis (IBM) and overlap myositis (OM) including anti-synthetase syndrome (ASS). Classification criteria were debated, diversified and re-established in recent years and are essential for the diagnostic workup, in which muscle biopsy is often the core, as well as for therapeutic approach and clinical research.

Matherials and methods

In this presentation we review the major advances in IM classification and describe, exemplify and discuss the different myopathological patterns of changes identified during evaluation of open muscle biopsies diagnosed in the last five years in the Pathology Department of Colentina Clinical Hospital. We also registered age, sex, clinical signs, levels of myositis-specific and myositis-associated autoantibodies and serum creatine-kinase activity, extra-muscular organ involvement and co-morbidities, electromyographic data and other available relevant parameters.

Results

Significant histopathological data for IM diagnosis are active myopathic changes with myofiber size variation, perifascicular atrophy, degeneration and regeneration of fibers, muscle fiber necrosis, vacuolar changes, presence of rimmed vacuoles in IBM, mitochondrial abnormalities and fibrosis. Inflammatory infiltrates are key features, with their particular perivascular-perimysial or predominantly endomysial distribution and distinct cell composition suggesting different pathogenic mechanisms in the major IM subtypes. Immunohistochemical assessment of membrane-attack complex MAC, cytochrome-c oxidase-negative fibers, CD4/CD8+T-cells, CD 68 and Major histocompatibility complex class I overexpression are valuable in the diagnostic algorithm of IM.

Conclusion

Typical patterns of muscle involvement in the various IM are recognised, but none are pathognomonic, changes are known to be patchy, may be minimal, unspecific and sometimes overlapping. Detailed clinico-sero-pathological correlations are crucial to avoid misinterpretation of biopsy, rule out non-inflammatory myopathies, identify the subset of IM and thus improve the outcome of patients.

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

IDIOPATHIC INFLAMMATORY MYOPATHIES- PARTICULAR FEATURES IN CHILDREN.

Niculina Butoianu[1,2], Dana Șurlică[1], Diana Bârcă[1,2], Catrinel Iliescu[1,2], Alexandra Bastian[3]

1 Spitalul Clinic de Psihiatrie Prof. Dr. Alexandru Obregia, Secția de neurologie pediatrică București, Romania
2 Universitatea de Medicină și Farmacie „Carol Davila” , București, România
3 Anatomopatologie, Spitalul Clinic Colentina

Introduction

Juvenile dermatomyositis, the most frequent idiophatic inflammatory myopathy, is a systemic autoimmune disease characterized by muscle inflammation.

The most common clinical manifestations are muscle weakness, mostly proximal, and characteristic cutanat rash. It can also associate myalgia/arthralgia, dysphagia, anorexia or fever. During the disease course, there can be severe complications like interstitial lung disease, calcinosis, cardiovascular and cerebrovascular comorbidities. The discovery of myositis-specific antibodies and their association with relatively specific clinicopathological aspects led to the modification of the classification based on clinicoseropathological criteria.

Establishing the diagnosis of juvenile dermatomyositis is not difficult, but can be delayed because it is a rare disease and because it is difficult to asses muscle weakness in children. Currently, are available the EULAR/ACR criteria which can be used even when the muscle biopsy was not performed.

The diagnosis is mostly clinical, but the paraclinical test helps confirming the disease. After the clinical evaluation we perform the muscle enzume lever, followed by muscle biopsy and antibody testing.

Juvenile dermatomyiositis benefits from specific treatment wit immunosuppressive agents like glucocorticoids, methotrexate, clclosporine. Also, the studies have demonstrated that intravenouse immunoglobulin can bring some improvement. The treatment must be initiated as quicly as possible because it improves the quality of life and lowers the associated mortality and morbidity.

Conclusions

Juvenile dermatomyositis is a rare disease in pediatric population which affects the patient life and which, untreated, has a raised grade of mortality and morbidity. The early diagnosis is essential in order to benefit from rapid treatment and a better prognosis.

