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SOMS | Scientific Organisation of Medical Students

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ABSTRACT BOOK

(and Meeting Program)

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CONTENTS

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PROGRAM

THURSDAY, NOVEMBER 22

08:30 – 10:00 REGISTRATION (permanent for all meeting days)

10:00 – 10:30 **Opening Ceremony** (Victor Babeș Auditorium)

10:30 – 11:30 **Session 1:** ***Tradition, Continuity, Innovation*** (Victor Babeș Auditorium)
Chair: Dr Gina Manda

Molecular Profiles in Alzheimer Disease - The REDBRAIN Project After Two Years
Antonio Cuadrado[1,7,8,9], Elena Milanesi[1], Bogdan Ovidiu Popescu[1,2], Gabriel Prada[2,3], Ovidiu Bajenaru[2,4], Catalina Tudose[2,5], Luiza Spiru[2,6], Gina Manda[1]
[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]National Institute of Gerontology and Geriatrics Ana Aslan, Bucharest; [4]Emergency University Hospital, Bucharest; [5]Clinical Hospital of Psychiatry Alexandru Obregia, Bucharest; [6]Ana Aslan International Foundation, Bucharest; [7]Centro de Investigacion Biomedica en Red sobre Enfermedades Neurodegenerativas CIBERNED ISCIII, Madrid; [8]Instituto de Investigaciones Biomadicas Alberto Sols UAM CSIC Department of Biochemistry Faculty of Medicine Autonomous University of Madrid, Madrid; [9]Instituto de Investigacion Sanitaria La Paz, Madrid, Spain

High Dose-Rate Radiation and Endogenous Biomarkers – Perspectives for Early Imaging of Biological Effects
T. Asavei, M. Bobeica, V. Nastasa, M. O. Cernaianu, P. Ghenuche, D. Savu, D. Stutman, M. Radu, K. Tanaka, D. Doria, P. R. Vasos
Horia Hulubei National Institute for Physics and Nuclear Engineering, Extreme Light Infrastructure - Nuclear Physics ELI-NP, Bucharest-Magurele

11:30 – 11:45 COFFEE BREAK

11:45 – 13:00 **Plenary Lecture 1** (Victor Babeș Auditorium)

Bench to Bedside Research on Critical Illness Myopathy and Ventilator Induced Diaphragm Muscle Dysfunction

Prof. Lars Larsson

Karolinska Institute & Karolinska Hospital Stockholm, Sweden

13:00 – 14:00 LUNCH BREAK

14:00 – 16:00 **Session 2: *Experimental and Clinical Neurosciences*** (Victor Babeș Auditorium)
Chairs: Prof. Alexandru Babeș, Prof. Bogdan Ovidiu Popescu

Animal Models of Neuropsychiatric Disorders Focusing on Some Oxidative Stress Markers
Alin Ciobica
Alexandru Ioan Cuza University, Iași

The Role of Transient Receptor Potential Channels in the Pathophysiology of Genetic Diseases Associated with Painful Photosensitivity
Alexandru Babeș
University of Bucharest

Endovascular Interventional Radiology Techniques in Acute Ischemic Stroke - A Single Emergency Center Experience
Florina Anca Antochi, Raluca Stefania Badea, Bogdan Dorobat
Emergency University Hospital, Bucharest

Phosphorylation of Amyloid Precursor Protein Depends on Caveolin 1 Expression in the Murine Brain
Bianca Pătrănoiu[1], Ana-Maria Enciu[2]
[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest

Microbiota, Enteric Nervous System and Neurodegenerative Diseases
Bogdan Ovidiu Popescu
Victor Babeș National Institute of Pathology, Bucharest

16:00 – 16:15 COFFEE BREAK

16:15 – 18:00 **Session 3A: *New Insights in Pathology*** (Victor Babeș Auditorium)
Chair: Prof. Emil Iancu Pleșea

Difficulties of Staging pT1 Colonic Adenocarcinoma Arising on Adenoma
Cristiana Popp[1], Mirela Cioplea[1], Liana Sticlaru[1], Gianina Micu[1], Sabina Zurac[1,2], Florica Staniceanu[2], Patricia Stinga[1], Theodor Voiosu[1,2], Bogdan Mateescu[1,2], Luciana Nichita[1,2]
[1]Colentina University Hospital, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest

Clinical, Endoscopic and Pathological Particularities of 25 Cases, Including Immunohistochemical Aspects of Inflammatory Fibroid Polyps, Rare Mesenchymal Tumors of the Digestive Tract

Andrei-Mihai Borcan[1], Laura Ioana Florea[1], Alexandra Rosulescu[2], Simona Enache[2], Florina Vasilescu[2], Emma Marcelle Burke[2], Florin Andrei[2], Emil Plesea[2], Valentin Enache[2], Vlad Herlea[3], Gabriel Becheanu[1]
[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest; [3]Fundeni Clinical Institute, Bucharest

Subcellular Localization of Calreticulin Mutants in Myeloproliferative Neoplasms
Tudor Emanuel Fertig[1], Daciana Marta[1], Silvia-Diana Prelipcean[1], Anita Roy[2], Ștefan N. Constantinescu[2], Mihaela Gherghiceanu[1]
[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Universite Catholique de Louvain and de Duve Institute, Brussel, Belgium

16:15 – 18:00 **Session 3B:** ***Nephropathology*** (Ioan Moraru Auditorium)
Chair: Dr Gener Ismail, Dr Mihaela Gherghiceanu

The Role of Kidney Biopsy in the Management of Patients with IgA Nephropathy
Bogdan Obrisca[1], Roxana Jurubita[1], Andreea Andronesi[1], Bogdan Sorohan[1], Alexandru Procop[1], Vlad Herlea[1], Mihaela Gherghiceanu[2], Gener Ismail[1]
[1]Fundeni Clinical Institute, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest

Importance of Kidney Biopsy in Monoclonal Gammopathies of Renal Significance
Andreea Andronesi[1,2], Mihaela Gherghiceanu[3], B. Obrișcă[1,2], B. Sorohan[1,2], Cristina Cristache[1,2], Gener Ismail[1,2]
[1]Nephrology Department, Fundeni Clinical Institute, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Victor Babes National Institute of Pathology, Bucharest

A Case of Late Onset Antibody Mediated Rejection and IgA Nephropathy Recurrence
Bogdan Sorohan[1], Dorina Tacu[2], Mihaela Gherghiceanu[1,3], Gener Ismail[2,4]
[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Department of Uronephrology and Kidney Transplant, Fundeni Clinical Institute, Bucharest; [3]Carol Davila University of Medicine and Pharmacy, Bucharest; [4]Department of Nephrology, Fundeni Clinical Institute, Bucharest

Genetic Testing for Pediatric Kidney Disease – International Collaboration and Ethics
Adrian Catalin Lungu, Cristina Stoica
Pediatric Nephrology Department, Fundeni Clinical Institute, Bucharest

Kidney Biopsy Processing for Immunofluorescence and Electron Microscopy
Mihaela Gherghiceanu[1,2], Gener Ismail[3,1]
[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Department of Uronephrology and Kidney Transplant, Fundeni Clinical Institute, Bucharest

18:00 – 18:30 **Posters mounting**

FRIDAY, NOVEMBER 23

09:30 – 11:30 **Session 4:** ***Knowledge Transfer on Natural Products towards Industry for Human Health Benefits – Project G*** (Ioan Moraru Auditorium)
Chair: Prof. Cristiana Tănase

Red Things from Red Sage for a Green Pharmacy - Potential of Tanshinones and Related Diterpenoids as Natural Drugs or Drug Leads
Adam Matkowski
Department of Pharmaceutical Biology, Medical University of Wroclaw, Poland

Safety and Benefit Assessment of Food Supplements in the European Union
Valeriu Curtui
Nutrition Unit, European Food Safety Authority, Parma, Italy

Food Supplements Made in Romania with Multiple Benefits for Human Health
Elvira Gille
NIRDBS/Stejarul Biological Research Centre, Piatra Neamt; Faculty of Chemistry, Alexandru Ioan Cuza University of Iasi

COP – G Project Overview
Cristiana Tănase
Victor Babeș National Institute of Pathology, Bucharest

11:30 – 11:45 COFFEE BREAK

11:45 – 13:00 **Plenary Lecture 2** (Victor Babeș Auditorium)

Lipid-Dependent Mechanisms in Cell-Cell Signaling

Prof. Adrian Salic

Harvard Medical School, Boston, MA, USA

13:00 – 14:00 LUNCH BREAK

14:00 – 16:00 **Session 5: *Omics Technologies for Precision Medicine*** (Victor Babeș Auditorium)
Chairs: Prof. Cristiana Tănase, Dr Aurora Arghir

The Involvement of E6 and E7 HPV16 Oncogenes in Chromatin Remodelling through Components of NuRD Complex
Anca Botezatu, Iulia Iancu, Adriana Plesa, Alina Fudulu, Adrian Albulescu, Marinela Bostan, Mirela Mihaila, Gabriela Anton
Stefan S. Nicolau Institute of Virology, Bucharest

Combined Approach of Next Generation Sequencing and Microarray Technologies for Characterization of Molecular Signatures in Acute Myeloid Leukemia
Aurora Arghir[1,2], Sorina Mihaela Papuc[1], Alina Erbescu[1], Raluca Colesniuc[1], Diana Cisleanu[2,3], Dan Sebastian Soare[2,3], Viola Maria Popov[4], Daniela Georgescu[4], Nicoleta Berbec[2,5], Ana-Maria Vlădăreanu[2,3], Horia Bumbea[2,3]
[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Emergency University Clinical Hospital, Bucharest; [4]Colentina Clinical Hospital, Bucharest; [5]Coltea Clinical Hospital, Bucharest

A Multidisciplinary Approach of Patients with Schizophrenia – from Deep Phenotyping to Genotyping and Back
Magdalena Budisteanu[1], Sorina Mihaela Papuc[1], Dan Riga[2], Sorin Riga[2], Aurora Arghir[1]
[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Prof Alexandru Obregia Clinical Hospital of Psychiatry, Bucharest

From Cilia to Complex Disorders: The Ciliopathies
Ina Ofelia Focsa[1], Laurentiu Camil Bohiltea[1], Mihaela Balgradean[1,2]
[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Children Clinical Emergency Hospital Marie Curie, Bucharest

The Epigenome Targeted by Bioactive Components from Diet: Facts and Perspective in Cancer Prevention
Sevinci Pop[1], Eleonora Codorean[1], Violeta Alexandra Ion[2], Elvira Gille[3], Cristiana Tanase[1,4]
[1]Victor Babeș National Institute of Pathology, Bucharest; [2]SC Cromatec Plus SRL, Bucharest; [3]NIRDBS Stejarul Biological Research Centre, Bucharest; [4]Titu Maiorescu University Faculty of Medicine, Bucharest

Proteome Profiling of Bone Healing in a Rat Tibial Defect Model Using Titanium Implant with Functionalized Surface
Raluca Maria Boteanu[1], Viorel Iulian Suica[1], Luminita Ivan[1], Florentina Safciuc1, Elena Uyy[1], Sorin Croitoru[2], Valentina

Grumezescu[3], Livia Sima[4], Constantin Vlagioiu[5], Gabriel Socol[3], Felicia Antohe[1]
[1]Institute of Cellular Biology and Pathology N Simionescu, Bucharest; [2]Faculty of Engineering and Management of Technological Systems, Bucharest; [3]National Institute for Lasers Plasma and Radiation Physics, Bucharest; [4]Institute of Biochemistry, Bucharest; [5]Faculty of Veterinary Medicine, Bucharest

Green Synthesis of Nanoparticles and their Biomedical Applications; Proteomic Analysis
Elena Codrici[1], Simona Mihai[1], Ionela Daniela Popescu[1], Ana-Maria Enciu[1,2], Lucian Albulescu[1], Radu Albulescu[1], Mircea Leabu[1,2], Alina Butu[3], Cristiana Tanase[1,4]
[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Institute of Biological Sciences, Bucharest; [4]Titu Maiorescu University, Faculty of Medicine, Bucharest

Proteomic Investigations of Natural Products in Cancer Prevention and Therapy
Cristiana Tanase[1,2], Ana-Maria Enciu[1,3], Elena Codrici[1], Simona Mihai[1], Ionela Daniela Popescu[1], Lucian Albulescu[1], L.G. Necula[1,4], Sevinci Pop[1], Radu Albulescu[1,5]
[1]Biochemistry-Proteomics Department, Victor Babeș National Institute of Pathology, Bucharest; [2]Titu Maiorescu University, Bucharest; [3]Carol Davila University of Medicine and Pharmacy, Bucharest; [4]St. S. Nicolau Institute of Virology, Bucharest; [5]National Institute for Chemical Pharmaceutical R&D, Bucharest

16:00 – 16:15 COFFEE BREAK

16:15 – 18:00 **Helping Research – Industry Partners' Session** (Ioan Moraru Auditorium)
Chair: Dr Elena Codrici

Clinical Decision Support in the New Diagnostics Era
Violeta Dragos
Roche Diagnostic Romania

16:15 – 18:00 **Posters Session**

SATURDAY, NOVEMBER 24

10:00 – 12:00 **Session 6: Varia** - mainly for Students, PhD Students and Postdocs (Victor Babeș Auditorium)
Chair: Dr Ana-Maria Enciu, Dr Cătălin Gabriel Manole

The Effect of Nanoparticles Obtained by Green Synthesis on Cell Proliferation

Alexandra Cătălina Vîlceanu[1], Simona Mihai[2], Elena Codrici[2], Lucian Albulescu[2], Ionela Daniela Popescu[2], Mihaela Lupu[3], Crina Karmezan[3], Alina Butu[4], Ana-Maria Enciu[2,1]
[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest; [3]SC Sanimed International Impex, Bucharest; [4]The National Institute of Research and Development for Biological Sciences, Bucharest

Eight-Arm Radial Maze Test for Assessment of Memory Performance in Nrf2 Mice
Ștefania Grigoraș[1], Radu Ioan Tiron[1], Andreea Elena Ștefan[1], Ana-Maria Enciu[2,1], Ionela Victoria Neagoe[2]
[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest

Detection and Quantification of Rare Mutant Alleles by Digital Droplet PCR
Victor Ionescu, Gisela Găină, Ștefania Rogozea, Cristina Niculițe, Valeriu Cișmașiu
Victor Babeș National Institute of Pathology, Bucharest

High Resolution Microarray Analysis of Epilepsy-Linked Genomic Regions
Artsiom Klimko[1], Sorina Mihaela Papuc[2], Magdalena Budisteanu[3,2,4], Dana Craiu[4], Aurora Arghir[1,2]
[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest; [3]Titu Maiorescu University Bucharest; [4]Prof Dr Alex Obregia Clinical Hospital of Psychiatry, Bucharest

Cytokines and Growth Factors as Potential Biomarkers for Evaluation of Pituitary Adenoma Aggressiveness
Dana Tapoi[1], Elena Codrici[2], Linda Maria Popa[1], Ionela Daniela Popescu[2], Simona Mihai[2], Ancuta Augustina Gheorghisan Galateanu[1], Cristiana Tanase[2]
[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest

12:00 – 12:30 COFFEE BREAK

12:30 – 13:30 **Closing Ceremony** (Victor Babeș Auditorium)

THURSDAY, NOVEMBER 22

SESSION 1

TRADITION, CONTINUITY, INNOVATION

Chair: Gina Manda

MOLECULAR PROFILES IN ALZHEIMER DISEASE - THE REDBRAIN PROJECT AFTER TWO YEARS

Antonio Cuadrado[1,7,8,9], Elena Milanesi[1], Bogdan Ovidiu Popescu[1,2], Gabriel Prada[2,3], Ovidiu Bajenaru[2,4], Catalina Tudose[2,5], Luiza Spiru[2,6], Gina Manda[1]

[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]National Institute of Gerontology and Geriatrics Ana Aslan, Bucharest; [4]Emergency University Hospital, Bucharest; [5]Clinical Hospital of Psychiatry Alexandru Obregia, Bucharest; [6]Ana Aslan International Foundation, Bucharest; [7]Centro de Investigacion Biomedica en Red sobre Enfermedades Neurodegenerativas CIBERNED ISCIII, Madrid; [8]Instituto de Investigaciones Biomadicas Alberto Sols UAM CSIC Department of Biochemistry Faculty of Medicine Autonomous University of Madrid, Madrid; [9]Instituto de Investigacion Sanitaria La Paz, Madrid, Spain

Keywords: Alzheimer, MCI, leukocytes, monocytes, gene expression, cytokines

Introduction. Mild cognitive impairment (MCI) is recognized as an intermediate clinical stage that often evolves towards Alzheimer disease (AD), the most common cause of dementia. There is an urgent need to identify biomarkers in the blood of MCI/AD patients for better monitoring of the disease and therapy, while using minimally invasive procedures. The present study started from the hypothesis that blood leukocytes bear a molecular fingerprint specific for MCI/AD, related to oxidative stress and inflammation, which may be associated with increased susceptibility of AD patients to infection.

Aim: The study is focused on detecting blood biomarkers related to the transcription factors NRF2 and NFkB, which control oxidative stress and inflammation, for early diagnosis and prognosis of MCI.

Methods. The expression levels of 168 genes involved in oxidative stress and inflammation were analyzed by qRT-PCR in the blood of 30 MCI/AD patients and 22 non-demented controls (CTRL), age- and sex-matched and grouped according to the Mini-Mental State Examination (MMSE) score, adjusted by age and education. Additionally, the functional status of monocytes (Mo) was investigated regarding cytokine release by using unstimulated and lipopolysaccharide (LPS)-stimulated cells in connection with the molecular fingerprint of NRF2. A parallel study relevant for Endotoxin Tolerance (ET) was done using transgenic mice mimicking AD (APP+TAU+Nrf2+/+ mice), and the involvement of NRF2 was demonstrated in APP+TAU+Nrf2-/- mice.

Results. The gene expression study revealed that only a few of the genes involved in oxidative stress and inflammation were over-expressed in the MCI/AD group, and far more genes were under-expressed in patients versus CTRL. Some of these under-expressed genes were correlated with the disease score (MMSE), therefore being promising candidates for disease monitoring.

We found that Mo isolated from AD/MCI patients exhibited a decreased basal release of pro-inflammatory cytokines, which could explain the increased susceptibility to infection observed in AD. In animal models of AD, NRF2 was activated in response to LPS, and an even higher NRF2 activity was observed in tolerant human and mouse monocytes/macrophages (Mo/Mφ). Moreover, NRF2 deficiency was associated with a tolerogenic state in mouse Mφ, accompanied by an increased pro-inflammatory activity induced by LPS.