Key words

idiophatic inflammatory myopathies, juvenile dermatomyositis

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

THE INVOLVEMENT OF PARKIN IN THE SKELETAL MUSCLE PHENOTYPE IN PARKINSON'S DISEASE

Manole E.[1,2], Gaina G.[1,3], Lambrescu I.[1,3], Ceafalan L.[1,3]

[1]“Victor Babes” National Institute of Pathology
[2]Colentina Clinical Hospital, Pathology Department
[3]“Carol Davila” University of Medicine and Pharmacy

Emilia Manole e-mail: emilia_manole@yahoo.com

Parkin and α-synuclein are two key proteins involved in the pathophysiology of Parkinson’s disease (PD). α-synuclein alteration lead to the formation of toxic oligomers and fibrils (Lewy bodies) which contribute to PD pathology. In patients with PD, skeletal muscles are affected. They have impaired motor activity, which is associated with loss of skeletal muscle mass, muscle weakness, fatigue, and resistance to exercise but the molecular mechanisms behind the skeletal muscle phenotype in PD are not well understood. In approximately half of the cases, the recessively inherited early-onset PD is caused by mutations in the PARK2 gene that encodes PARKIN (E3-ubiquitin ligase) and PINK1 gene that encodes PTEN-induced kinase 1 (PINK1). They are involved in the clearance of misfolded and aggregated proteins by the ubiquitin-proteasome system and regulates mitophagy and mitochondrial biogenesis. Biochemical and genetic studies revealed that in autosomal recessive parkinsonism, PINK1 and PARKIN, normally work together in the same pathway to govern mitochondrial quality control.

Under physiological conditions, PINK1 passes across outer and inner mitochondrial membranes, it accumulates on mitochondria as full-length form and becomes activated. Active PINK1 autophosphorylates itself and phosphorylates PARKIN and ubiquitin, further enhancing PARKIN activity in a feed-forward mechanism. These phosphorylation cascades lead to the recruitment of selective autophagy receptors and subsequent degradation of mitochondrial proteins.

There are *in vitro* and *in vivo* studies showing that knock down of PARKIN significantly increases proteolytic activities in skeletal muscle. PARKIN deficiency exacerbates fasting-induced skeletal muscle wasting, through upregulating genes involved in catabolic activities in skeletal muscle. Skeletal muscle atrophy is mainly regulated through the activity of atrophy-related genes (atrogenes), under the control of FOXO transcription factors that are required among others for the control of atrogenes upon fasting and denervation in mouse model.

PARKIN could be a potential therapeutic target in cases of Parkinson's disease with muscle atrophy. In vitro studies revealed that knockdown of Parkin led to myotubular atrophy suggesting that PARKIN function is required for post-natal skeletal muscle growth and development. PARKIN had been shown to ameliorate the atrophy phenotype observed in sarcopenic mice, through improving mitochondrial quality, which further underscores the role of Parkin in skeletal muscle function. PARKIN overexpression attenuates ageing-related loss of muscle mass, increases in mitochondrial content and enzymatic activities.

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

NICOTINAMIDE MONONUCLEOTIDE (NMN) EFFECTS IN TYPE 2 DIABETES MUSCLE: A DEEP PROTEOMICS STUDY

Marinescu GC[1,2*], Popescu RG[1,2]

[1]Independent Research Association, Bucharest, Romania
[2]Blue Screen SRL, Bucharest, Romania

β-Nicotinamide mononucleotide (NMN), a precursor of nicotinamide adenine dinucleotide (NAD+), has proven effective in several age-related disorders, including Type 2 diabetes (T2D). It has been proposed that NMN works in T2D by inducing mitochondrial biogenesis.

In this study, we examined how NMN treatment influenced glucose absorption processes in C2C12-derived myotubes and mouse muscle tissue. We identified specific proteome-level changes using data-independent acquisition (DIA) mass spectrometry, specifically SWATH-MS, followed by label-free and library-free peptide mapping with neural networks and interference correction.

Contrary to previous hypotheses, our data clearly indicates a down-regulation of mitochondrial proteins, including those associated with oxidative phosphorylation, TCA cycle, and NAD mitochondrial ADP/ATP antiporter Slc25a5. We also observed down-regulation of proteins involved in energy metabolism, amino acid metabolism, and the regulation of the actin cytoskeleton. Ucp1 was notably increased in the thermogenesis pathway, supporting enhanced glucose uptake in muscle tissue.