Conclusion. The study highlights that genes critically involved in inflammation and in oxidative stress have impaired expression in MCI/AD, some of them being correlated with disease stage. Results will be further validated on a larger cohort by a longitudinal study. A potential role of NRF2 in maintaining the hypo-responsive state of Mo/Mφ in AD was evidenced in animal models, hence singling out NRF2 as a promising drug target for modulating Mo reactivity in AD patients.

Acknowledgement: The study was funded by the European Regional Development Fund, Competitiveness Operational Program through the grant P_37_732/2016 REDBRAIN.

HIGH DOSE-RATE RADIATION AND ENDOGENOUS BIOMARKERS – PERSPECTIVES FOR EARLY IMAGING OF BIOLOGICAL EFFECTS

T. Asavei, M. Bobeica, V. Nastasa, M. O. Cernaianu, P. Ghenuche, D. Savu, D. Stutman, M. Radu, K. Tanaka, D. Doria, P. R. Vasos

Horia Hulubei National Institute for Physics and Nuclear Engineering, Extreme Light Infrastructure - Nuclear Physics ELI-NP, Bucharest-Magurele

Keywords: proton radiation, high-power lasers, hyperpolarised magnetic resonance, free radicals

Development of new radiotherapeutic strategies in cancer is a major focus of research: treatment plans with improved efficacy and limited side-effects are sought. We highlight herein the recent advent of short-pulsed laser sources, which afford garnering high dose-rate beams of particles and photons to kill cancer cells while sparing normal tissues. Existent results show that the biological impact of high dose-rate beams differs from the impact of radiation generated using classical accelerators or sources. High-power lasers can deliver particles to cells in doses of up to a few Gy on time scales on the order of nanoseconds, and radiation delivery to tissues is under study. At ELI-NP, we anticipate that protons of up to 200 MeV will be accelerated by the laser.

The interaction of laser-accelerated particles with cells is different from the traditional, prolonged radiation exposure, and perturbs cellular function via different molecular pathways. This happens mainly because the time scales on which reactive molecules, notably free radicals, are generated and persist differ between the two regimes. Sensitive biomolecular markers have to be identified to follow radiation effects, ideally using endogenous molecules that can also be imaged *in vivo*, in the clinical setting.

This presentation introduces the advantages of using endogenous biomarkers enriched with stable magnetic nuclear isotopes that can be used to follow radiation effects. Such biomarkers are available since the introduction of Dynamic Nuclear Polarisation-enhanced Magnetic Resonance (DNP-MR) for biomolecular observations *in vivo*, fifteen years ago. The sensitivity of magnetic resonance *in cells* and *in vivo* has been significantly increased, and the personalised follow-up of radiotherapy plans becomes possible in real time via metabolic imaging.

Acknowledgement: The authors acknowledge support from the core project of the Romanian Ministry of Research, projects PN 18 09 01 05 / 2018, UEFISCDI project number PN-III-P4-ID-PCE-2016-0887, and PN 18 21 01 02 / 2018. Work has been supported by the Extreme Light Infrastructure Nuclear Physics (ELI-NP) Phase II, a project co-financed by the Romanian Government and the European Union through the European Regional Development Fund and the Competitiveness Operational Programme (1/07.07.2016, ID 1334).

PLENARY LECTURE 1

BENCH TO BEDSIDE RESEARCH ON CRITICAL ILLNESS MYOPATHY AND VENTILATOR INDUCED DIAPHRAGM MUSCLE DYSFUNCTION

Lars Larsson
Department of Physiology & Pharmacology, Department of Clinical Neuroscience, Clinical Neurophysiology, Karolinska Institute, Stockholm, Sweden

Keywords: myosin, intensive care, mechanical ventilation

Significant improvements in modern critical care related to technological advances, improved understanding of the pathogenesis of disease process, new therapies and the removal of inefficient/harmful interventions have led to improved survival from critical illness. However, the improved survival is associated with an increased number of patients with complications related to modern critical care. Severe muscle wasting and impaired muscle function are frequently observed in immobilized and mechanically ventilated intensive care unit (ICU) patients. Approximately 30% of mechanically ventilated and immobilized ICU patients for durations of 5 days and longer develop generalized muscle paralysis of all limb and trunk muscles, a condition known as critical illness myopathy (CIM). Mechanical ventilation is a lifesaving treatment in critically ill ICU patients; however, the being on a ventilator creates dependence, and the weaning process occupies as much as 40% of the total time of mechanical ventilation. Furthermore, 20-30% of patients require prolonged intensive care due to VIDD, resulting in poorer outcomes, and greatly increased costs for health care providers. CIM and VIDD in ICU patients may be related to the primary disease, but there is heterogeneity of underlying disease and pharmacological treatment among patients exhibiting similar outcomes. Thus, it is highly likely that a common component of ICU treatment *per se* is directly involved in the progressive impairment of muscle function and muscle wasting during long-term ICU treatment. The specific mechanisms underlying the muscle wasting and impaired muscle function associated with the ICU intervention are poorly understood in the clinical setting. There is, accordingly, compelling need for experimental animal models closely mimicking the ICU condition, including long-term exposure to mechanical ventilation and immobilization. In this project, the muscle dysfunction, which by far exceeds the loss in muscle mass in limb and respiratory muscles in patients with CIM and VIDD have been investigated in detail at the cellular and molecular levels in rodent and porcine experimental ICU models, allowing detailed studies in immobilized and mechanically ventilated animals for long durations. Results demonstrate that the motor protein myosin is highly involved in the pathogenesis of both CIM and VIDD, but mechanisms are different. In CIM there is a preferential loss of myosin due to transcriptional down-regulation and enhanced degradation while post-translational modifications of myosin play a significant role for the diaphragm muscle dysfunction in VIDD. Specific intervention strategies targeting the mechanisms underlying CIM and VIDD will be presented and the translation of these interventions to the clinic.

SESSION 2

EXPERIMENTAL AND CLINICAL NEUROSCIENCES

Chairs: Alexandru Babeș, Bogdan Ovidiu Popescu

ANIMAL MODELS OF NEUROPSYCHIATRIC DISORDERS FOCUSING ON SOME OXIDATIVE STRESS MARKERS

Alin Ciobica

Alexandru Ioan Cuza University, Iași

Keywords: schizophrenia, anxiety, depression, autism, irritable bowel syndrome

Although neuropsychiatric disorders are specifically human pathologies, with complex symptomatology which cannot be perfectly replicated on animals, the assessment of these pathological conditions in animals can still be done in several ways, including the manipulation of the main neurotransmitters and brain areas or the usage of various behavioral tests. We will describe here some of our animal models for Alzheimer's and Parkinson's disease, schizophrenia, anxiety, depression, autism and irritable bowel syndrome, as well as the relevance of the oxidative stress in this context.

Acknowledgement: CA is supported by an UEFISCDI TE grant no. PN-III-P1-1.1-TE-2016-1210 called "Complex study regarding the interactions between oxidative stress, inflammation and neurological manifestations in the pathophysiology of Irritable bowel syndrome (animal models and human patients)".

THE ROLE OF TRANSIENT RECEPTOR POTENTIAL CHANNELS IN THE PATHOPHYSIOLOGY OF GENETIC DISEASES ASSOCIATED WITH PAINFUL PHOTOSENSITIVITY

Alexandru Babeș

University of Bucharest

Keywords: pain, TRP channel, photosensitivity

Pain can be produced by mechanical, thermal and chemical stimuli, but we generally do not associate it with light exposure. However, there are certain rare genetic diseases in humans in which exposure for even a couple of minutes to sunlight or even artificial light produces excruciating pain, itch and erythema. Cutaneous porphyrias, for example, are a family of hereditary diseases caused by deficits in enzymes involved in the heme biosynthetic pathway. Downregulation of certain enzymes in the late steps of the heme pathway lead to accumulation of powerful endogenous photosensitizers, such as protoporphyrin IX. In another genetic disease, deficits in the 7-dehydrocholesterol reductase gene lead to accumulation of 7-dehydrocholesterol in the plasma and tissues of patients with the Smith-Lemli-Opitz (SLO) syndrome. These patients also complain of painful and itchy light hypersensitivity. Our work was aimed at unraveling the molecular mechanisms behind these symptoms and we demonstrate that the Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) channels play major roles in nociceptor activation by light, under pathological conditions associated with porphyrias and SLO syndrome. These findings may lead to improved therapeutic strategies to alleviate the suffering of these patients.

Acknowledgement: Financial support was from CNCS/UEFISCDI grant PN-III-P4-ID-PCE-2016-0475.

ENDOVASCULAR INTERVENTIONAL RADIOLOGY TECHNIQUES IN ACUTE ISCHEMIC STROKE - A SINGLE EMERGENCY CENTER EXPERIENCE

Florina Anca Antochi, Raluca Stefania Badea, Bogdan Dorobat

Emergency University Hospital, Bucharest

Keywords: endovascular stroke, acute interventional radiology

Introduction: Cerebral ischemic infarction is the second cause of death and third cause of disability worldwide. The key for improving the neurological outcome and for reducing the mortality of patients diagnosed with acute ischemic stroke is early treatment, capable of restoring brain blood flow. At the moment, the main treatment for patients presenting to the hospital within 4.5 hours of stroke onset is thrombolysis using intravenous alteplase and/or mechanical thrombectomy.

Endovascular therapy (EVT) for acute ischemic stroke has recently been adopted into routine clinical practice. Patients with symptoms onset at less than 6 hours prior are eligible for treatment with mechanical thrombectomy if they had minimal disability before the stroke, if they have a National Institutes of Health stroke scale score of ≥6, an ASPECT score ≥6 or if there are objective arguments for occlusion of a large cerebral artery (internal carotid artery or M1 segment of middle cerebral artery). EVT can also be performed within 6 to 24 hours, but only in selected patients. When selecting the patients for EVT, clinicians must respect the contraindication to this procedure. The mTICI (modified thrombolysis in cerebral infarction) scale is the primary way to assess angiographic reperfusion after mechanical thrombectomy and thrombaspiration. The goal is to achieve an mTICI score of 2b/3 (at least 50% recanalization of the previous occluded artery or complete recanalization), in less than 60 minutes from arterial puncture.

Objectives and methods: The aim of this presentation is to offer an overview on the endovascular methods and techniques used to treat patients with acute ischemic stroke, information emphasized by several case presentations.

Conclusion: When selecting the appropriate patients and when performed by experienced interventional specialists, EVT is the solution for successful revascularization and a better outcome in patients with ischemic stroke.

PHOSPHORYLATION OF AMYLOID PRECURSOR PROTEIN DEPENDS ON CAVEOLIN 1 EXPRESSION IN THE MURINE BRAIN

Bianca Pătrănoiu[1], Ana-Maria Enciu[2]

[1]*Carol Davila University of Medicine and Pharmacy, Bucharest*
[2]*Victor Babeș National Institute of Pathology, Bucharest*

Keywords: Alzheimer's disease, amyloid precursor protein, APP phosphorylation

Introduction: β-amyloid precursor protein (bAPP) is a transmembrane protein which generates the amyloid-β peptide (Ab) through proteolytic cleavage. This peptide aggregates in the brain as senile plaques, a key pathologic feature of Alzheimer's disease (AD).
Aim: In the present study we investigated whether caveolin-1, a membrane scaffold protein, could affect phosphorylation and proteolytic cleavage of APP.
Material and methods: Laboratory animals were dived in two groups: control group (n=13) and Caveolin1-knock-out (cav-1 KO) group (n=14). Mice brains were harvested and lysed for a standard Western Blot analysis. We used antibodies directed against β-site APP cleaving enzyme 1 (BACE-1), A Disintegrin and Metalloproteinase 17 (ADAM-17), APP-P-E668 and APP-P-T743. All of the samples subsequently underwent a multiplex assay for analysis of kinase activity.
Results: Phosphorylation of APP at T743 was intensively expressed in the KO group, whereas APP-P-E668 was predominantly detected in the control group. Furthermore, the control group expressed less Ab peptide than the KO group, although BACE activity was not different between groups. Instead ADAM expression, as detected by WB, was increased in the control group.
Conclusions: Our findings suggest that APP metabolization relies on Cav-1, possibly due to its ability to form cholesterol-dependent microdomains. Moreover, Cav-1, a known inhibitor of various signaling pathways, modifies the APP phosphorylation profile, with possible impact on its gene-transduction role.

MICROBIOTA, ENTERIC NERVOUS SYSTEM AND NEURODEGENERATIVE DISEASES

Bogdan Ovidiu Popescu

Victor Babeş National Institute of Pathology, Bucharest

Keywords: neurodegeneration, microbiota, aggregated proteins

Human beings are populated by trillions of bacteria, used to live in a mutually advantageous symbiosis with our bodies. However, studies from recent years suggest that this microbiota could influence triggers for different human pathologies, including neurodegeneration. Neurodegenerative diseases have still no elucidated etiology and no disease-modifying treatment. Brain and gut are connected through a massive enteric nervous system, which might accumulate aggregated proteins and transport them to the brain. In the present work I will try to evaluate the most recent data regarding neurodegenerative disorders and microbiota.

SESSION 3A

NEW INSIGHTS IN PATHOLOGY

Chair: Emil Iancu Pleșea

DIFFICULTIES OF STAGING pT1 COLONIC ADENOCARCINOMA ARISING ON ADENOMA

Cristiana Popp[1], Mirela Cioplea[1], Liana Sticlaru[1], Gianina Micu[1], Sabina Zurac[1,2], Florica Staniceanu[2], Patricia Stinga[1], Theodor Voiosu[1,2], Bogdan Mateescu[1,2], Luciana Nichita[1,2]

[1]Colentina University Hospital, Bucharest
[2]Carol Davila University of Medicine and Pharmacy, Bucharest

Keywords: Haggit classification, Kikuchi staging, epithelial misplacement

Background: Staging of early invasive colonic adenocarcinoma arising on adenoma is a challenge for the pathologist, and has great impact on the therapeutical approach and patient outcome. Besides TNM staging, the Haggit classification for pedunculated malignant colorectal polyps (dividing pT1 in four levels of invasion) and the Kikuchi classification for sessile malignant colorectal polyps (dividing pT1 in three levels of invasion) are proving useful in practice.

Materials and method: We present 36 consecutive cases of pT1 adenocarcinoma arising on adenomas, with various degrees of intraepithelial neoplasia, emphasizing the difficulties of staging when using the above classifications. Problems associated with differential diagnosis in cases of epithelial misplacement are also included. All cases are endoscopically resected polypoid lesions, 24 pedunculated and 12 sessile, and were diagnosed by the Pathology Department of Colentina University Hospital in the last two years.

Results: Most of pedunculated cases were classified as Haggit level 1 (14), only one lesion being Haggit level 4. From the sessile lesions, 6 were Kikichi sm1, 4 were sm2 and 2 sm3. Epithelial misplacement diagnostic challenge in 15 lesions.

Conclusion: Use of the Haggit and Kikuchi staging classifications might be difficult in common practice, but it is very useful for personalizing therapeutical management of the patient, avoiding unnecessary surgical procedures and modifying the short and long term outcome for patients.

CLINICAL, ENDOSCOPIC AND PATHOLOGICAL PARTICULARITIES OF 25 CASES, INCLUDING IMMUNOHISTOCHEMICAL ASPECTS OF INFLAMMATORY FIBROID POLYPS, RARE MESENCHYMAL TUMORS OF THE DIGESTIVE TRACT

Andrei-Mihai Borcan[1], Laura Ioana Florea[1], Alexandra Rosulescu[2], Simona Enache[2], Florina Vasilescu[2], Emma Marcelle Burke[2], Florin Andrei[2], Emil Plesea[2], Valentin Enache[2], Vlad Herlea[3], Gabriel Becheanu[1]

[1]*Carol Davila University of Medicine and Pharmacy, Bucharest*
[2]*Victor Babeș National Institute of Pathology, Bucharest*
[3]*Fundeni Clinical Institute, Department of Pathology, Bucharest*

Keywords: inflammatory fibroid polyp, mesenchymal tumor, digestive tract

Inflammatory fibroid polyps (IFP) are clinically benign, rare lesions of the mesenchymal tissues with the highest incidence in the 6th to 7th decades of life, in the digestive tract. They are mostly met in specimens collected from the stomach, most common in the antrum.

For this research, 25 specimens were confirmed in the Victor Babeș National Institute of Pathology in Bucharest, collected from patients aged between 30 and 79. The specimens were collected by methods such as polypectomy (4 specimens), segmental or total enterectomy (5 specimens), gastric resection (1 specimen), and colectomy (2 specimens).

Our study indicates an almost equal incidence between males and females (13:12), an overwhelmingly high incidence in the 6th decade of life (30-40 - 4 IFP, 41-50 - 3 polyps, 51-60 - 2 IFP, 61-70 - 11 IFP, 71-80 - 5 IFP) and also a high incidence in the upper digestive tract, especially in the antrum (stomach - 13 IFP, small intestine - 9 IFP, large intestine - 3 IFP). From an immunohistochemical point of view, the tumors were selectively positive for CD34 where angiogenesis was present, negative for CD117, but positive in the peritumoral mast cells and eosinophils in two cases and diffusely positive for vimentin (25/25). As expected, numerous eosinophils were identified with certainty in over half of the specimens (16/25) and atypia and mitoses were scarce.

In conclusion, the research assesses the anatomical, histological and immunohistochemical variability of inflammatory fibroid polyps among the 25 cases presented. IFP is a benign tumor with a characteristic phenotype and differential diagnosis with other mesenchymal tumors, especially GIST, is essential.