C2C12-derived myotubes exhibited a distinct response characterized by reduced Txnip expression, resulting in improved glucose uptake, and increased reactive oxygen species (ROS) production. Protein synthesis was clearly up-regulated in all NMN-treated muscle cells, while the proteasome pathway was suppressed under conditions of high glucose and high insulin levels during NMN treatment. Up-regulation of ribosomal and lysosomal proteins, coupled with down-regulation of the spliceosome pathway were observed, potentially leading to slightly impaired and less energy-efficient protein synthesis machinery.

Our findings strongly suggest that the positive effects of NMN in T2D are more likely attributed to the induction of thermogenesis and fasting-mimicking effects, rather than mitochondrial biogenesis, as NMN treatment modestly inhibits the expression of mitochondrial proteins in muscle.

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*e-mail of the corresponding author: Catalin.Marinescu@independent-research.ro

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

DAY 3 - SATURDAY, NOVEMBER 4

Course – Electron microscopy for study and diagnosis of viruses
Session 11: Short communication - young researchers
Session 12: Short communication - students
Communication (K)now

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Course – Electron microscopy for study and diagnosis of viruses

Mihaela Gherghiceanu, Emanuel Fertig
`Victor Babeș` National Institute of Pathology, Bucharest, Romania

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Session 11: Short communication - young researchers

IN VITRO RECONSTITUTION OF HUMAN RQT DEPENDENT RIBOSOME DISSOCIATION REACTION

Shuangshuang Niu[1], **Leona Chitoiu[1]**, Ken Ikeuchi[1], Timo Denk[1], Yoshitaka Matsuo[2], Toshifumi Inada[2], Roland Beckmann[1]
[1] Gene Center and Department of Biochemistry, University of Munich LMU, Germany
[2]Division of RNA and gene regulation, Institute of Medical Sciences, The University of Tokyo, Japan

Ribosome-associated Quality Control (RQC) is a critical pathway that detects and resolves stalled ribosomes to prevent the accumulation of truncated proteins. In mammals, the E3 ubiquitin ligase ZNF598 plays a key role in recognizing various types of stalls that lead to ribosomal collisions. ZNF598 labels the leading stalled ribosome by adding ubiquitin chains on the eS10 and uS10 proteins of the 40S subunit. Subsequently, these ubiquitinated collided ribosomes are split into monosomes and ribosomal subunits by the Ribosome-associated Quality Control Trigger (RQT) complex. In mammals, the RQT complex contains the RNA-helicase ASCC3, the stabilizing factors ASCC2 and TRIP4, and ASCC1 with unclear contributions to ribosomal splitting activity. However, the mechanism behind the assembly and splitting activity of mammalian RQT remains unknown.

For an in depth biochemical and structural analysis we aim at the complete in vitro reconstitution of this process. First, we report the successful purification of preparative yields of collided human ribosomes, active human ZNF598 and RQT proteins. Next, we were able to optimize the ubiquitination reaction of eS10 and uS10 on collided ribosomes by ZNF598, with the majority of both proteins being poly-ubiquitinated. Subsequently, we also succeeded in reconstituting the hRQT dependent splitting reaction. These systems offer now new opportunities to control and query the mechanisms of hRQT assembly and ribosomal splitting, as well as to elucidate the structural basis of the hRQT-ribosome interaction in different reaction states.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

IN VITRO GENERATION OF FUNCTIONAL MELANOMA MODELS BY CRISPR-CAS9 GENE EDITING TECHNOLOGY COUPLED WITH LENTIVIRAL TRANSDUCTION

Dobre EG[1,2], Koumantou D[3], Boisel E[3], Constantin C[1,4], Neagu M[1,2,4], Saveanu L[3]

[1] Immunology Department, "Victor Babes" National Institute of Pathology, Bucharest, Romania;
[2] Faculty of Biology, University of Bucharest, Bucharest, Romania;
[3] Centre de Recherche sur l'Inflammation, Faculté de Médecine Paris Diderot, Paris, France;
[4] Pathology Department, Colentina Clinical Hospital, Bucharest, Romania.

Introduction

In cutaneous melanoma (CM), specific tumor driver mutations may affect the antitumor immune response through changes in expression of tumor antigens or checkpoint molecules, or production of immune-suppressive cytokines. For instance, the highly prevalent BRAF V600E mutations, may promote immune evasion, either by directly altering the surface expression and intracellular distribution of MHC class I molecules in melanoma cells or by hindering the activity of anti-tumoral CD8+ T-cells [1]. Recent data also indicates that patients with BRAF-mutant melanoma tend to respond better to immunotherapy than those with NRAS-mutant or wild-type tumors [2]. This implies that the immune landscape in treatment-naive melanoma with BRAF mutations is distinct from that in melanoma without BRAF mutations. This underscores the importance of investigating the relationship between the immune response and mutational status in determining the clinical benefit of immunotherapy in CM patients. The purpose of the study was to develop a work-flow for specific knockout (KO) clone generation in melanoma cell lines to be further used in functional cellular tests.

Materials and methods

The pSpCas9(BB)-2A-GFP(PX458)-gRNA editing system was delivered via electroporation to disrupt NRAS and BRAF genes in mouse melanoma cell lines B16 and B16-OVA. Melanoma KO cells were transduced with lentiviral plasmids carrying human (h)NRAS wt/G12D/Q61K and hBRAF wt/V600E gene variants. Lentiviral vectors encoding human (h)BRAF wt and human BRAF V600E proteins were obtained from Addgene (Plasmid #116719 and Plasmid #116204). Lentiviral vectors encoding hNRAS wt/G12D/Q61K variants were constructed through genetic engineering. The cDNA sequence of hNRAS wt/G12D/Q61K was amplified from a pEX-A128 plasmid and cloned into the pLVX-IRES-ZsGreen1 plasmid (Clontech) using EcoRI and NotI. Lentiviral plasmids encoding the desired human variants were transfected into HEK293T cells to produce lentiviral particles, which were concentrated from the supernatant by ultracentrifugation. Melanoma KO cells were transduced with the viral concentrate in the presence of 8 µg/mL polybrene (Sigma). Post-transduction, cells that integrated pHAGE-BRAF wt were selected using puromycin (2 µg/mL, 24 hours after transduction), while cells with other integrated lentiviral plasmids were identified by GFP expression (48 hours). Lentiviral transduction was confirmed by Western Blot analysis.

Results

In our experimental model, we achieved KO clone generation with an efficiency ranging from 5% to 55%. KO of NRAS and BRAF genes were confirmed by flow cytometry and Western Blot analysis. Efficient production and successful delivery of lentiviral vectors were also accomplished. The steps and principles of this protocol could be used to edit and study the functions of any gene of biomedical interest, both in adherent 2D cultures of human or animal cells and in 3D organoid cultures.

Conclusions

Our study demonstrates that CRISPR-Cas9 and lentiviral transduction can be used together to establish stable mouse melanoma cell lines that incorporate different human genetic variants which are of great interest for CM *in vivo* immunological and therapeutic studies. The genetic ''scissors'' are expected to reinvigorate cancer research in many ways, enabling the identification of novel immune-oncology gene targets, generation of cancer animal models, and hence facilitating better cell design and manufacture for adoptive cellular therapies.

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

IMPACT OF GUT MICROBIAL MOLECULES ON THE ONSET OF PARKINSON'S DISEASE – AN IN VITRO STUDY

Octavian Costin Ioghen[1], Gisela Gaina, Ioana Lambrescu, Emilia Manole, Sevinci Pop, Oana Mosoia, Teodora Niculescu, Laura Cristina Ceafalan, Bogdan Ovidiu Popescu

[1]"Victor Babes" National Institute of Pathology, Bucharest, Romania

Parkinson's disease (PD) is a neurodegenerative disorder in which a critical pathological finding is the deposition of the alpha-synuclein protein into the dopaminergic neurons. A recent hypothesis of PD pathogenesis is the prion-like dissemination of alpha-synuclein aggregates that starts in the gastrointestinal tract and ascends into the central nervous system. Moreover, gut microbiota appears to influence the initiation of alpha-synuclein aggregation via bacterial functional amyloids.