SUBCELLULAR LOCALIZATION OF CALRETICULIN MUTANTS IN MYELOPROLIFERATIVE NEOPLASMS

Tudor Emanuel Fertig[1], Daciana Marta[1], Silvia-Diana Prelipcean[1], Anita Roy[2], Ștefan N. Constantinescu[2], Mihaela Gherghiceanu[1]

[1]*Victor Babeș National Institute of Pathology, Bucharest*
[2]*Universite Catholique de Louvain and de Duve Institute, Brussel, Belgium*

Keywords: calreticulin, immuno-electron microscopy, electron tomography, myeloproliferative neoplasms

Introduction: Polycithemia vera, essential thrombocythemia (ET) and primary myelofibrosis (PMF) are Philadelphia chromosome-negative myeloproliferative neoplasms, induced by mutations in the hematopoiesis signalling pathways. Some of the mutations identified in patients with ET and PMF are of the calreticulin gene (CALR). Calreticulin (CRT) is a Ca^{2+}-binding endoplasmic reticulum (ER) chaperone protein, with a wide array of functions both inside and outside the cell. There are two main mutations of CALR, type I being a 52-base pair deletion (del52) and type II a 5-base pair insertion, both of them leading to the loss of a KDEL C-terminal aminoacid sequence in the protein. This structural motif serves as the retention signal for CRT in the ER. Loss of its anchoring sequence will lead to CRT being no longer held in the ER, thus allowing it to bind and activate the thrombopoietin receptor and induce JAK2-STAT5 signalling. However, the exact subcellular distribution of the mutants and their routes of trafficking is currently unknown. Here we show confocal microscopy and immunoelectron microscopy (IEM) data on the comparative localization of wild-type and the del52 mutant CRT in a Ba/F3 cell model, suggestive for potential routes of intracellular traffic.

Materials and methods: For electron microscopy and electron tomography, Ba/F3 cells were fixed 2% paraformaldehyde and immunolabeled for CRT using 10nm gold-conjugated secondary antibodies. This was followed by Epon-embedding and sectioning according to standard protocols. Specimens were viewed and imaged using a FEI Talos F200C TEM, equipped with a 4x4k Ceta camera. Tomography single axis tilt series were recorded at 1º intervals, between -60º and +60º, then aligned and segmented using freely available software (IMOD/eTomo).

Results and conclusion: Confocal microscopy revealed a different subcellular distribution for the wild-type protein, when compared to the del52 mutant. This was confirmed by IEM which showed that CRT mutants localize predominantly in the ER, Golgi, endo/exocytic vesicles and the nucleus. By contrast wild-type CRT localizes mostly at the plasma membrane, in accordance with the physiological activity of endogenous CRT. Revealing the distribution of mutant CRTs with nanometer precision paves the way for future co-localization studies and has the potential to facilitate targeted therapies in myeloproliferative neoplasms.

SESSION 3B

NEPHROPATHOLOGY

Chairs: Gener Ismail, Mihaela Gherghiceanu

THE ROLE OF KIDNEY BIOPSY IN THE MANAGEMENT OF PATIENTS WITH IGA NEPHROPATHY

Bogdan Obrisca[1], Roxana Jurubita[1], Andreea Andronesi[1], Bogdan Sorohan[1], Alexandru Procop[1], Vlad Herlea[1], Mihaela Gherghiceanu[2], Gener Ismail[1]

[1]*Fundeni Clinical Institute, Bucharest*
[2]*Victor Babeș National Institute of Pathology, Bucharest*

Keywords: IgA nephropathy, Oxford Classification, kidney biopsy

Introduction: IgA nephropathy (IgAN) is the most common glomerulonephritis worldwide and an important cause of chronic kidney disease. It usually follows a slowly progressive course with a 10-year renal survival between 60-90%. The short follow-up of most clinical trials makes difficult the assessment of progression of this disease only from a clinical standpoint and additional, more sensitive, markers of disease progression are needed. The possibility to predict the progression of IgAN after a short follow-up period has improved since the Oxford MEST-C score was developed. The MEST-C score comprises five histological parameters that are independently associated with renal outcome: mesangial hypercellularity (M), endocapillary hypercellularity (E), segmental glomerulosclerosis (S), tubular atrophy/interstitial fibrosis (T) and crescents (C). We sought to validate the Oxford Classification of IgA Nephropathy in our cohort of patients.

Material and methods: All patients with biopsy-proven IgA nephropathy followed at our department, between 1999 and 2017 were considered for study inclusion. Univariate and multivariate analysis were performed to identify clinical and histological predictors of renal outcome (doubling of serum creatinine and/or end-stage renal disease).

Result: This retrospective analysis included 123 patients followed for a median 23 months (IQR: 12 – 61 months). The mean age, estimated GFR and 24-hour proteinuria were 41± 12 years, 52 ± 30 ml/min/1.73m2 and 2.7 ± 2.7 g/day, respectively. The percentage of patients with M1, E1, S1, T1 and T2 were 81%, 27%, 57%, 25% and 18%, respectively. Additionally, 15% of patients showed crescents in less than 25% of examined glomeruli (C1), while 6,5% had more than 25% of glomeruli with crescents (C2). In multivariate Cox proportional hazard model, after adjusting for clinical variables, only T score was significantly associated with worse outcome [T1 (HR of 3.5; 95% CI 0.97 to 12.8, p=0.05) and T2 (HR 3.5; 95% CI 1.16 to 10.7, p=0.02)]. However, in models including only histological variables, when considering only those patients that received immunosuppressive therapy, T and C2 scores were independent predictors of worse outcome, while E score was associated with better outcome, thus suggesting that E lesions are responsive to IS therapy.

Conclusion: In addition to clinical variables, histological data provides important information regarding prognosis of IgA nephropathy patients and may offer the possibility to identify patients that could benefit from immunosuppressive therapy.

IMPORTANCE OF KIDNEY BIOPSY IN MONOCLONAL GAMMOPATHIES OF RENAL SIGNIFICANCE

Andreea Andronesi[1,2], Mihaela Gherghiceanu[3], B. Obrișcă[1,2], B. Sorohan[1,2], Cristina Cristache[1,2], Gener Ismail[1,2]

[1]Nephrology Department, Fundeni Clinical Institute; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Victor Babes National Institute of Pathology, Bucharest

Keywords: paraprotein, amyloidosis, light chains, immunoglobulin

Monoclonal gammopathies of undetermined significance (MGUS) are one of the most frequent premalignant condition in elderly patients from developed countries, with an increasing prevalence because of the aging population. The pathogenesis is based on the presence of a nonmalignant or premalignant plasma cell, B-cell or lymphoplasmacytic clone which secretes monoclonal proteins. Nevertheless, the risk of malignant transformation is usually small on long term, but a significant percent of patients with MGUS has the risk of developing progressive kidney injury up to advanced stages of chronic kidney disease, condition known under the name of monoclonal gammopathy of renal significance (MGRS). MGRS have also an important risk of recurrence on kidney graft unless patients do not receive proper treatment prior to transplantation. Although monoclonal proteins are usually secreted in very small quantities, since the secretion is continuous, on long term they are depositing in kidneys' structures and induce different types of histological lesions because of their intrinsic nephrotoxic properties. Hematological workup is important in identifying the responsible clone so the chemotherapy will address the pathological clone. Unfortunately, up to 30% of patients have no evidence of serum and urinary M-protein and undetectable clone on bone marrow and nodes because of the usually low or very low serum level of the paraproteins. For this reason, histological evaluation of kidney is essential for proper diagnosis of MGRS, with immunofluorescence being mandatory to prove and characterize the monoclonal deposits. Kidney biopsy also demonstrates the direct relationship between the presence of circulating monoclonal proteins and histological lesions. Special techniques, like immunofluorescence after pronase digestion, or laser microdissection with mass spectrometry may be needed in difficult cases in which masked deposits are not visible in ordinary immunofluorescence. Chemotherapy ameliorates patients' renal and general outcome and reduces the risk of recurrence after renal transplantation, but poses a significant systemic toxicity and may be unnecessary in patients with certain histological subtypes of MGRS, with low risk of progression towards advanced stages of chronic kidney disease. For this reason, kidney biopsy is also important to characterize the histological type of MGRS, the severity of kidney involvement and to evaluate the patient's prognosis in order to choose the proper treatment. The aim of the presentation is to present the case of a female patient with MGRS in which diagnosis was possible only after kidney biopsy was performed and to review the current knowledge regarding the role of pathology exam in evaluation of patients with MGRS.

A CASE OF LATE ONSET ANTIBODY MEDIATED REJECTION AND IgA NEPHROPATHY RECURRENCE

Bogdan Sorohan[1], Dorina Tacu[2], Mihaela Gherghiceanu[1,3], Gener Ismail[2,4]

[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Department of Uronephrology and Kidney Transplant, Fundeni Clinical Institute, Bucharest; [3]Carol Davila University of Medicine and Pharmacy, Bucharest; [4]Department of Nephrology, Fundeni Clinical Institute, Bucharest

Keywords: ABMR, IgA recurrence, DSA, kidney transplant

Introduction: Antibody-mediated rejection (ABMR) is an important post-transplant complication that plays a definite role in kidney allograft function and survival. ABMR could be classified as acute or chronic and according to a Banff report, acute ABMR could be divided as phenotype 1 (appears early after transplantation, in presensitized patients) and phenotype 2 (appears in the late transplant period, associated with de novo donor specific antibodies). Clinically evident recurrent IgA nephropathy is often quoted as 30%, more frequently at 5 years post-transplantation and histological recurrence occurs earlier. Patients with IgA nephropathy have excellent transplantation outcomes compared with other glomerulonephritis types or other diagnoses.

Case presentation: We reported the case of a 49-year-old male patient who was admitted after fifteen years from kidney transplantation for deteriorating graft function. He had received a kidney graft from his brother in 2002, but the primary cause for end stage renal disease was unknown. Between 2002 and 2017 renal function was stable, with a creatinine level around 1 mg/dl and proteinuria <0.3g/24h. No rejection or infections were reported in this period. At the current presentation, creatinine had increased to 2.08 mg/dl and proteinuria to 1.2 g/24h. Class I and II donor specific antibodies (DSA) were detected (HLA-A1, MFI 3600; HLA-DQ8, MFI 25000). A kidney graft biopsy was performed that showed signs of acute ABMR (peritubular capilaritis with linear moderate positive C4d2 staining, microvascular inflammation [g≥1], interstitial inflammation [i1]) and IgA nephropathy (mesangial proliferation, granular mesangial depositions of IgA and C3c on immunofluorescence and paramesangial deposits in electron microscopy). Treatment decision consisted of 5 applications of plasma exchange and intravenous immunoglobulin, followed by Rituximab (375mg/ m2). After the treatment, graft function was improved, proteinuria and DSA titer decreased.

Discussion: This is a rare case of late onset acute ABMR that appeared 15 years after kidney transplantation, with a good treatment response. Coexisting IgA nephropathy is suggestive for recurrence of the primary disease on kidney graft.

Conclusion: We described the case of a 49-year-old male with coexisting late onset ABMR and IgA nephropathy recurrence.

GENETIC TESTING FOR PEDIATRIC KIDNEY DISEASE – INTERNATIONAL COLLABORATION AND ETHICS

Adrian Catalin Lungu, Cristina Stoica

Pediatric Nephrology Department, Fundeni Clinical Institute, Bucharest

Keywords: genetic testing, kidney transplant

Charles Darwin's work on the origin of species in 1859 elicited a tremendous shock by presenting a new perspective on integrating life. This change in understanding from obscure and dogmatic ideas intensified the debate between new knowledge and ancient traditions (Lucas 1979).

In the 20th century, some social applications of the theory of evolution were negative (Stoddard 1920), whereas others were positive (Moreno 1934). The first one used genetics arguments to justify racism (Stoddard 1920), while the second integrated genetics with social perspectives (Moreno 1934).

The concept of critical knowledge, proposed by Van Renselaer Potter, refers to knowledge that accumulates more rapidly than the wisdom required to use it (Potter 1971).

Recognizing research in genetics as a source of dangerous knowledge does not entail avoiding or prescribing it. On the contrary, realizing the potential for perilous knowledge requires the need for extended discussion on the various aspects of research, including ethical, legal, and social issues (ELSI).

One of the major contributions of modernity was to put forth a reflection on what is universal and individual. Much more than fearing genetic advances, or questioning the need for ethical reflection on research to implement these advances in society, it is essential to rescue and update ethical aspects.

KIDNEY BIOPSY PROCESSING FOR IMMUNOFLUORESCENCE AND ELECTRON MICROSCOPY

Mihaela Gherghiceanu[1,2], Gener Ismail[3,1]

[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Department of Uronephrology and Kidney Transplant, Fundeni Clinical Institute, Bucharest

Keywords: kidney biopsy, direct immunofluorescence, electron microscopy

Percutaneous renal biopsy is an irreplaceable tool in the clinical practice of nephrologists to determine diagnosis, prognosis and treatment of several kidney diseases. This procedure is considered safe if it is performed in well-trained centers. Main indications are acute glomerulonephritis and nephrotic syndrome. Since bleeding is the major primary complication, careful evaluation of risks and benefits must be considered. The number of glomeruli is the main determinant of the biopsy adequacy. For accurate histological analysis the core biopsy should be processed differently for immunofluorescence, light microscopy and electron microscopy. These 3 techniques require different and mutually exclusive fixation techniques. The perfect core biopsy, perfectly divided for the 3 analyses is difficult and required technical steps should be known for correct sampling. This presentation focus on the difficulties in processing kidney biopsy samples for immunofluorescence and electron microscopy.

FRIDAY, NOVEMBER 23

SESSION 4

KNOWLEDGE TRANSFER ON NATURAL PRODUCTS TOWARDS INDUSTRY FOR HUMAN HEALTH BENEFITS – PROJECT G –

Chair: Cristiana Tănase

RED THINGS FROM RED SAGE FOR A GREEN PHARMACY - POTENTIAL OF TANSHINONES AND RELATED DITERPENOIDS AS NATURAL DRUGS OR DRUG LEADS

Adam Matkowski
Department of Pharmaceutical Biology, Medical University of Wroclaw, Poland

SAFETY AND BENEFIT ASSESSMENT OF FOOD SUPPLEMENTS IN THE EUROPEAN UNION

Valeriu Curtui
Nutrition Unit, European Food Safety Authority, Parma, Italy

FOOD SUPPLEMENTS MADE IN ROMANIA WITH MULTIPLE BENEFITS FOR HUMAN HEALTH

Elvira Gille
NIRDBS/Stejarul Biological Research Centre, Piatra Neamt
Faculty of Chemistry, Alexandru Ioan Cuza University of Iasi

COP – G PROJECT OVERVIEW

Cristiana Tănase
Victor Babeș National Institute of Pathology, Bucharest

PLENARY LECTURE 2

LIPID-DEPENDENT MECHANISMS IN CELL-CELL SIGNALING

Adrian Salic
Department of Cell Biology, Harvard Medical School, Boston MA, USA

Communication between cells is fundamental for the development and physiology of multicellular organisms. In animals, this process is carried out by a small number of signaling pathways, each consisting of a chain of biochemical interactions through which extracellular signals regulate the behavior of responding cells. One such communication system is the Hedgehog pathway, which is essential for embryonic development and for adult stem cell maintenance. Insufficient Hedgehog signaling leads to many birth defects, while uncontrolled activation is implicated in many human cancers. While the components of the Hedgehog pathway were identified by genetics more than a decade ago, our understanding of the molecular mechanisms involved in signaling is far from complete.

In the absence of signaling, the tumor suppressor membrane protein Patched suppresses the Hedgehog pathway, by inhibiting the seven transmembrane domain oncoprotein Smoothened. The pathway is triggered by the secreted Hedgehog ligand, which binds and inhibits Patched, leading to Smoothened activation. Active Smoothened then sets in motion the downstream cytoplasmic steps of the Hedgehog signal transduction cascade. I will present findings from our lab on the essential role that lipids play in the following steps of Hedgehog signaling:

1) Hedgehog ligand secretion. The Hedgehog ligand is covalently modified with two lipids, palmitate and cholesterol. Lipidated Hedgehog is very insoluble, yet it signals to distant cells. I will discuss the mechanism by which Hedgehog is released from producing cells in soluble form, via the concerted action of the membrane protein Dispatched and the secreted protein Scube.

2) Patched inhibition. The palmitate modification of the Hedgehog ligand is critical for activating the Hedgehog pathway. I will discuss the mechanism by which palmitate inhibits Patched, and its implications for disease.

3) Smoothened activation. When the Hedgehog pathway is stimulated, cholesterol binds to the extracellular domain of Smoothened, causing the protein to adopt an active conformation, which triggers downstream signaling. I will present findings from our lab, based primarily on X-ray crystallography, concerning the mechanism by which cholesterol controls Smoothened. I will also discuss how oncogenic mutations lead to Smoothened activation, and how various small molecules, including drugs approved for clinical use, inhibit Smoothened.

Our results elucidate important lipid-dependent mechanisms in Hedgehog signal transduction, and suggest strategies for blocking oncogenic Hedgehog signaling

SESSION 5

OMICS TECHNOLOGIES FOR PRECISION MEDICINE

Chairs: Cristiana Tănase, Aurora Arghir

THE INVOLVEMENT OF E6 AND E7 HPV16 ONCOGENES IN CHROMATIN REMODELLING THROUGH COMPONENTS OF NuRD COMPLEX

Anca Botezatu, Iulia Iancu, Adriana Plesa, Alina Fudulu, Adrian Albulescu, Marinela Bostan, Mirela Mihaila, Gabriela Anton

Stefan S. Nicolau Institute of Virology, Bucharest

Keywords: cervical cancer, epigenetics, chromatin remodelling complex, ChIP-Seq

Cervical cancer is the third most commonly diagnosed type of cancer and one of the most frequently occurring malignant tumours in women worldwide. Human papilloma virus (HPV) is considered the etiologic agent of cervical neoplasia. The study of HPV carcinogenic mechanisms continues to attract interest, especially regarding the molecular biology of cervical cancer, in order to find the most appropriate cellular or viral targets for this disease. The control of gene expression is complex and involves epigenetic changes (DNA methylation, histone modification, miRNA activity). The nucleosome remodelling and deacetylation complex (NuRD) is a group of associated proteins with ATP-dependent chromatin remodelling and histone deacetylase activities. MBD2 and MBD3 proteins from NuRD complex exhibit methyl-CpG-binding domains (MBD), which mediate an interaction with methylated DNA. Given the increasing data regarding the MBD2 and MBD3 functional differences and similarities, the current study aims to assess the influence of viral oncogenes on the MBDs overall binding pattern to CpG islands. To this purpose we developed an HPV 16 E6/E7 oncogene silencing experimental model in CaSki cell culture. We performed ChIP-Seq (Chromatin Immunoprecipitation Sequencing) for MBD2, MBD3 genome wide DNA binding pattern (e.g. promoters, gene control region, transcriptional enhancers etc.) in untreated and siRNA treated CaSki cell cultures and the results were analysed using Base Space Illumina apps.

In most cases, NuRD complex (MBD2/3) is localized at the level of intron or intergenic regions. The pattern is different depending on the exposure time and the silenced HPV oncogene (E6 or E7). When the two oncogenes are targeted, the binding regions are especially at the promoters, exon level or the transcription initiation site. To note the case of chromosome 10 in which both transcriptional initiation and exon sites are targeted, as in the case of chromosome 12 where promoters are targeted in both cases (E6 orE6/E7 gene silencing).