In this study we set up a dopaminergic differentiation protocol of the SH-SY5Y human neuroblastoma cell line, an accurate translational model for studying PD, and we assessed the effects of several bacterial compounds such as rhamnolipid, lipopolysaccharide, phenol soluble modulin and curli . We performed viability and cytotoxicity assays, we analyzed morphological changes by measuring surface impedance with xCELLigence Real-Time Cell Analysis system and we measured alpha-synuclein expression levels by qRT-PCR and Western Blot.

Our results demonstrated that the gut microbial compounds induced overexpression of alpha-synuclein and morphological changes in the dopaminergic-differentiated neurons.

In conclusion, our in vitro study showed that gut bacterial compounds play a major role in the onset of PD. The results can be further translated in vivo and in clinical research studies for potential more effective therapeutic strategies.

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

Session 12: Short communication - students

ANTI-TUMOR POTENTIAL OF NOVEL SYNTHESIZED THIOSEMICARBAZIDE DERIVATIVES

Antonia-Maria Stroe[1], Marian Zaroschi[1], Ștefania-Felicia Bărbuceanu[1] and Sevinci Pop[2]

[1] Department of Organic Chemistry, Faculty of Pharmacy, “Carol Davila” University of Medicine and Pharmacy
[2] “Victor Babes” National Institute of Pathology and Biomedical Sciences

Introduction

Thiosemicarbazides are versatile molecules with pharmacological properties, acting as potential anti-bacterial, anti-viral or anti-cancer agents. Nowadays, there is a great demand in pharmaceutical industry for synthesis of new thiosemicarbazides derivatives that target specific mechanisms in cancer. This study presents the biological activities of two novel thiosemicarbazide derivatives investigated on breast cancer adenocarcinoma cell line.

Materials and methods

Bromo-thiosemicarbazide derivate (TSBr) and the unsubstituted one (TSH) containing a diaryl-sulfone moiety and a cyclopropyl radical were obtained by a multi-step organic synthesis. They were characterized by physical constants (melting points, solubility), and the chemical structure was confirmed by spectral techniques (IR, UV-VIZ, NMR) and elemental analysis.

The cellular viability in the presence of different concentrations of compounds (1-200 µM) was assessed at 48 and 72 hours by MTS assay. The cytotoxicity was evaluated for the same interval of treatment on MCF-7 cells, using LDH test. The cells proliferation rate was real-time monitored by xCELLIgence platform and by video-microscopy the morphological changes induced by the two compounds on adenocarcinoma cells were analyzed.

Results

The viability of breast adenocarcinoma cells was affected by both compounds in a concentration- and time-dependent manner. The TSBr compound induced the highest cytotoxicity at 72 hours of treatment at all tested concentrations, in comparison with the unsubstituted compound (TSH). A strong anti-proliferative effect was observed for TSBr treatment at lower concentration 5-100µM and cellular death for 150-200 µM. The time-lapsing microscopy confirmed the morphological changes related with cellular death.

Conclusion

Our study has demonstrated that a novel Bromo- thiosemicarbazide derivate possesses a strong anti-proliferative, pro-apoptotic activity and significantly more pronounced cytotoxicity on breast adenocarcinoma cells than the unsubstituted compound. The Bromo- thiosemicarbazide derivate might have a potential anti-tumor activity on breast cancer cell line.

Acknowledgement

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

BIOLOGICAL ACTIVITIES OF TWO PLANT EXTRACTS RICH IN ISOFLAVONES -IN VITRO INVESTIGATION ON BREAST ADENOCARCINOMA CELL LINE

Ana-Maria Cîrjan[1], Elena Cerasela Gîrd[1], Liliana Popescu[1], Sevinci Pop[2]

[1] Department of Pharmacognosy-Phytochemistry-Phytotherapy, Faculty of Pharmacy, “Carol Davila” University of Medicine and Pharmacy
2 “Victor Babeș” National Institute of Pathology and Biomedical Sciences

Introduction

Nowadays, plant extracts are extensively used as nutritional supplements for prevention of chronic diseases. However, the relationship between a diet rich in isoflavones and breast cancer risk is still unveiled. The aim of the current study was to compare biological activities of red clover (Trifolium pratense) and alfalfa (Medicago sativa) ethanolic extracts using an estrogen-dependent breast cancer cell line.