Epigenetic gene control is a complex phenomenon that is guided by both internal, cellular and external factors as well as viral infections. As shown, both E6 and E7 viral oncogenes act synergistically on the gene transcription pattern by interacting with the NuRD complex and the MBD2/3 proteins, respectively. This mechanism can be modulated by targeting the expression of viral proteins and may represent a possible direction for a potential treatment.

Acknowledgement: PN-II-RU-TE- 2014-4-2502

COMBINED APPROACH OF NEXT GENERATION SEQUENCING AND MICROARRAY TECHNOLOGIES FOR CHARACTERIZATION OF MOLECULAR SIGNATURES IN ACUTE MYELOID LEUKEMIA

Aurora Arghir[1,2], Sorina Mihaela Papuc[1], Alina Erbescu[1], Raluca Colesniuc[1], Diana Cisleanu[2,3], Dan Sebastian Soare[2,3], Viola Maria Popov[4], Daniela Georgescu[4], Nicoleta Berbec[2,5], Ana-Maria Vlădăreanu[2,3], Horia Bumbea[2,3]

[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Emergency University Clinical Hospital, Bucharest; [4]Colentina Clinical Hospital, Bucharest; [5]Coltea Clinical Hospital, Bucharest

Keywords: genomic technologies, mutations, diagnosis, prognosis

Background. Acute myeloid leukemia (AML), the most common type of acute leukemia in adults, is a heterogeneous disorder characterized by excessive proliferation and accumulation of myeloid leukemic blasts. Tools currently used for AML diagnosis include cytomorphology/cytochemistry, immunophenotyping by flow cytometry, cytogenetic and molecular genetic investigations. Emerging techniques such as DNA microarray and next generation sequencing (NGS) are being implemented in clinical laboratories for diagnosis and measurable residual disease monitoring, proving informative as well as sensitive and robust.

Aim. We report on the results of genomic investigations in a group of 22 AML patients.

Material and methods. Bone marrow samples were analyzed at diagnosis by classical and molecular cytogenetics. PCR followed by fragment analysis on a microfluidic electropherogram system (Agilent Bioanalyzer 2100) and capillary electrophoresis (ABI 3500 Genetic Analyzer) were used for FLT3 gene internal tandem duplication testing. CGH+SNP microarray 4x180k cancer design platforms (Agilent Technologies) were used for genomic profiling. Targeted NGS with a panel covering 19 genes was performed on an Ion PGM System (ThermoFisher Scientific).

Results and discussion. Twenty patients had intermediate risk cytogenetics (normal karyotype – 18 patients, other anomalies – 2 patients) and 2 patients had adverse risk cytogenetics (complex chromosomal anomalies and KMT2A gene rearrangement, each in one patient). Five patients had copy-neutral loss of heterozygosity (CN-LOH), larger than 10 Mb, on chromosomes 2q, 3q, 6p and 13q. The mutational screening revealed that NPM1 had the highest mutation prevalence (14 patients), followed by DNMT3A (10 patients), FLT3 (2 patients FLT3-D835, 6 patients FLT3-ITD), NRAS (6 patients), IDH1 (4 patients) and IDH2 (3 patients). Co-occurrences of mutations in different genes or different types of genetic lesions (karyotype changes and mutations, CN-LOH and mutations) were observed in our patient group, some associations being previously unreported.

Conclusions. Targeted NGS and CGH+SNP array testing proved successful for molecular profiling of AML in cytogenetically characterized patients. This combined approach highlights the utility of sequential use of various techniques, ranging from classical karyotyping to NGS. Unraveling the molecular architecture in AML patients leads to accurate diagnostic, improved prognosis and better therapeutic decisions.

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A MULTIDISCIPLINARY APPROACH OF PATIENTS WITH SCHIZOPHRENIA – FROM DEEP PHENOTYPING TO GENOTYPING AND BACK

Magdalena Budisteanu[1], Sorina Mihaela Papuc[1], Dan Riga[2], Sorin Riga[2], Aurora Arghir[1]

[1]*Victor Babeș National Institute of Pathology, Bucharest*
[2]*Prof Alexandru Obregia Clinical Hospital of Psychiatry, Bucharest*

Keywords: schizophrenia; synaptic dysfunction; molecular genetics

Schizophrenia (SZ) is a severe mental illness characterized by a wide range of defective cognitive function and a complex set of symptoms. SZ represents one of the major challenges for society, with large unmet patient needs and substantial health care costs. Various studies on the genetic risk architecture and aberrant brain functional connectome of SZ suggested a synaptic dysfunction in SZ pathophysiology, yet the precise mechanisms remain elusive.

We present the aim and scientific objectives of an ERA-NET NEURON (SYNSCHIZ) project, started in May 2018.

The main aims of SYNSCHIZ are to uncover the genetic architecture that increases the risk for synaptic dysfunction in SZ using large international cohorts; to integrate the identified genes into the development of novel computational models of synapse dysfunction; to experimentally validate these models in neuronal cell cultures derived from stem cells; and to link gene- and neuron-level discoveries to brain network level in SZ patients focusing on the SZ prodrome and in individuals at ultra-high risk for psychosis in a clinical setting.

Obregia Clinical Hospital of Psychiatry is partner in a consortium of 6 institutions coordinated by the Norwegian Centre for Mental Disorders Research in Oslo. The Romanian partner has already implemented a comprehensive diagnostic approach consisting of a standardized set of clinical tools for deep neuro-psychiatric phenotyping, high resolution bioimaging (3T brain MRI), and biochemical investigations. The genome-wide genotyping of the cohort and subsequent functional studies will be performed at other consortium partner sites.

SYNSCHIZ implements a strong translational component that will transfer scientific discoveries into clinical application. By identifying biomarkers useful for early detection and prognostic predictions, and by gaining a deep insight into SZ neurobiology, the project aims to improve the treatment and care of SZ patients.

FROM CILIA TO COMPLEX DISORDERS: THE CILIOPATHIES

Ina Ofelia Focsa[1], Laurentiu Camil Bohiltea[1], Mihaela Balgradean[1,2]

[1]*Carol Davila University of Medicine and Pharmacy, Bucharest*
[2]*Children Clinical Emergency Hospital Marie Curie, Bucharest*

Keywords: Mendelian disorders, oligogenic inheritance, signalling, Bardet Biedl

The motile cilium is an ancient organelle, observed ever since the early microscopy era, in protozoa. Two hundred years later a similar, immotile structure has been described in kidneys and the thyroid gland- primary cilia. The two type of cilia differ by structure, function and localization. Structural defects, dysfunction of cilia or an abnormal cilio-genesis gives rise to an extremely heterogeneous group of diseases: the ciliopathies.

Motile cilia line the respiratory tract, fallopian tube, the efferent ducts of the testis and brain ventricle so that their defects cause a range of diseases, whose clinical features include: bronchiectasis and chronic respiratory tract, ear and sinus infections, situs inversus and infertility.

Considering the presence of primary cilia in almost all tissue and organs, their impairment leads to a broad spectrum of clinical manifestation, ranging from single organ involvement to multi-systemic disorders. Whereas the retina, the kidneys, the CNS and skeletal system are the main organs and system affected in ciliopathies, involvement of liver, heart or endocrine system has also been described, complicating the clinical pictures. Therefore, extensive efforts have been done in order to establish a clinical diagnosis and to classify ciliopathies. A clinical algorithm was proposed by Beales and al. in 1999 starting with the presence of renal, retinal involvement and/or polydactyly, which is still applicable in the case of the 35 ciliopathies known to date.

The first gene shown to be linked with pathogenesis of ciliopathic disorders was identified in 2000 by two different research groups. Since then, as a result of the huge advance in sequencing technologies over 180 genes have been identified. Other 240 genes are proposed as candidate genes for ciliophatic disorders. The genetic mechanism is complex and, although they are considered Mendelian disorders, oligogenic inheritance, multiple allelism, epistatic interactions or genetic modifiers it seems to play an important role in phenotype variability.

Predicting an organ involvement and, consequently, a phenotype severity based on genetic defect, continue to be a great challenge. In the coming years, combined efforts in proteomics, cell biology and model organism will give us new insights that could reorganize the field of ciliopathies.

THE EPIGENOME TARGETED BY BIOACTIVE COMPONENTS FROM DIET: FACTS AND PERSPECTIVE IN CANCER PREVENTION

Sevinci Pop[1], Eleonora Codorean[1], Violeta Alexandra Ion[2], Elvira Gille[3], Cristiana Tanase[1,4]

[1]Victor Babeș National Institute of Pathology, Bucharest; [2]SC Cromatec Plus SRL, Bucharest; [3]NIRDBS Stejarul Biological Research Centre; [4]Titu Maiorescu University Faculty of Medicine, Bucharest

Keywords: epigenomics, nutraceuticals, anti-cancer properties, DNA methylation

The World Health Organization reports indicate that one-third of all cancer deaths are preventable by life-style changes, including a healthy diet based on natural products. Also, scientific studies demonstrated that there is a positive correlation between caloric or dietary restriction and the prevention of 13 types of cancer, such as those involving mammary gland, colon, liver, pancreas, skin, and pituitary gland. Therefore, the impact of bioactive components from diet on human health has started to be studied at the molecular level, in order to understand their chemopreventive action and anti-cancer properties.

Cancer initiation and development are driven by genetic mutations and epigenome dysregulation. The main epigenetic regulators of gene expression are: DNA methylation, histone modifications, chromatin structure remodelling and expression of non-coding RNA (microRNAs and lncRNAs). All epigenetic marks at the chromatin level and the proteins involved in epigenetic mechanisms represent the epigenome. Different external environmental factors (including diet) can lead to altered gene function and malignant cellular transformation through disruption of the normal epigenome pattern. Accumulating evidence showed that bioactive components from nutrients have the ability to modulate and reverse epigenome alterations which occurred in early malignancy stages. Many of these bioactive nutrients have the capacity to target more than one epigenetic event. Consequently, more knowledge is needed to better understand the interaction of nutraceuticals with the epigenome in order to improve their chemopreventive and anti-cancer effects.

Here, we reviewed the latest findings on Sea buckthorn extracts with anti-cancer properties and presented their potential mechanism of interaction with the epigenome. The Sea buckthorn is an arbuscular species cultivated at industrial scale throughout the world, including Romania, appreciated for the nutritional value of its juice and oil extracted from berries. Also, all its plant components are rich in nutraceuticals that have been used in traditional medicine since ancient times. By understanding the nature of epigenetic modulation induced by every bioactive components of Sea buckthorn we can evaluate its chemoprevention potential in cancer.

Acknowledgement: This work was supported by the grant COP A 1.2.3., ID: P_40_197/2016.

PROTEOME PROFILING OF BONE HEALING IN A RAT TIBIAL DEFECT MODEL USING TITANIUM IMPLANT WITH FUNCTIONALIZED SURFACE

Raluca Maria Boteanu[1], Viorel Iulian Suica[1], Luminita Ivan[1], Florentina Safciuc[1], Elena Uyy[1], Sorin Croitoru[2], Valentina Grumezescu[3], Livia Sima[4], Constantin Vlagioiu[5], Gabriel Socol[3], Felicia Antohe[1]

[1]Institute of Cellular Biology and Pathology N Simionescu, Bucharest; [2]Faculty of Engineering and Management of Technological Systems, Bucharest; [3]National Institute for Lasers Plasma and Radiation Physics, Bucharest; [4]Institute of Biochemistry, Bucharest; [5]Faculty of Veterinary Medicine, Bucharest

Keywords: bone healing, growth factors, MS-based proteomics

Introduction: Titanium and some of its alloys are bioinert materials used in orthopaedic implants that replace hard tissue. However, impaired fracture healing can have different causes, leading to delayed unions or non-union fractures. Our aim was to test the regenerative potential of titanium implants with functionalized surfaces and to identify the downstream protein effectors involved in bone healing in a diaphyseal tibial defect in rat.

Methods and Results: We developed a composite implant of titanium coated with a poly(ethylene glycol) (PEG) matrix containing FGF2, VEGF and Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) microspheres embedded with BMP4. The biocompatibility assessment of the functionalized titanium plates showed that implants were well tolerated, exhibiting a high rate of viability (up to 100%) of the implanted animals. Histological and histomorphometric analysis showed that the incorporation of the three growth factors in the titanium adsorbed polymer matrix induced an improved healing process when compared with the individual action of the biomolecules. The shotgun proteomic approach allowed the high-confidence identification of 1614 proteins, of which 113 were differentially expressed. Additionally, the GO Slim Biological Processes analysis of differentially expressed proteins indicated a large number of proteins clustered in the response to stimulus class. The collinearity between these proteins was performed using the Pearson correlation matrices. Next, the STRING database (version 10.5) was used to reveal whether correlated proteins could generate possible protein networks. Based on confidence prediction of interactions between proteins, we built a protein network which was visualized using Cytoscape 3.5.1. For the titanium implant designed with a PEG surface containing optimized proportions of FGF2, VEGF and BMP4, the general interaction was very well organized in hub-based networks related to chemical, wound healing and response to stress pathways.

Conclusion: The proteomics results of this study allow for a new and in depth insight of the complex healing process, but further investigations are required to truly understand the roles and how the interactions of differentially expressed proteins exert their impact on the repair process of bone fracture.

Acknowledgement: The study was supported by Romanian Academy and Research grant CNCSIS-UEFISCSU PN-II-PCCA-2011-3 no. 153/2012.

GREEN SYNTHESIS OF NANOPARTICLES AND THEIR BIOMEDICAL APPLICATIONS; PROTEOMIC ANALYSIS

Elena Codrici[1], Simona Mihai[1], Ionela Daniela Popescu[1], Ana-Maria Enciu[1,2], Lucian Albulescu[1], Radu Albulescu[1], Mircea Leabu[1,2], Alina Butu[3], Cristiana Tanase[1,4]

[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Institute of Biological Sciences, Bucharest; [4]Titu Maiorescu University, Faculty of Medicine, Bucharest

Keywords: green synthesis, nanoparticles, proteomic analysis

In science and technology, one of the rapidly developing concepts in recent years is nanotechnology, which has brought remarkable development. Metal nanoparticles based on nanotechnology have earned global attention due to their expansive applications in biomedical, agricultural, environmental and physiochemical fields.

Lately, phytonanotechnology has offered new insights for the synthesis of nanoparticles and represents an ecofriendly, simple, rapid, stable, and cost-effective technology. Thus, plant-derived nanoparticles produced by accessible plant materials and the nontoxic nature of plants are suitable for achieving the high demand for nanoparticles with applications in various biomedical areas. Successfully synthesized gold and silver nanoparticles using different medicinal herbal plant parts and their extracts proposed the use of these bio-products as appropriate resources. It has been suggested that proteins, amino acids, vitamins, organic acid, as well as secondary metabolites, such as flavonoids, alkaloids, polyphenols, terpenoids, heterocyclic compounds, and polysaccharides are significant players in metal salt reduction and, furthermore, act as capping and stabilizing agents for synthesized nanoparticles. Green nanoparticles have been applied in many biomedical contexts, including anticancer, antimicrobial and wound healing applications. The cytotoxicity, respectively biocompatibility tests are required for nanoparticles with biomedical applications. Deciphering the precise mechanisms and the components responsible for plant-mediated synthetic nanoparticles remain to be elucidated; proteomics analysis could offer the key to understand molecular mechanisms of action. A comprehensive analysis of global proteomic changes induced in cells of green nanoparticles could provide useful data for understanding the toxic and pathological responses and also to identify candidate toxicity biomarkers, including apoptotic, reactive oxygen species, or inflammatory proteins.

Green technology is rising as a nontoxic, and safe option in biomedical applications, thus improving nanomaterials in terms of biodegradability, functionalization, and biocompatibility.

Acknowledgement: This work was partially supported by the grants COP A 1.2.3., ID: P_40_197/2016, no 52/2016 and PN 18.21.01.06.

PROTEOMIC INVESTIGATIONS OF NATURAL PRODUCTS IN CANCER PREVENTION AND THERAPY

Cristiana Tanase[1,2], Ana-Maria Enciu[1,3], Elena Codrici[1], Simona Mihai[1], Ionela Daniela Popescu[1], Lucian Albulescu[1], L.G. Necula[1,4], Sevinci Pop[1], Radu Albulescu[1,5]

[1]Biochemistry-Proteomics Department, Victor Babeș National Institute of Pathology, Bucharest; [2]Titu Maiorescu University, Bucharest; [3]Carol Davila University of Medicine and Pharmacy, Bucharest; [4]St. S. Nicolau Institute of Virology, Bucharest; [5]National Institute for Chemical Pharmaceutical R&D, Bucharest

Research into natural products has experienced certain resurgence recently. One reason for this interest is due to the growing evidence supporting the potential application of natural products as agents for cancer prevention and treatment. Natural products can regulate cellular signaling pathways, as well as down-regulate the expression of oncogenic miRNAs and up-regulate the expression of tumor suppressive miRNAs. By modulating these key processes, natural products can inhibit cancer cell growth and cancer stem cell (CSC) renewal, therefore deterring tumor progression and development. Furthermore, by targeting and inhibiting CSC, natural products could prevent the emergence of drug-resistant tumors. However, additional in vitro and in vivo studies and clinical trials are required to achieve the true value of natural products for the prevention and/or treatment of cancer.

Acknowledgement: Partially supported by the grant COP A 1.2.3., ID: P_40_197/2016, Ctr. 52/2016 and PN 18.21.01.06.

HELPING RESEARCH

- INDUSTRY PARTNERS' SESSION -

Chair: Elena Codrici

CLINICAL DECISION SUPPORT IN THE NEW DIAGNOSTICS ERA

Violeta Dragos

Roche Diagnostic Romania

The statistics associated with lung cancer clearly demonstrate the aggressive nature of this deadly disease. Roche Diagnostics offers a robust menu of lung cancer diagnostic tools. Our portfolio of IHC diagnostic assays, IHC staining products, and more, deliver the high sensitivity and specificity healthcare specialists need. Roche proven portfolio of lung cancer assays can assist in the stratification of disease, including non-small cell lung carcinoma (NSCLC), neuroendocrine carcinoma and their various subtypes.

SATURDAY, NOVEMBER 24

SESSION 6

VARIA

Chairs: Ana-Maria Enciu, Cătălin Gabriel Manole

THE EFFECT OF NANOPARTICLES OBTAINED BY GREEN SYNTHESIS ON CELL PROLIFERATION

Alexandra Cătălina Vîlceanu[1], Simona Mihai[2], Elena Codrici[2], Lucian Albulescu[2], Ionela Daniela Popescu[2], Mihaela Lupu[3], Crina Karmezan[3], Alina Butu[4], Ana-Maria Enciu[2,1]

[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest; [3]SC Sanimed International Impex, Bucharest; [4]The National Institute of Research and Development for Biological Sciences, Bucharest

Keywords: nanoparticles, green synthesis, toxicity assay

Introduction: Nanoparticles of noble metals have gained important interest in the biomedical field due to their uses, ranging from antimicrobial agents to anti-inflammatory effects, in vivo imaging, biosensors, and drug-delivery systems.
Conventional methods to produce nanoparticles employ toxic chemicals, prohibiting their use in the biomedical field. Green synthesis has emerged in the past years as a more efficient and environmentally-friendly method that relies on a variety of plant extracts to produce noble metal nanoparticles.
Materials and Methods: We used several cell types: normal epithelial cells, dysplastic epithelial cells and aggressive tumour cells from the U87 glioblastoma cell line. We used metallic nanoparticles of silver, gold, palladium and selenium, with a plant-based coating. Our methods involved cytotoxicity measurements using LDH assays, viability measurements by MTS assays and electrical impedance measurements using the xCELLigence system.
Results and Discussions: Regarding cytotoxicity, the assays showed that metal salts alone are the most toxic and the plant extract reduces the toxicity. Out of the metals tested, selenium showed very little variation in toxicity with dilution. The vegetal extracts alone had proliferative effects. To study biological effects, non-toxic dilutions ranging from 1/200 to 1/800 were tested in the xCELLigence system. For the selected concentrations, impedance measurements showed a dose-dependent pro-proliferative effect for some of the studied nanoparticles.
Conclusions: The plant-based coating reduces nanoparticle toxicity. However, it also decreases the cytotoxic effect, if they are to be used as anti-cancer agents. Taking into account their pro-proliferative effect, these nanoparticles could prove of greater use in the field of regenerative medicine.

Acknowledgment: This work has been partially supported by Core Program, contract no. 18.21.01.06 and Program COP Axis 1 Action 1.2.3., Grant ID P_40_197, Contract no. 52/05.09.2016, implemented with the support of Ministry of Research and Innovation.

EIGHT-ARM RADIAL MAZE TEST FOR ASSESSMENT OF MEMORY PERFORMANCE IN Nrf2 MICE

Ștefania Grigoraș[1], Radu Ioan Tiron[1], Andreea Elena Ștefan[1], Ana-Maria Enciu[2,1], Ionela Victoria Neagoe[2]

[1]*Carol Davila University of Medicine and Pharmacy, Bucharest*
[2]*Victor Babeș National Institute of Pathology, Bucharest*

Keywords: video tracking, cognitive impairment, behavior

Introduction: Disturbances in cognitive functions are among the most debilitating problems experienced by patients with neuropsychological disorders.
Investigations of these deficits in animals help extend and refine our understanding of human disorders, while at the same time providing valid tools to study higher executive functions in animals. We employ a genetic mouse model of dementia to study working memory, reference memory, anxiety and exploratory behavior.
Materials and methods: We compared the performance of two lots of laboratory animals (mice), a control lot and an Nrf2-/- lot, each containing 6 animals, 3 males and 3 females, aged between 1 and 3 months. The test was performed using the Panlab 8-arm radial maze and the results were interpreted using two software packages: Mazesoft 8 and SMART v.3.0 for video tracking. The training had three stages: 1) adaptation (2 days); 2) training (18 days); 3) actual testing (3 days). The parameters assessed were reference and working memory, time spent in different parts of the maze and average speed.
Results: We tested short-term and long-term memory and compared them between the two lots, as well as between males and females. No differences were found for memory errors between the two groups, but there were differences in levels of anxiety and exploratory behavior among genders.
Conclusions: The two methods proved to be useful in the analysis of behavior of laboratory animals, although the age of the lots was too young for development of cognitive impairment.

Acknowledgement: This work has been partially supported by the European Regional Development Fund; Operational Program Competitiveness 2014-2020; implemented with the help of Ministry of Research and Innovation, Priority Axis 1, Action 1.1.4, contract no. P_37_732/2016 REDBRAIN.

DETECTION AND QUANTIFICATION OF RARE MUTANT ALLELES BY DIGITAL DROPLET PCR

Victor Ionescu, Gisela Găină, Ștefania Rogozea, Cristina Niculițe, Valeriu Cișmașiu

Victor Babeș National Institute of Pathology, Bucharest

Keywords: optimization, TaqMan, gDNA, lncRNA gene

Introduction: Droplet digital PCR (ddPCR) is a novel method for molecular diagnostics, shown by many groups to enhance the sensitivity and specificity of standard real-time PCR. It enables absolute quantification of targets, as well as detection of rare mutations or copy number variations. We describe the ddPCR optimization procedure for the quantification of very low copy numbers of a mutant non-coding RNA in a strong background of wild-type copies.

Materials and methods: Primers and TaqMan probes were designed to detect two deletion/insertion polymorphisms (DIPs) between mouse strains C57BL/6 and BALB/c, hosted in lncRNA genes. Genomic DNA samples were isolated from total bone marrow. The PCR was initially set up as per manufacturer specifications – Bio-Rad ddPCR Supermix for Probes (no dUTP). The C57 and BALB gDNA samples were first individually tested with their specific probes, followed by cross-reactivity tests with both probes combined into the same PCR reaction. Both samples (C57 and BALB) were combined in subsequent reactions. Next, the temperature and duration of the annealing-and-extension step were modified (56-62.2°C, 1-2 minutes). The number of PCR cycles (40-50), primer concentration and probe concentration (1-10 μM and 250-500nM, respectively) were tested as well. The selection criteria were: highest fluorescence level, lowest nonspecific droplet numbers, absence of droplet 'rain pattern'.

Results and discussion: Lower annealing-and-extension temperature provided stronger fluorescence, with a maximum at 58°C for both polymorphisms. Higher concentrations of primers translated to a little signal increase, on their own. However, increasing both primer and probe enhanced fluorescence, with 2.5μM primers showing the highest level. At constant primer concentrations, a better signal was provided by the 500nM probe concentration. Increasing PCR cycle number only improved fluorescence modestly. The same effect was observed when raising annealing-and-extension time. Across the entire range of parameters, we were able to detect 100ng wild-type gDNA as a mean of 29272 target copies per well, and 1ng mutant as 181 copies. We found the data highly reproducible and conclude that reaction conditions afford greater flexibility compared with real-time PCR, while improving accuracy – an outcome consistent with other studies describing ddPCR optimization. This can be attributed to the system end point rather than real-time data collection.

Conclusions: Optimization procedures for ddPCR follow the same steps as traditional PCR. The best overall conditions for the selected DIPs are 58°C and 1 minute for the annealing-and-extension step, 40 cycles, 2.5μM primer and 500nM probe concentration.

Acknowledgement: This work was done through project PN-III-P2-2.1-PED-2016-1932 (UEFISCDI) and project PN 18.21.01.04 (PN18/2018 MCI).

HIGH RESOLUTION MICROARRAY ANALYSIS OF EPILEPSY-LINKED GENOMIC REGIONS

Artsiom Klimko[1], Sorina Mihaela Papuc[2], Magdalena Budisteanu[3,2,4], Dana Craiu[4], Aurora Arghir[1,2]

[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest; [3]Titu Maiorescu University, Bucharest; [4]Prof Dr Alex Obregia Clinical Hospital of Psychiatry, Bucharest

Keywords: CNV, aCGH, epilepsy, SCNA2, KANSL1

Introduction: Copy number variations (CNV) analysis is an integral part of the study of human genomes in both research and clinical settings. In the last years, the use of high resolution microarray assays and next generation sequencing has led to significant progress in understanding the intricate genetic profile of numerous diseases, revealing new genomic regions and genes involved in pathogenesis. For example, today, more than 700 genomic regions and genes are proved to be involved in different types of epileptic disorders. The aim of this study was to review the clinical significance of CNVs detected by aCGH (Microarray-based Comparative Genomic Hybridization) in a previously analyzed cohort of epilepsy patients.

Material and Methods: The study group included 32 patients presenting seizures, as part of complex phenotypes, previously analysed by aCGH with different Agilent platforms (44K, 60K, 105K, 180K). The CNVs were reviewed against different databases, like UCSC Genome Browser, Database of Genomic Variants (DGV), Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources (DECIPHER), Online Mendelian Inheritence in Men (OMIM).

Results: Pathogenic and likely pathogenic CNVs, ranging from 117 kb to more than 68 Mb, were detected in 14 out of 32 patients. Several of those pathogenic CNVs overlapped syndromic regions (2q22.3, 4p16, 8p23, 10q26, 1q43-q44), thus contributing to clinical diagnosis. Two previously variants of uncertain significance CNVs were reclassified as pathogenic, a 2q24.3 deletion (879 kb) affecting two epilepsy genes (SCN2A and SCN3A) and a 17q21.31 deletion (117 kb) disrupting KANSL1 gene.

Conclusion: Our data illustrates the importance of revisiting previous cases for update of clinical significance of CNVs, especially of focal, gene poor variants.

Acknowledgement: Partial funding UEFISCDI Project 249PED/2017 and Ministry of Research and Innovation grants PN16.22.05.01 and PN09.33.02.03.

CYTOKINES AND GROWTH FACTORS AS POTENTIAL BIOMARKERS FOR EVALUATION OF PITUITARY ADENOMA AGGRESSIVENESS

Dana Tapoi[1], Elena Codrici[2], Linda Maria Popa[1], Ionela Daniela Popescu[2], Simona Mihai[2], Ancuta Augustina Gheorghisan Galateanu[1], Cristiana Tanase[2]

[1]Carol Davila University of Medicine and Pharmacy, Bucharest
[2]Victor Babeș National Institute of Pathology, Bucharest

Keywords: cytokines, growth factors

As oncology still relies on histopathological examination, in order to establish the diagnosis in pituitary adenomas, it is important to discover new biomarkers that can serve as a non-invasive tool for detection and monitoring. Cytokines and growth factors have been already recognized as important players in tumourigenesis. It has already been demonstrated that IL-6 plays a key role in triggering pituitary cell senescence, but it also appears to be produced by tumour cells to promote their proliferation. This dual effect of IL-6 may be an explanation for the usually benign nature of pituitary adenomas. Growth factors are involved in the normal development of the pituitary gland, but they also seem to be involved in the development of pituitary adenomas. Moreover, since most pituitary tumours (such as pituitary adenomas) are benign, it is important to determine if these biomarkers can be correlated with the tumour invasiveness. In this respect, we discussed the influence of cytokines or growth factor panels, such as IL-1β, IL-2, IL-6, IL-8 and TNFα, TGF β, FGF, EGF and VEGF on pituitary adenoma behaviour and their potential use as biomarkers for accurate diagnosis of pituitary adenoma.

POSTERS

MOUNTING: THURSDAY 18:00 - 18:30

VIEWING AND PRESENTATION: FRIDAY 16:15 - 18:00

AGE-RELATED VASCULAR CHANGES IN HUMAN THYMUS

Radu Stănescu, Olivia-Garofița Mateescu, Mihaela Culescu, Dana-Maria Albulescu, Mihail-Relu Stănescu

University of Medicine and Pharmacy, Craiova

Keywords: lymphatic structure, involution, regeneration

Introduction: Thymus, as the central lymphoid organ, plays a key role in T cell development, and is essential in immune defense against pathogens or neoplasia. The thymus undergoes rapid degeneration as part of the aging process, but it is however capable of regenerating in peculiar conditions. The aim of this presentation is to reveal the vascular features of thymus degeneration and regeneration in all its human ontogenesis.

Material and methods: The 17 retrospective cases consisting of fetuses, infants, adults and old human subjects were autopsied in Pathology Service of Philanthropia Clinical Hospital in Craiova. Thymus specimens were routinely processed: fixed in 10% buffered formalin, embedded in paraffin, then stained with hematoxylin-eosin, van Gieson, and Nonidez silver stain.

Results and discussions: Thymus specimens were histologically classified in three classes as follows: (a) normal, in which lobulation and sharp demarcation of the cortex from the medulla were clearly present and postcapillary venules related; (b) slightly to moderately involuted thymus; and (c) markedly to extremely involuted thymus. The vascular changes in each class are epithelio-stromal related, and correlations with imagistic data are discussed.

Conclusion: The vascular pattern helps the thymus to regenerate and restore its function to a degree. This paves the way to strategies to therapeutically restore thymus function.

AGE-RELATED GENE EXPRESSION CHANGES: OXIDATIVE STRESS, INFLAMMATION, ENVIRONMENT AND COGNITION

Elena Milanesi[1], Maria Dobre[1], Ionela Victoria Neagoe[1], Mihaela Surcel[1], Gheorghita Isvoranu[1], Bogdan Ovidiu Popescu[1,2], Antonio Cuadrado[1,3,4,5]

[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Instituto de Investigaciones Biomédicas "Alberto Sols" UAM-CSIC, Department of Biochemistry, Faculty of Medicine, Autonomous University of Madrid, Madrid; [4]Instituto de Investigación Sanitaria La Paz (IdiPaz), Madrid; [5]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED) ISCIII, Madrid, Spain

Keywords: aging, dementia, gene expression, age-correlation

Introduction: Oxidative stress seems to contribute to age-related physical and cognitive decline and plays a key role in inflammation processes contributing to the pathophysiology of neurodegenerative disorders. Age-related gene expression levels both in the CNS and in peripheral tissues are influenced by multiple environmental factors, dietary habits and toxicity exposure.

Aim: In this study we investigated how the expression levels in a panel of genes related to inflammation and the oxidative stress pathway (1) correlated with age; (2) how these correlations changed in normal aging and dementia and (3) how environmental factors could influence the aging process.

Methods: Gene expression analysis of 168 genes involved in the NFK-B pathway and oxidative stress and response has been performed in whole blood from sixty-five individuals (age range 43-87 years). The following socio-demographical, environmental and clinical features have been collected: age, sex, BMI, education, diabetes, coffee assumption, smoking, toxicity exposure, childhood environment, pre-diagnostic decade environment and Mini-Mental State Examination (MMSE) score. Twenty-nine out of 65 individuals were diagnosed with mild cognitive impairment (MCI) or Alzheimer disease (AD) (age range 62-85) and 26 elderly individuals (age range 63-87) showed not-demented aging.

Results: We found that the expression of 39 genes correlated with age in the entire cohort. In particular, Metallothionein 3 (MT3) levels seemed to correlate with age independently of the disease status. When analyzing the two cohorts of elderly individuals (not-demented and demented) we did not find significant differences in terms of socio-demographic and environmental characteristics except for education, which was higher in the not-demented group ($p=0.001$). Interestingly, we found that Superoxide Dismutase 1 (SOD1) levels positively correlated with age ($p= 0.02$ Pearson's $r=0.431$) and negatively with MMSE score ($p=0.003$, Pearson's $r=-0.53$) in individuals with dementia, but not in individuals showing normal aging.

Conclusion: Reactive oxygen species within cells mediate changes in memory function, synaptic plasticity and neuronal death. SOD1 is known to play a significant role in catalyzing the breakdown of highly reactive O2- playing a protective role in neurodegeneration. In our demented cohort we found that blood levels correlate with age and cognitive function decline, suggesting that activation of Sod1 may be a therapeutic strategy for the inhibition of AD progression.

Acknowledgement: The study was funded by the European Regional Development Fund, Competitiveness Operational Program through the grant P_37_732/2016 REDBRAIN.

ASSAY OF ALPHA SMA EXPRESSING MELANOMA ASSOCIATED FIBROBLASTS IN 34 CASES

Patricia Irina Stîngă, Cristiana Popp, Mirela Cioplea, Alexandra Cioroianu, Oana Barbuceanu, Sabina Zurac

Colentina University Hospital, Bucharest

Keywords: tumor microenvironment; Breslow thickness; metastasis

Introduction: Our knowledge of melanoma-associated fibroblasts (MAFs) is narrow in comparison with that of malignant melanoma (MM) cells. MM stroma represents the microenvironment fueling MM cells and facilitating tumor growth, invasion, and metastasis. MM progression depends to some extent on the ability of melanoma cells to recruit a variety of stromal cells and to take advantage of them. It is believed MAFs originate in dermal fibroblasts; they become activated, acquiring myofibroblastic properties. Unlike the normal myofibroblasts found in wound healing for instance, MAFs are perpetually activated, and neither revert to a normal phenotype nor undergo apoptosis and elimination. They play a role in the melanoma ”odyssey” by secreting multiple chemical factors, by remodeling the extracellular matrix, by controlling angiogenesis and the immune response. MAFs are heterogeneous, but a large percentage of them is characterized by increased expression of alpha-smooth muscle actin (α SMA).

Methods: To partially fill in this gap of knowledge, we aimed to investigate MAF presence in 34 selected cases of MM, diagnosed at our hospital in the year 2017: 3 in situ MM, 2 acral lentiginous MM, 7 nodular MM, 17 surface spreading MM and 5 cutaneous metastases of MM. In regard to tumor thickness, 7 MM were thinner than 1 mm (group A); the Breslow thickness (Bt) of 5 MM was between 1 and 2 mm (group B); 9 MM Bt was between 2 and 4 mm (group C); 8 MM were thicker than 4 mm (group D). MM metastases (group E) thickness was not considered relevant. We used α SMA immunohistochemical marker in order to identify MAFs in the tumor stroma of all 34 cases included in the study. The abundance of α SMA positive MAFs found in the stroma was assessed for each case as follows: none (0*), sparse (1*), moderate (2*), numerous (3*).

Results: Regarding the cases of thin MM (groups A and B), α SMA positive MAFs were not found (0*). In group C (Bt between 2 mm and 4 mm), we identified α SMA positive MAFs in 4 cases (44.4%); in 3 cases, MAFs were quantified as sparse (1*); in one case, we found a moderate number of MAFs (2*). As for the thick MM cases (group D), we spotted MAFs in 7 of them (87.5%); in one case we identified a moderate number (2*) of MAFs; in all the others 6 cases, they were quantified as being sparse (1*). Referring to the cases of MM metastases at the skin (group E), numerous MAFs (3*) with α SMA positive immunohistochemical expression were found in all 5 of them (100%). Of all 7 cases of nodular MM, we observed MAF absence in only one.