Materials and methods

The biological activity of each extract was evaluated in order to determine their cytotoxicity profile on breast adenocarcinoma cells (MCF-7) by using end-point assays (MTS, LDH). The cells proliferation rate was real-time monitored by measuring the cellular impedance (xCELLingence system) in presence or in the absence of plant extract treatment. The estrogenic activity of extracts was investigated by E-SCREEN test. Their capacity to reduce or activate reactive oxygen species (ROS) were evaluated by CellROX green staining and was observed at fluorescent microscope.

Results

The plant extracts induced a moderate cytotoxicity on breast adenocarcinoma cells, and the red clover extract had affected cells viability at high concentration and for 72 hours of treatment. Moreover, a biphasic dose-response was observed: high-dose of ethanolic extract triggered the cell death via oxidative stress; and low-doses of both extracts had a cytoprotective activity against ROS generation. The *Trifolium pratense* extract had the more significant estrogenic activity in comparison with *Medicago sativa* extract. Overall, the aflalfa extract exerted a less potent effect on breast adenocarcinoma cell line compared with red clover extract.

Conclusion

Our study has demonstrated that Trifolium pratense extract could induce anti-cancer effect, by reducing the cellular proliferation rate and by affecting the viability of tumour cells at higher concentration. Besides, red clover extract could have a cytoprotective activity against ROS generation in tumour cells.

Acknowledgement

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Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

A VACCINE ADENOVIRUS IN 3D

Anamaria Teodora Ciobotea[1], Lucian Ionuț Antone-Iordache[1], Mihaela Gherghiceanu[1,2], Tudor Emanuel Fertig[1,2]
[1]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[2]Ultrastructural Pathology and Bioimaging Lab, Victor Babeș National Institute of Pathology, Bucharest, Romania

Introduction

SARS-CoV-2 vaccines have been key for preventing hospitalization and death during the COVID-19 pandemic. mRNA and recombinant adenovirus vaccines were the main types of vaccines available. In this study, we obtained a three dimensional (3D) model of the adenoviral nucleocapsid from one COVID-19 vaccine, to use the resulting structure and the image processing protocol for teaching purposes.

Methods

Vaccine was thawed and pipetted on carbon-coated electron microscopy grids, then 2% uranyl-acetate was used for staining. The 4x4k Ceta camera of a Talos 200keV transmission electron microscope was used to acquire 435 images. Single-particle analysis (SPA) was done in RELION. Around 3000 particles were involved in 2D classification and 3D rendering.

Results

We obtained a 3D model with a resolution of around 6 nm. Using visualization software Chimera X, the structure was compared and docked with similar models and the symmetry was shown to fit the literature .

Conclusions

Negative stain electron microscopy (NS-EM) is still a quick and efficient method to assess various biological samples, including vaccines and viruses, giving valuable insights into their structure.

Keywords

3D model; SARS-COV-2 vaccines; electron microscopy; adenovirus; SPA.

Splaiul Independenței 99-101, București 5, 050096, România; Tel: +40 21 319 45 30, +40 21 319 27 32, +40 21 319 27 33; Fax: +40 21 3192734 / 3194528; E-mail: info@ivb.ro

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ÎMBUNĂTĂȚIREA ABILITĂȚILOR DE RELAȚIONARE ȘI COMUNICARE PRIN JOC TEATRAL LA COPIII ȘI TINERII DEFAVORIZATI / IMPROVEMENT OF THE RELATIONAL AND COMMUNICATION SKILLS THROUGH THEATRICAL PLAY IN DISADVANTAGED CHILDREN AND YOUTH

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Andreea-Diana Jicman, PhD, Georgiana Adelina Dobrea, Ioana-Mădălina Lixăndroaia, Ana Maria Victoria Vicovan