Conclusion: Our work endorses the idea that MAFs may play a major part in MM growth, invasiveness and metastasis.

Acknowledgement: This work was supported by a grant of Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number 61PCCDI/2018 PN-III-P1-1.2-PCCDI-2017-0341, within PNCDI-III.

CEACAM1 EXPRESSION IN MALIGNANT MELANOMA WITH REGRESSION

Mirela Cioplea[1], Cristiana Popp[1], Luciana Nichita[1,2], Liana Sticlaru[1], Alexandra Cioroianu[1], Patricia Stinga[1], Monica Neagu[1], Carolina Constantin[1], Roxana Nedelcu[2], Sabina Zurac[1,2]

[1]Pathology Department, Colentina University Hospital, Bucharest
[2]Carol Davila University of Medicine and Pharmacy, Bucharest

Keywords: malignant melanoma, regression, CEACAM1

Objective: CEACAM1 is a transmembrane glycoprotein from the carcinoembryonic antigen (CEA) gene family. It is expressed on epithelial cells and mediates complex homophilic intercellular interactions involved in cellular growth, cellular immunity, and tissue morphogenesis. CEACAM1 can act as a tumour suppressor in some types of malignancies, including malignant melanoma. This study evaluates CEACAM1 expression in regressing melanomas, since regression is considered a model for interaction between tumor cells and host immunity. In melanoma, CEACAM1 inhibits natural killer (NK) cells activity and effector functions of tumor infiltrating lymphocytes (TILs).

Methods: We present a retrospective study including 21 consecutive cases of melanoma with regression and maximal Breslow index of 2 mm (stages T1 and T2). Comparative analysis of CEACAM1 expression in regressed and non-regressed areas was performed, using three different clones of CEACAM1: AA 1-428, extracellular domain, rabbit, AA 1-428, mouse, clone 8B6E2F4 and AA 1-468, full length, mouse, clone 2F6.

Results: All three clones had similar reactivity. Tumor cells in non-regressed areas in melanomas were CECAM1 positive, while the remaining tumor cells in regressed areas were mostly CEACAM1 negative.

Conclusions: In regressed melanomas, we found a polymorphism of CEACAM1 expression: in areas of regression tumor cells lose CEACAM1 expression, probably correlated with the presence of natural killer cells, while in non-regressed areas, CEACAM1 expression was observed, probably correlated with a higher resistance to immune mechanisms against tumor cells.

*Mirela Cioplea and Cristiana Popp are first authors in equal proportion.

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CHRYSIN AS A POTENTIAL THERAPY TO REVERSE LIVER FIBROSIS

Simona Ignat[1], Sorina Dinescu[1], Andreea Stan[1], Andreea Lazar[1], Aida Selaru[1], Ferenc Fenyvesi[2], Anca Hermenean[3], Marieta Costache[1]

[1] Department of Biochemistry and Molecular Biology, University of Bucharest
[2]Department of Pharmaceutical Technology, University of Debrecen, Hungary
[3]Vasile Goldis Western University of Arad

Keywords: liver, chrysin, drug delivery, hepatic stellate cells

Liver fibrosis is characterized by scar tissue accumulation following chronic liver injury and it can progress to liver cirrhosis and hepatocellular carcinoma. The potential of different natural compounds to prevent or treat liver fibrosis is investigated. Chrysin (5,7-dihydroxyflavone, Chr) shows anti-inflammatory, antioxidant and hepatoprotective effects that recommend it for liver fibrosis treatment. However, it has poor solubility in water that affects its bioavailability and requires complexation with cyclodextrins (CD). The aim of this study was to investigate the potential of a new drug system based on Chr complexed with different CD to reverse liver fibrosis.

For biocompatibility evaluation, hepatic carcinoma cells from a Huh-7 cell line were seeded and treated with two different types of CD, Random methyl-beta cyclodextrin (RAMEB) and (2-Hydroxypropyl)-beta-cyclodextrin (HPBCD), alone or associated with Chr in 1:1 and 2:1 ratios. The treatment was added in a 1-100 μM range of concentrations for 24 hours. Cells untreated with CD-Chr were maintained as control. Cell viability was evaluated by an MTT test, by measuring absorbance at 550 nm. Cytotoxicity was determined by the LDH assay, by measuring absorbance at 490 nm. The LiveDead assay allowed simultaneous visualisation of dead and alive cells by fluorescence microscopy. To evaluate the effect of Chr on liver fibrosis reversion, hepatic stellate cells (HSCs) were seeded and activated by treatment with TGF-β to simulate liver fibrosis. The activation of HSCs was validated at gene level (qPCR) by investigating the expression of fibrosis markers, collagen type I (col I) and smooth muscle actin α (α-SMA), before and after treatment with CD-Chr complexes.

The MTT test showed the highest viability for RAMEB-, followed by HPBCD-treated cells suggesting they are biocompatible. The 2:1 complexes induced lower cell viability indicating a better Chr bioavailability in these complexes. The LDH test showed the cytotoxicity was higher for concentrations >30 μM. These results were confirmed by fluorescence microscopy, where the number of dead cells was higher when treated with concentrations >30 μM of CD-Chr complexes. As for the potential of these complexes to reverse liver fibrosis, gene expression levels for both markers of fibrosis were significantly decreased after treatment, with better results for the HPBCD-Chr complex. The two complexes investigated showed good biocompatibility. Higher concentrations than 30 μM of RAMEB-Chr and HPBCD-Chr in 2:1 ratio induced lower cell viability. The CD-Chr complexes showed potential to reverse liver fibrosis. The RAMEB and HPBCD complexes may be good Chr delivery systems that induce a positive effect on the diseased liver.

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DIFFICULTIES OF DIFFERENTIAL DIAGNOSIS IN A CASE OF HIGH GRADE SARCOMA OF THE SPERMATIC CORD

Patricia-Irina Stîngă[1], Simona Iacob[2], Luciana Nichita[1], Alexandra Cioroianu[1], Florentina Hanu[3], Gabriel Pop[1], Liana Sticlaru[1], Sabina Zurac[1]

[1]Colentina University Hospital, Bucharest
[2]Personal Genetics, Bucharest
[3]Emergency University Hospital, Bucharest

Keywords: myxofibrosarcoma; liposarcoma; CDK4; MDM2 gene amplification

A 47-year-old man without significant medical history noticed a painless swelling in the left scrotum in June 2018. He had an MRI examination that revealed a 11.5/55/45mm left spermatic cord (SC) tumor mass. A surgical procedure was performed and a 10/7-cm tumor was excised. Gross assessment sets out a compact, whitish, nodular mass with smooth margins, elastic consistency and relatively homogenous aspect on the cut section. A diagnosis of malignant mesenchymal cell proliferation was firstly given at another hospital. We received the case in consultation in order to perform immunohistochemistry (IHC) tests and to establish a more specific diagnosis. Follow-up revealed that the tumor relapsed 2 months following the surgical procedure.

The microscopic examination revealed a proliferation of fusiform cells arranged in sheets; hypocellular areas interspersed with hypocellular, myxoid areas; the cytonuclear pleomorphism was striking, with nuclear monstrosities, multinucleated cells, floret-like cells and nuclear pseudoinclusions, with up to 50 mitotic figures on 10HPs. No lipoblast or well-differentiated liposarcomatous areas were spotted after examination of 5 sections obtained from 5 different paraffin blocks. Limited areas of necrosis were observed. The tumor stroma included relatively large myxoid areas, with prominent, curvilinear vessels; perivascular condensation of tumoral cells was observed. The accompanying inflammatory infiltrate was composed of lymphocytes and plasma cells.

The IHC evaluation revealed diffuse positivity for vimentin and p16; rare cells were positive for αSMA, EMA. Staining for S100, Adipophilin, Myogenin, CD34, and CDK4 was negative in the tumor cells. The Ki-67 index was high (80%).

The differential diagnosis spectrum consists of the following high grade sarcomas: MFS (extremely rare SC); dedifferentiated liposarcoma (DDL); undifferentiated pleomorphic sarcoma (UPS); pleomorphic liposarcoma (PL).

A clear cut between these entities is desirable, because of the disparities regarding treatment; if complete surgical excision with negative margins is required in all entities, radiotherapy is efficient in MFS, UPS and PL.

Regardless the rarity of the entity, we made a diagnosis of spermatic cord MFS, based on the histomorphological appearance of the tumor, correlated with the results of the IFC tests.

Acknowledgement: We gratefully thank Dr. Paul Corici from City Hospital, Medgidia for his contribution to the diagnosis process.

DISTRIBUTION OF SQUAMOUS INTRAEPITHELIAL LESIONS AND HPV INFECTION BETWEEN AGE GROUPS

Mihaela Ioana Enache, Anca Poteca

Carol Davila University of Medicine and Pharmacy, Bucharest

Keywords: human papillomavirus, cervical screening, cytology, cervical cancer

Cervical screening by Babeș Papanicolaou cervico-vaginal cytology and histopathological analysis are effective detection methods of squamous intraepithelial lesions (SIL). The Bethesda System for Reporting Cervical Cytology classifies SIL as low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL), the same as the 2014 WHO Classification of Tumours. Development of both types of lesions is due to HR and LR human papillomavirus (HPV) infections. SIL can progress to cervical cancer and Romania has the highest incidence and mortality in Europe for this type of malignancy.

The aim of this study was to analyze the histopathological and cytological spectrum of lesions, the presence of HPV infection and parity by age group. We gathered data from patients who underwent histopathological and cytological testing at the "Prof. Dr. Panait Sârbu" Clinical Hospital of Obstetrics and Gynecology from January 2018 to June 2018 (173 patients, aged 19-76). We only included patients diagnosed with LSIL and/or HSIL. Most patients included in the study were in the 30-39 age group (n=63), followed by those aged 20-29 (n=40) and 40-49 (n=38). From 173 cases, 140 had LSIL (80.92%) and 58 HSIL (19.08%). A peak in LSIL was found in the 30-39 age group (51 out of 63 patients), followed by 20-29 (37/40). Presence of LSIL decreased with age. Most HSIL cases were found in the 30-39 age group (19/58) and increased with age. 50 women were known to have HPV infection (28.9%), 19 of them aged between 20-29 years. We had more residents from urban areas than rural areas (78.03% vs 21.96%, respectively). A likely explanation is the hospital location, with rural areas having lower healthcare access. Forty-two patients had previous pregnancies and 36 were nulliparous.

Our findings underline the necessity of regular cervical screening for women from the beginning of sexual activity, followed by HPV testing if necessary. The small number of patients and lack of information regarding HPV status for most patients represent limitations of this study. It is important to promote and facilitate screening for cervical cancer for the whole female population. Early detection of SIL reduces the incidence of invasive cervical carcinoma.

EVALUATION OF DNMTS ACTIVITY AND DNA METHYLATION PATTERN ON HUMAN TUMOR CELL LINES AFTER PROLONGED TREATMENT WITH UNSATURATED FATTY ACIDS

Sevinci Pop[1], Victor Stefan Ionescu[1], Ioana Maria Lambrescu[1], Emilia Manole[1,2], Daciana Marta[1]

[1]Victor Babeș National Institute of Pathology, Bucharest
[2]Research Center, Colentina Clinical Hospital, Bucharest

Keywords: epigenetic, gene activation, promoter methylation, transcriptionally inactive

The epigenetic activation or inactivation of genes by DNA methylation plays a major role in several human diseases, including cancer. In carcinogenesis there are abnormal DNA methylation events: large repetitive DNA sequences and multiple gene promoters are hypomethylated and transcriptionally active, whereas many tumor suppressor promoters are hypermethylated and transcriptionally inactive. Methylation of genomic DNA is controlled by particular DNA methyltransferases (DNMTs) proteins. DNMT1 is involved in the maintenance of the DNA methylation pattern, whereas DNMT3a and DNMT3b are de novo methyl-transferases. These proteins transfer a methyl group from a donor, such as S-adenosyl L-methionine (SAM), to the 5' position of cytosine from a CpG island. Emerging evidence suggests that omega 3 fatty acids might influence global and local DNA methylation patterns, due to their role in one-carbon metabolism, the major pathway to generate methyl group donor.

Here we present results after the evaluation of DNMT proteins activity and DNA methylation status on human tumor cell lines, before and after prolonged treatment with an unsaturated fatty acid. The DNMT proteins activity was measured using ELISA test kits and the proteins expression level was quantified by immunofluorescence and Western Blot. The specific gene promoter methylation patterns were measured using Real-Time PCR and the global genomic DNA methylation level was assessed by ELISA and immunofluorescence. The global DNA methylation level showed ~ 25% decrease on adenocarcinoma cell line treated with unsaturated fatty acid in comparison with untreated cells. Interestingly, the DNMT3a protein activity increased under unsaturated fatty acid treatment on tumoral cells compared with control cells. This process was correlated with higher protein expression showed by Western Blot and immunofluorescence. The methylation pattern at the promoter of SIRT1 and BRAC1 genes was significantly changed under omega 3 acid treatment compared with untreated samples.

Conclusion: The prolonged treatment of tumoral cell lines with an unsaturated fatty acid affected DNMT proteins activity, which induced modifications of the methylation status at specific gene promoters and promoted changes of the global genomic DNA methylation pattern.

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FOUR CASES OF THYMIC PATHOLOGY

Liliana Parascan[1], Florina Vasilescu[2], Doina Mihaela Pop[2]

[1]IUBCV CC Iliescu, Bucharest
[2]Victor Babeș National Institute of Pathology, Bucharest

Keywords: thymolipoma, mediastinal liposarcoma, B2 thymoma, thymic carcinoma

Introduction: In the past two years, there have been 4 cases with thymic mediastinal pathology: a unilateral thymic cyst, a thymolipoma associated with mediastinal liposarcoma, a type B2 thymoma and a thymic carcinoma.

Material and methods: In the first case, a 28-year-old female presented with a mediastinal cyst, and a 5/2/0.5-1 cm, oblong, translucent, yellow-red, tall, slightly granular mass was extracted. The second case, male, 28 years old, presented 2 pieces: 1. Tumor fragment of 1.5/1.5/1 cm, un-encapsulated, hard, of yellow-brown-red color and 2. Thymus of 8/6/2 cm surrounded by and interspersed with adipose tissue. The third case, a 45-year old woman with myasthenia gravis and mediastinal tumor, presented an encapsulated tumor of 5.5/3.5/2 cm, of high consistence, white-gray-and-gray color. The last case, a 53-year-old patient with a mediastinal tumor, presented a mass with dimensions of 5.5/4/3 cm as sectioned by the surgeon, with high consistence, gray, uncoated. The harvested fragments were included in paraffin and stained with the usual techniques: HE, VG, PAS-Alcian, Gomory. Malignant tumors were stained for immunohistochemistry (IHC).

Results: In the first case, stained with common dyes (HE, VG), the diagnosis was unilocular thymic cyst. The second case, stained with HE, VG, and additionally with PAS-Alcian, Gomory, Argentic Impregnation, PM (Movat Pentachrome), was immunohistochemically S 100-positive diffuse in the lesion, CD34-positive in vessels and focal in stromal cells, SMA-positive in vascular walls, CK7-negative, AE 1/3-negative, CD 45-positive in the inflammatory infiltrate and had a Ki-67-positive approx. 1-3%, leading to a diagnosis of well-differentiated liposarcoma (lipomatous atypical tumor). The third case, female, 45 years, required IHC: CD57-positive staining in small lymphocytes, CK19-positive in tumor cells, CD117-negative, TdT and CD3-positive in thymocytes, KI67-positive 10%. The diagnosis was of B2 type. The fourth case, female, 53 years of age, also required IHC: CD5 was positive in small lymphocytes and rare tumor cells, CD117 was positive in tumor cells, TTF1 was negative in tumor cells, P63 was positive in tumor cells dispersed, Ki-67-positive 60% in tumor cells, negative ER in tumor cells, supporting the diagnosis of squamous cell carcinoma of thymic origin.

Conclusions: The thymic cyst was of embryonic origin. In the case of well-differentiated liposarcomas, tumor location, histology, size and subtype are the most important prognostic factors. The prognosis of the predominantly cystic thymoma (the particularity of the case diagnosed in our clinic) depends, as with other types of thymomas, on: stage, microscopic type, complete excision, myasthenia gravis, proliferation index, and DNA ploidy. The complete loss of thymus-specific architecture, as well as the absence of immature lymphocytes can be considered an unfavorable prognostic factor, as they are found in various types of thymic carcinomas.

GLOBAL GLOMERULOSCLEROSIS IN FABRY'S DISEASE - A MORPHOLOGICAL STUDY OF EIGHT CASES -

Alexandru Procop, Monica Hortopan, Lucia Ciobotaru, Bogdan Obrisca, Ismail Gener, Vlad Herlea

Fundeni Clinical Institute, Bucharest

Keywords: genetic, renal disease, glycoside accumulation

Introduction: Fabry's disease is an X linked recessive lysosomal storage disease which causes a deficiency of an enzyme (alpha-galactosidase A) responsible for catabolizing neutral sphingolipids. The absence of this enzyme leads to intracellular accumulation of ceramides (trihexoside and digalactosyl) in the skin, kidney (glomerular, tubular, interstitial), blood vessels, corneal epithelium and heart, leading to multiple organ disease and death.

Objective: The aim of this study was to present a series of 8 cases of Fabry's disease diagnosed at Fundeni Clinical Institute between 2017 and 2018, with their distinctive morphological characteristics.

Materials and methods: Samples were biopsied at the Nephrology Department of the Fundeni Clinical Institute, processed by conventional histopathological methods with Hematoxylin – Eosin stain but also special stains (Periodic Acid Schiff – PAS and Masson trichrome) and then diagnosed at the Department of Pathology of the same institute.

Results: Out of 8 cases diagnosed as Fabry's disease, 4 were male patients with a median age of 35.25 (the youngest patient was 30 years old and the oldest was 43 years old) and 4 were female patients (median age 55 years) with the youngest being 50 years old and the oldest 62 years old thus confirming the later in life presentation in female patients (chromosome X linked disease). The first symptoms were cutaneous, diagnosed in childhood (angiokeratomas on skin, hypohidrosis and acroparaesthesia of the extremities) mostly ignored by the patients or misdiagnosed (due to the rarity of the disease) followed by heart disease due to the accumulation of glycolipids in heart cells (high blood pressure, hypertrophic cardiomyopathy leading to heart failure), ocular symptoms (clouding of the cornea – cornea verticillata) and last but not least kidney failure. The histopathological evaluation revealed glomerular changes – honeycomb pattern – enlarged podocytes with multiple cytoplasmic clear vacuoles positive to PAS examination, glycoside accumulation, found also in the parietal epithelium, mesangial cells and mesangial matrix depending on the extent of the disease. Patients with end stage disease had glomerulosclerosis (segmental and global), vacuolation of distal tubular cells, interstitial fibrosis with the accumulation of interstitial foam cells and vascular involvement (tunica intima and tunica media) with vacuolation of endothelial cells and empty spaces in tunica media. The degree of glomerulosclerosis in patients with Fabry's disease ranged from minimal glomerulosclerosis to segmental and global glomerulosclerosis. Diffuse global glomerulosclerosis was reached in male patients in the third and fourth decades of life, whilst female patients had a slower disease progression: the oldest female patient (62 years old) had minimal glomerulosclerosis changes and two female patients (in the fifth decade) had advanced glomerulosclerosis (global and segmental)

Conclusion: Fabry's disease is a rare genetic X-linked disorder which progresses to renal failure, recurring acroparaesthesia, heart failure and corneal dystrophy. Our study reveals the importance of early diagnosis and substitution treatment in male patients (hemizygous and heterozygous) especially given the early onset of glomerulosclerosis with end stage renal disease (third and fourth decade of life).

HISTOLOGICAL ASPECTS IN CARDIAC MYXOMA

Liliana Parascan

IUBCV CC Iliescu, Bucharest

Keywords: myxoma, lepidic cells, proteoglycans

Introduction: Cardiac myxomas are the most frequent heart tumors that surgeons of the IUBCV "C.C. Iliescu" Institute have removed over the past two decades.

Material and methods: One hundred and three myxomas were analyzed for this study. The patient age ranged from 27 to 86 years. Seventy of them were women and 33 men. The symptoms most of them suffered from ranged from fatigue, arrythmia, precordial pain, weight loss, myalgia, muscular pain and arthralgia to fever and embolic symptoms. Echocardiograms revealed the presence of intracardiac tumoral formations, predominantly in the left atrium. After the tumors were removed, histopathological examinations were performed. Ninety-two of them were localized in the left atrium, eight in the right atrium, two in the left ventricle and one in the right ventricle. Macroscopically, the tumors ranged from 0.8/0.7/0.4 cm to 11/5/5. They were polypoid, ovoid or spherical. The color was not homogeneous. Some of them were incapsulated (16), others were not (33), while still others were relative incapsulated. HE, VGE, PAS-Alcian staining was performed and on some occasions immunohistochemistry (IHC) for Calretinin, S100, NSE, CD34, vimentin. Electron microscopy was performed at Victor Babeș National Institute of Pathology.

Results: Microscopically, the presence of diagnostic criteria was the general rule that was taken into account. The number of cases showing a (generally recent) superadded blood clot was around 15 percent. In 20 percent of the cases, an IHC was also performed: 75 percent of the cases were Calretinin positive, 60 to 80 percent of cases were S1000 positive, 50 percent NSE positive, 65 percent CD34 positive and 50 percent vimentin positive.

Conclusions: A myxoma diagnosis is based on the identification of lepidic cells in background proliferations within the myxomas. The myxoid matrix is made up of proteoglycans, elastin, fibrinogen, fibrin and collagen that can be evidenced by specific staining (PAS-Alcian for proteoglycans, VGE for elastin and collagen fibres). The myxoma (lepidic) cells are generally reactive to anti-Calretinin, S100, NSE, CD34 and vimentin antibodies.

IDENTIFICATION OF NEUROTROPIC VIRAL INFECTIONS IN CENTRAL NERVOUS SYSTEM TUMORS

Dorel Eugen Arsene[1,2], Elena Milanesi[1], Ioana Ruxandra Pîrvu[1], Maria Neagu[1], Maria Dobre[1]

[1]*Victor Babeș National Institute of Pathology, Bucharest*
[2]*National Institute of Neurology and Neurovascular Diseases, Bucharest*

Keywords: CNS tumors, neurotropic viruses

Introduction: Approximately 12% of human cancers show viral etiology. Among these viruses, in particular, cytomegalovirus and Epstein-Barr have been proven to have a tumorigenic role. Tumors of the central nervous system (CNS) represent a relatively small part of human tumor pathology, with just over 100 different histological types. Of note, several types of viral infections are known to be potentially associated with the development of gliomas. The aims of this study were: to conduct a reclassification of CNS tumors according to the new 2016 WHO guidelines and detect the presence of viruses previously linked with cancer in these tumors.

Materials and methods: Ninety-nine samples representing the most frequent tumor types in neurological practice have been selected from more than 200 CNS FFPE tumoral tissues, stored in the National Institute of Neurology and Neurovascular Diseases archives. Their characterization and classification was performed according to the new 2016 WHO guidelines, by immunohistochemistry. DNA was isolated from 40 samples and the detection of HSV 1 and 2, HV6, HV7, HV8, JCV, SV40 was performed with commercial kits based on real-time PCR.

Results and discussions: The tumor reclassification confirmed the initial diagnosis in most cases. Interestingly, a tumor originally classified as gliosarcoma has been diagnosed as fibrosarcoma because of its negative reaction to OLIG2. KIR7.1 and PHH3 have shown a particular sensitivity for choroid plexus tumors and the somatostatin receptor (SSTR2) a good sensitivity for the diagnosis of meningioma of any degree. No viral DNA has been detected in the studied samples. This can be due to the small sample size of the cohort analyzed so far and/or to the long-term formalin fixation of the tissue which may influence the quality of the isolated DNA.

Conclusions: Further studies on larger cohorts will be useful to better understand the role of viruses in CNS tumors. Moreover, new and more sensitive methods are needed for virus detection in FFPE samples. Another interesting option is represented by the use of fresh-frozen tissues.

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IMMUNOHISTOCHEMICAL AND MOLECULAR PROGNOSTIC FACTORS IN GASTRIC CARCINOMA

Alexandru Procop, Monica Hortopan, Mihaela Mihai, Catalin Pechianu, Elena Stoica Mustafa, Andreea Iorgescu, Simona O. Dima, Irinel Popescu, Vlad Herlea

Fundeni Clinical Institute, Bucharest

Keywords: microsatellite profile, immunohistochemistry, molecular classification, stomach

Introduction: Gastric carcinomas are malignant epithelial neoplasms and account for 8% of all cancers worldwide, with a declining incidence over the past two decades. The Lauren classification divides gastric carcinomas **into diffuse, intestinal, mixed and indeterminate types**, and a recent phenotype based on immunohistochemistry and molecular biomarkers – **microsatellite unstable** carcinomas.

Objective: The aim of this study was to present a series of nine cases of gastric carcinomas diagnosed at the Fundeni Clinical Institute, with their distinctive immunohistochemical and molecular characteristics and to classify them into prognostic groups according to their molecular phenotype.

Materials and methods: Samples were processed by conventional histopathological methods with Hematoxylin – Eosin staining; the immunohistochemical profile included a series of diagnostic biomarkers (microsatellite instability profile – MLH1, PMS2, MSH2, MSH6, p53, E-cadherin, Muc2, Muc5AC, Muc 6, CDX2, CD10, Her2Neu(c-erbB2))

Results: Out of 9 cases diagnosed at the Fundeni Clinical Institute, three were gastric adenocarcinomas well/moderately (G1/G2) differentiated with a microglandular, papillary and tubular pattern, 4 were gastric adenocarcinomas moderately/poorly (G2/G3) differentiated with a microglandular, microtrabecular, solid and tubular pattern and 2 cases were diagnosed as diffuse poorly differentiated carcinomas (G3) with a diffuse growing pattern and signet ring cells; five were male patients with a median age of 61.8 years and four were female patients with a median age of 46 years. All nine cases had a microsatellite stable phenotype with nuclear intense diffuse staining for MLH1, PMS2, MSH2, MSH6 between 60% and 90% and were E-cadherin positive (cytoplasmic and membranous staining). The difference in staining patterns was observed with p53 – a nuclear biomarker with intense aberrant staining in two gastric adenocarcinomas (85 and 90%) thus placing them in the fourth prognostic group. We tried to subdivide them based on the staining with the other biomarkers and compiled a **null phenotype (MUC2 -, MUC5AC -, MUC6 -, CDX2 – and CD10)** for a poorly differentiated (G3) microsatellite stable gastric adenocarcinoma, with normal E-cadherin and another with an intestinal phenotype (MUC2+, CDX2 +) for a well differentiated (G1) microsatellite stable adenocarcinoma, with a tubular pattern and normal E-cadherin. The gastric carcinomas were stained for Her2neu (c-erbB2) with 4 cases established as 3+ (positive staining) and 2 cases as 2+ (equivocal) eligible for Trastuzumab (Herceptin) treatment.

Conclusions: The classification of gastric carcinomas based on molecular and diagnostic biomarkers is a proper tool for establishing new phenotypes of gastric cancer, in order to compile more targeted future courses of treatments for a better survival rate (nowadays the five-year survival rate in gastric carcinomas is 20%).

METASTATIC COLONIC CANCER INVOLVING BLADDER - A CASE REPORT -

Mihaela Farcas, Larisa Zamfir, Bogdan Harsan, Luciana Nichita, Mirela Cioplea, Cristiana Popp

Colentina Clinical Hospital, Bucharest

Keywords: secondary bladder tumors, hematuria, bladder neck, immunohistochemistry

Background and objective: Bladder cancer is usually primitive (urothelial carcinoma being the fourth most common cancer in men), but 2% of bladder malignancies represent secondary neoplastic growths. In patients with primary colorectal adenocarcinomas, bladder involvement is most likely to be secondary to direct invasion from adjacent segments (e.g. recto-sigmoid in 21% of cases). Distant metastases from colorectal primary tumours have been reported very rarely. Secondary tumoral deposits are in 96.7% of cases solitary and are located in the trigone or bladder neck.

Methods: Here we present the case of a 76-year old man admitted for intermittent gross hematuria and pain in the lower abdomen. His medical history included a hemicolectomy for cancer of the transverse colon and removal of metastatic nodes adjacent to the stomach wall and epiploic apron, 3 years earlier.

Results: Cystoscopy detected a semi-pedunculated, nonpapillary (~2cm diameter), greyish tumour situated in the bladder neck. The histological evaluation of the resected specimen revealed a moderately differentiated intestinal-type adenocarcinoma. Immunohistochemical staining was performed and the tumour was positive for CDX2 and CK20, and negative for CK7 and cytokeratin 34βE12.

Conclusion: The bladder is an uncommon site for cancer metastases and, in the clinical follow up, it often goes undiagnosed. When bladder adenocarcinomas are identified histologically, suspicion of metastatic cancer from a distant site must be considered.

MPNST WITH UNKNOWN PREVIOUS HISTORY OF NEUROFIBROMATOSIS - CASE REPORT

Sorin Deacu[1], Liliana Mocanu[2], Liviu Mocanu[2], Elena Lupu[2], Alexandru Gavrila[3], Vladimir Fricatel[3]

[1]University Ovidius, Constanta, Faculty of Medicine
[2]Mangalia County Hospital
[3]Harsova County Hospital

Keywords: MPNST, neurofibromatosis, immunohistochemistry

We present a case of Malignant Peripheral Nerve Sheath Tumor localised on the left thigh of a 60-year old man, with an otherwise normal anamnesis. The tumor had 9.5/5cm and was gray-whitish and fasciculated, with hard consistency. On conventional stain, the tumor had alternating light and dark areas, the cells were spindled serpentine shaped; there were large gaping vascular spaces, non-branched, with focal perivascular plump tumor cells, geographic necrosis with tumor palisading at edges, resembling glioblastoma multiforme; numerous mitoses were noted.

Immunohistochemistry was performed on 5 μm thick sections from formalin fixed paraffin-embedded specimens, was done on automate platform and internal and external controls were used for establishing the results. It showed a 3+ intense and diffuse positive reaction to S-100 and vimentin, CD34 positive in about 5% of tumoral cells and negative reaction to AE1/3, CD99, SMA, desmin. Ki-67 was intensely positive in 40% of tumoral nuclei and p53 was overexpressed in 80% of tumoral nuclei. CD57 (leu7) was positive in neurofibroma-like areas.

We concluded that the intense and diffuse positivity to S-100, correlated with conventional aspects (non-branched vessels and areas of palisading necrosis glioblastoma-like), were consistent with the diagnosis of MPNST NF1-related.

PD-L1 EXPRESSION IN BREAST CARCINOMA

Oana Cristina Voinea

Carol Davila University of Medicine and Pharmacy, Bucharest

Keywords: breast carcinoma, PD-L1, prognostic factor

Objectives: Breast carcinoma, the most frequent malignant tumor among women worldwide, represents a pathologic condition that encompass a large scale of diseases, defined by histologic, molecular or prognostic criteria. Since the 2013 St Gallen consensus, substantial progress was done regarding the pathological characterization of breast cancer subtypes, by defining four distinct surrogate models. This paradigm allows, considering the therapeutic options, the assessment of prognosis by adding to the TNM and histological criteria the expression of the hormone receptor, HER2neu oncoprotein and proliferation index. Considering the latest advances in the treatment of oncological disease, we tested the presence of PD-L1 in breast cancers.

Materials and Method: Retrospectively, 36 malignant breast tumor specimens selected from the Pathology Department of the University Emergency Hospital Bucharest were analyzed. Different histotypes, molecular surrogate subtypes, stages and ages were comprised. ER, PR, Ki67, HER2neu, CD8 and PD-L1 were immunohistochemically tested. For statistical analysis SPSS was used.

Results: A positive correlation between the infaust prognostic factors (young age, advanced stage of disease, HER2-enriched or triple negative subtype) and PD-L1 expression was observed. CD8 and PD-L1 were also correlated.

Conclusions: In light of PD-1 PD-L1 monoclonal antibody results, we suggest that PD-L1 expression could be regarded as an individual prognostic marker and also, we encourage its testing as a therapeutic option in breast cancer also, as it is already successfully used in other malignancies.

PRIMARY HYPERTENSION AND RENAL DISEASE - A MORPHOLOGICAL STUDY OF FIVE CASES -

Alexandru Procop, Monica Hortopan, Sonia Balanica, Elena Georgia Micu, Ismail Gener, Vlad Herlea

Fundeni Clinical Institute, Bucharest

Keywords: kidney, nephrosclerosis, solidified glomeruli, hyalinosis

Introduction: Primary hypertension is a major disease worldwide, with a prevalence of over one billion people. The renal changes in primary hypertension are codependent on the blood pressure values and can be divided in benign nephrosclerosis (the most frequent) and malignant nephrosclerosis, a hypertensive emergency (less than 1% of patients).

Objective: The aim of this study was to present a series of five cases of renal disorders associated with high blood pressure diagnosed at Fundeni Clinic Institute between 2017 and 2018, with their distinctive morphological characteristics.

Materials and methods: Samples were biopsied at the Nephrology Department at the Fundeni Clinical Institute and processed by conventional histopathological methods with Hematoxylin-Eosin staining; in order to assess vascular hyalinosis and interstitial fibrosis, special stains were performed (Periodic Acid Schiff - PAS and Masson Trichrome)

Results: Out of five cases diagnosed by the Department of Pathology, all were male patients with a median age of 59 years (the youngest being 45 years old and the oldest 71 years old). Light microscopy revealed glomerular, tubular, interstitial and vascular involvement of varying degrees depending on the blood pressure values and the frequency of blood pressure spikes. We identified segmental and global glomerulosclerosis of varying degrees, mesangial hypercelullarity, the thickening of the basement glomerular membrane and also ischemic glomeruli with glomerular collapse of the capillary loops (in early disease) and solidified glomeruli in advanced disease. The tubules affected by hypertension had a thickened tubular basement membrane, atrophy of varying degrees and tubular thyroidization (luminal colloid casts). The interstitium revealed increased Trichrome Masson positive collagen and a chronic diffuse inflammatory process with frequent lymphoid aggregates in the scarring areas. The most frequent changes (5/5) were of vascular origin with medial thickening and intimal fibrosis of medium and large size vessels and diffuse circumferential PAS positive hyalinosis.

Conclusion: The renal disease in essential hypertension can be revealed by conventional histopathological methods and special stains. Prolonged high blood pressure without subsequent treatment will cause target kidney damage and end stage renal failure.

REFRACTORY CELIAC DISEASE CHALLENGES OF DIAGNOSIS AND POSSIBLE COMPLICATIONS

Cristiana Popp[1], Alexandra Bastian[1,2], Eliza Gramada[1], Luciana Nichita[1,2], Liana Sticlaru[1], Alexandra Cioroianu[1], Andrei Voiosu[1,2], Mirela Cioplea[1]

[1]Colentina University Hospital, Bucharest
[2]Carol Davila University of Medicine and Pharmacy, Bucharest

Keywords: gluten-free diet, ulcerative jejunitis, villous atrophy

Background: Celiac disease is a chronic malabsorbtive inflammatory condition of the gastro-intestinal tract, triggered by dietary gluten. It is a common disease, diagnosed at any age, usually with a good response to a gluten-free diet. Refractory celiac disease is defined by persistence of symptoms and histologically proven inflammation and villous atrophy despite strict adherence to the diet for at least 6-12 months. Patients with refractory celiac disease require aggressive treatment and have a high incidence of severe complications: malabsorption syndrome, ulcerative jejunitis and enteropathy-associated lymphoma.

Materials and method: We present two cases of refractory long-standing celiac disease. Both patients are middle-aged women, with very good adherence to the gluten-free diet and variable evolution.

Results: One of the patients had minor persistent symptoms with periodic recurrences of severe diarrhea and malabsorption and persistent histological inflammation and villous atrophy. The other patient had a more aggressive disease, with extended ulcerative jejunitis and oligoclonal selection of lymph cells.

Conclusion: Refractory celiac disease is a big challenge for the gastroenterologist and as well as the pathologist, requiring aggressive treatment, thorough surveillance and, sometimes, advanced techniques for the early diagnostic of forerunners of lymphoid malignancies.

ROLE OF EPITHELIAL TO MESENCHYMAL TRANSITION IN INVASIVE SQUAMOUS CELL CARCINOMA ARISING IN ACTINIC KERATOSIS

Alexandra Ioana Cioroianu, Patricia-Irina Stîngă, Cristiana Popp, Luciana Nichita, Mirela Cioplea, Liana Sticlaru, Răzvan Andrei, Sabina Zurac

Colentina Clinical Hospital, Bucharest

Keywords: E-cadherin, vimentin, beta-catenin, cutaneous cancer

Background: Actinic keratosis (AK) is the most common precursor of invasive squamous cell carcinoma (iSCC), but many AKs will either persist at the same stage or regress, while only a few will progress into iSCC following two main pathways, classical and differentiated. Epithelial to mesenchymal transition (EMT) is an intricate process by which epithelial cells loose epithelial characteristics and acquire a mesenchymal-like phenotype, being involved in tumour invasion, dedifferentiation and metastasis. Recently, EMT was found to be involved in the transformation from AK into iSCC (differentiated pathway), whereas a higher proliferative capacity facilitates intraepidermal extension in the classical pathway. Our objective was to determine the expression pattern of E-cadherin, vimentin and beta-catenin in normal skin, AKs, iSCCs arising from AKs, and de novo iSCCs.

Methods: We evaluated by immunohistochemistry (checking for E-cadherin, vimentin and beta-catenin) 20 consecutive cases of iSCC arising from AKs, 10 of AKs without any evidence of iSCC, 10 of de novo iSCCs and 30 samples of normal skin. Levels of membranous or cytoplasmic staining and the presence of aberrant expression were examined.

Results: In 50% of cases, E-cadherin expression was reduced in the same fashion in iSCCs and superjacent AKs. In 40% of cases the expression was progressively reduced in the epidermis, with solar elastosis through solar keratosis to SCC, independent of AK type, differentiation grade, tumor thickness or level of invasion. Reduced expression was also found in cases of AK without evidence of iSCC and more clearly separates atypical areas from normal skin. E-cadherin was better preserved in de novo iSCCs and progressively reduced expression correlated better with tumor thickness and SCC differentiation than in iSCCs arising in AKs. Diffuse pattern of loss of membranous beta-catenin staining correlated better with SCC differentiation than reduced expression in general or aberrant cellular localization of beta-catenin in iSCCs arising in AKs. De novo SCCs presented a decreased expression of beta-catenin from superficial to deeper tumor areas and from better to poorly differentiated areas. Also, a good correlation was observed between beta-catenin and E-cadherin loss/reduced expression in both types of SCC and in AKs. The number of vimentin positive cells correlated with tumor thickness and SCC differentiation and they were usually located at the periphery of the tumor and adjacent to the stroma.

Conclusion: We suggest that cutaneous SCC shows EMT, but E-cadherin, beta-catenin and vimentin are not eligible markers to evaluate the risk of progression from AK into iSCC. It is also possible that the loss of intercellular adhesion is only one of the stages required for the occurrence of progression and transformation.

Acknowledgement: This work was supported by a grant of Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number 61PCCDI/2018 PN-III-P1-1.2-PCCDI-2017-0341, within PNCDI-III.

THE IMPORTANCE OF BIOLOGICAL FLUIDS AS HISTOPATHOLOGICAL AND MOLECULAR MATERIAL SOURCE IN DIAGNOSIS AND COMPLEX EVALUATION OF LUNG CANCER

Liliana Mocanu[1], Laura Mazilu[1], Marius Militaru[1], Doina Tofolean[1], Ariadna-Petronela Fildan[2], Liviu Mocanu[3], Sorin Deacu[4], Catalina Muntean[2], Paul Corici[5], Ana Maria Cretu[1], Valentina Oancea[6]

[1]Constanta County Clinical Emergency Hospital; [2]Constanta Clinical Hospital of Pneumology; [3]Mangalia Municipal Hospital; [4]Constanta County Service of Forensic Medicine; [5]Medgidia Municipal Hospital; [6]Cernavoda City Hospital

Keywords: cytobloc, immunotherapy, EGFR

We present a case of fulminant lung cancer that clinically presented with dyspnoea, thoracic pain and massive pericardial effusion. The patient initially presented in the emergency room in a severe condition and he was quickly transferred to the cardiovascular surgery department, where the pericardic fluid was evacuated (about 0.7 l of liquid). The fluid was sent to our cytology and histopathology department. Conventional slides were made, stained with Giemsa and BPN. Cytological diagnosis was positive for malignancy and 2 cytoblocks were made, for immunohistochemical evaluation of tumour phenotype and for molecular tests. Subsequently, the patient underwent a transbronchial biopsy that was also evaluated histopathologically in our laboratory. The immunohistochemical tests were done on the same platform and with the same antibodies for both the cytoblock and bronchial sample, the results being identical. Immunohistochemically, the tumoral cells were positive for TTF1, negative for P40. A diagnosis of malignant pericardic fluid positive for cells of pulmonary adenocarcinoma was made. One of the 2 cytoblocks was sent for molecular tests. No EGFR mutation was detected. Imaging confirmed a huge lung tumor. Oncologic evaluation established a T4 lung adenocarcinoma and the patient received immunotherapy (anti-pd-l1 agent) for 1 month. Unfortunately, the patient died after one month. Our case revealed the utility of the cytoblock method in establishing the positive diagnosis of malignancy and tumoral phenotype, and in obtaining good material for molecular tests.

TP53 DELETION IN CHRONIC LYMPHOCYTIC LEUKEMIA

Raluca Mihaela Colesniuc[1,2], Sorina Mihaela Papuc[1], Ioana Borcan[1], Diana Cisleanu[3,2], Horia Bumbea[3,2], Ana Maria Vlădăreanu[3,2], Emilia Severin[2], Aurora Arghir[1,2]

[1]Victor Babeș National Institute of Pathology, Bucharest
[2]Carol Davila University of Medicine and Pharmacy, Bucharest
[3]Emergency University Clinical Hospital, Bucharest

Keywords: chronic lymphocytic leukemia, TP53 aberrations, prognosis

Chronic lymphocytic leukemia (CLL), the most common leukemia in adults, is characterized by a clonal expansion of mature, apoptosis resistant B-cells. TP53 gene aberrations, due to chromosome 17p deletion and/or gene mutations, are hallmarks of aggressive disease with an adverse prognosis and poor response or even resistance to treatment. Analysis of TP53 aberrations has, thus, been incorporated into routine clinical diagnostics in order to improve patient stratification and therapeutic decisions. Furthermore, in the era of targeted therapies with high efficacy in TP53-defective patients, such as inhibitors of B-cell receptor signaling and BCL2 family members, the identification of these anomalies has become even more significant.

We present the results of a study aiming to identify the presence of the 17p deletion in a CLL group consisting of 60 patients. Fluorescence in situ hybridization (FISH) tests on peripheral blood samples were performed using Vysis CLL FISH Probe Kits (Abbott Molecular) with probes specific for TP53 and ATM loci, 13q and 6q regions and chromosome 12 centromere.

Six out of 60 CLL patients showed deletion of 17p13.1 locus. In 5 patients positive for TP53 deletion at least one additional genetic defect co-occurred. For one CLL patient TP53 deletion was the sole anomaly detected with the FISH assays used. These results are concordant with literature data, highlighting the fact that TP53-mutated patients have an increased genomic instability and thus a propensity for accumulation of various genetic lesions.

The screening for del 17p/TP53 mutations stands as a critical step in the management of CLL patients. Identifying TP53 gene defects leads to improved risk stratification and optimized treatment decisions for CLL patients.

VARIATION OF THE GENE EXPRESSION PROFILE OF ADHESION AND EXTRACELLULAR MATRIX MOLECULES DURING SKELETAL MUSCLE REGENERATION

Laura Cristina Ceafalan[1,4], Maria Dobre[1], Elena Milanesi[3], Emilia Manole[3], Andrei Niculae[4], Mihail Eugen Hinescu[1,4]

[1]Department of Pathology, Victor Babeș National Institute of Pathology, Bucharest; [2]Department of Cellular, Molecular Biology and Histology, Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Department of Cellular and Molecular Medicine, Victor Babeș National Institute of Pathology, Bucharest; [4]Histopathology Laboratory, Victor Babeș National Institute of Pathology, Bucharest

Keywords: muscle injury, cell–matrix interactions, cell migration

Skeletal muscle has an exceptional regeneration capacity relying on the careful coordination of the dynamics of recruited myeloid cells with specific stages of the myogenic process and extracellular matrix remodelling. However, severe muscle injury produces persistent pain and severe dysfunction. There are no specific biomarkers to diagnose the severity and prognosis of muscle lesions and the effectiveness of stem cell based therapies is still very low, even after intramuscular injection. An analysis of cellular and molecular microclimate during normal regeneration is mandatory for deciphering interactions that lead to efficient cell differentiation and functional recovery.

We used Mouse Extracellular Matrix & Adhesion Molecules RT2 Profiler PCR Array to analyse the profile of adhesion and extracellular matrix molecules as premise for cellular cooperation and migration pattern and extracellular matrix remodelling. We also compare the gene expression changes induced by crushing with those reported by previous studies on different experimental injury models.

The gene expression study was conducted on samples from the injured area of the crushed and contralateral gastrocnemius muscle, 3 and 5 days after inflicting an extensive mechanical trauma in adult C57BL/6J mice. These time-points correspond to the peak activity of different phenotypes of the recruited myeloid cells. The results indicated that the expression profile differs from other experimental injury models, previously reported.

In our model 22 of 84 genes investigated on the array were differentially expressed, 9 at 3 days post-injury and 19 at 5 days post-injury. ITGAM upregulation at day 3 post-injury demonstrates the high proportion of pro-inflammatory macrophage in the injured area. Only 6 genes were found differentially expressed both at 3 and 5 days post-injury. One group of genes comprising FN1, THBS2 and VCAN were constantly upregulated. This small group of transcripts encode for proteins that play important roles in cell–matrix interactions for adhesion and migration during inflammatory responses and were shown to influence the immune cell phenotype. The second group including genes that are generally expressed by myogenic cells - LAMA2, TIMP3 and VTN - were downregulated, but more drastically at 3 days post-injury, the time-point when myogenic differentiation starts.

This study identifies a gene expression pattern for further testing in order to elucidate the dynamics of the mechanisms of scar-free healing.

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THE MLPA ASSAY IMPROVES DIAGNOSTIC IN GENETIC DISEASES

Gisela Gaina[1], Magdalena Budisteanu[2], Emilia Manole[1]

[1]*Victor Babeș National Institute of Pathology, Bucharest*
[2]*Clinical Hospital of Psychiatry Doctor Alexandru Obregia, Bucharest*

Keywords: MLPA, muscular dystrophy, molecular diagnostics.

Introduction: The development of molecular biology techniques in the past few years has led to improved diagnosis for several genetic diseases. For all genetic diseases with pathogenesis related to the presence of a large rearrangement such as deletions or duplications in human specific genes, the MLPA (Multiplex Ligation-Dependent Probe Amplification) assay seems to represents the gold standard for molecular analysis and diagnosis.
The aim of the study was to assess the diagnostic and prognostic utility of genetic analysis in different types of muscular dystrophy, like Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD) and Limb Girdle muscular dystrophy (LGMD2A).
Materials and Methods: The study was conducted on 30 D/BMD patients identified with a dystrophin deficiency, based on abnormal immunofluorescence staining and reduced to absent dystrophin bands at 427kDa by Immunofluorescence (IF) and multiplex western blot (WB), respectively, and 10 patients with clinical diagnosis of LGMD 2A and a reduced intensity of calpain3 band at 94kDa by WB. For the molecular analysis of DMD and CAPN3 gene, MLPA was performed on DNA samples from normal and dystrophic patients.
Results: Here, we compared results obtained by IF and WB with those by MLPA in order to establish the value of the MLPA assay in muscular dystrophy diagnosis. Based on the pattern expression of dystrophin by qualitative and quantitative methods, 25 DMD and 5 BMD subjects were identified. Based on the mutational pattern of the dystrophin gene (DMD), 23 DMD and 7 BMD patients were identified. According to the frame shift theory and protein analysis results another 25 DMD cases and 5 BMD cases were diagnosed. Out of 10 samples identified with a reduced 94-kDa calpain3 specific band on western blot, 5 cases did not present mutations in the CAPN3 gene. Our results demonstrate the important role of genetic analysis for a precise diagnosis in LGMD2A, taking in consideration false positive results on WB. In D/BMD only correlations between molecular and protein data could establish with accuracy the phenotype and disease severity. Comprehensive genetic testing for dystrophinopathy could detect 95% of pathogenic variants in the DMD gene and is often the preferred diagnostic approach, as it avoids the risks of an invasive procedure like muscle biopsy of a seriously affected muscle. More than two-thirds of affected patients have large rearrangements which can be detected by MLPA. The remaining cases include small mutations, which cannot be easily identified by routine techniques.
Conclusions: Given the value of the information provided by genetic analyses, MLPA is therefore a critical tool in the accurate diagnosis of muscular dystrophy, as well as of other diseases which involve large deletions and duplications. Due to this ability, the MLPA assay can be used in the molecular diagnosis of several diseases and could be implemented in routine diagnosis.

GUT MICROBIOTA-RENAL AXIS: EXPLORATION OF NOVEL THERAPEUTIC AVENUES IN RENAL DISEASES

Simona Mihai[1], Elena Codrici, Ionela Daniela Popescu[1], Ana-Maria Enciu[12], Lucian Albulescu[1], Radu Albulescu[1], Eleonora Codorean[1], Cristiana Tanase[1]

[1]Victor Babeș National Institute of Pathology, Bucharest
[2]Carol Davila University of Medicine and Pharmacy, Bucharest

Keywords: inflammation, microbiota, omics

Introduction: Accumulating evidence over the recent years revealed that chronic inflammation represents a nontraditional risk factor in chronic kidney disease (CKD) and highlighted that intestinal tract is a major player that orchestrates systemic inflammation. Gut microbiota can be considered a recently discovered "organ," being involved in many pathological axes, including kidneys. Intestinal inflammation and gut microbiota dysbiosis are now recognized as important contributors in chronic inflammation and other CKD complications, thus explaining the gut-therapeutic novel avenues taken into consideration in designing CKD interventions.

Dietary patterns in CKD - Deciphering the role of gut microbiota in CKD progression needs a complex comprehension regarding its composition, function, and homeostasis. Gut microbiota composition shows great variations, representing a unique signature with each individual harboring, consisting mainly of Gram-negative Bacteroidetes and the Gram-positive low-GC Firmicutes. Key mechanisms to preserve gut microbiota balance are considered to include special diets, enriched in non-digestible carbohydrates, subject to fermentation by gut microbiota, with low quantities of proteins or fats. It was also revealed that dietary content and their metabolites, such as advanced glycated end products (AGEs) could be closely linked to CKD. Promising therapeutic targets based on nutrition approaches include uremic toxin absorbents and inhibitors of AGEs or the receptor for AGEs.

Promising alternative therapy - Another area of potential beneficial therapies in CKD relies on administration of prebiotics and probiotics, and the combination of both therapies into "synbiotic" preparations. Gut dysbiosis in CKD was correlated with an increase in pathogenic flora compared to symbiotic flora, which, along with enhanced intestinal permeability, increases absorption of endotoxins, with harmful consequences in the organism. The gut-derived uremic toxins, along with an expanded permeability of the intestinal barrier, have been correlated with an increased inflammatory state and oxidative stress, constant features of advanced CKD. Considering the potential of all these preparations in shifting the uremic toxin expression and also in delaying the CKD progression, the exploration of these inoffensive nutritional therapy provides promising therapeutic avenues.

Omics approaches in CKD – Gut dysbiosis represents an underappreciated cause of inflammation, and subsequently could lead to malnutrition, accelerated cardiovascular disease and CKD. In this scenario, a huge step forward was made by the increasing progression of Omics approaches, providing novel insights in deciphering CKD pathophysiology, thus identification of specific circulating biomarker panels could improve CKD prediction, progression and outcome.

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COLLAGEN, A VERSATILE BIOMATERIAL: ITS SOURCES AND POTENTIAL BIOMEDICAL APPLICATIONS

Ionela Daniela Popescu[1], Simona Mihai[1], Elena Codrici[1], Ana-Maria Enciu[1,2], Lucian Albulescu[1], Radu Albulescu[1], Eleonora Codorean[1], Mihaela-Adi Lupu[3], CristianaTanase[1,4]

[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]SC Sanimed International Impex SRL, Bucharest; [4]Titu Maiorescu University Faculty of Medicine, Bucharest

Keywords: collagen, biomaterials, regenerative medicine

Introduction: The use of collagen-based biomaterials in regenerative medicine has achieved a remarkable increase over the recent years. Collagen represents the most abundant protein found in the animal kingdom, and there are different identified types that exert specific functions in our body; based on their structural features, each type of collagen exhibits different distinctiveness. Collagen, as one of the main proteins in the human body, has been expansively used in the development of biomaterials, which can be used as substitutes or can assist in regenerative medicine. Collagen is broadly used in tissue engineering because it can be extracted in large quantities, and has excellent biocompatibility, good biodegradability, and weak antigenicity. The use of biomaterials in biomedical areas can change the direction of research in tissue engineering; the goal is the self-repair of the organism based on collagen scaffolds that may contain different bioactive molecules.

Characterization of collagen as a biomaterial - The structure and remodeling of collagen is critical to the pathology and healing of various major diseases. Collagen structure is complex because of both diversity of source materials, chemistry, and structural hierarchy. Characterization of collagen includes: structural detail-mainly focused on molecular mass, purity, helical content, bulk thermal properties; chemical features-mainly focused on surface elemental analysis and hydrophobicity; and morphological features at different length scales.

Different types of collagens application - Collagen bio-scaffolds can be used in a wide variety of medical fields, and is important to develop a process that makes them as robust and viable as possible for their end purposes. The collagen-based biomaterials can be used in vivo - to support the regrowth of tissue or bone after surgery or an injury and in vitro - for example, growing new organs from adult stem cells.

Conclusion: Due to collagen excellent biocompatibility, weak antigenicity and biodegradability, collagen-based biomaterials are expected to become beneficial scaffolds for various biomedical applications in the future.

Acknowledgement: This work was partially supported by the grants COP A 1.2.3., ID: P_40_197/2016 and PN 18.21.01.06.

FLT3 MUTATIONAL SCREENING AND CHARACTERIZATION IN ACUTE MYELOID LEUKEMIA PATIENTS AT DIAGNOSTIC

Alina Erbescu[1], Sorina Mihaela Papuc[1], Ana-Maria Vlădăreanu[2,3], Horia Bumbea[2,3], Aurora Arghir[1,2]

[1]Victor Babeș National Institute of Pathology, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest; [3]Emergency University Clinical Hospital, Bucharest

Keywords: D835 mutation, internal duplications, polymerase chain reaction

Background: The Fms-like tyrosine kinase 3 (FLT3) gene, located on chromosome 13q12, is a receptor tyrosine kinase involved in hematopoietic stem/progenitor cell survival and proliferation. The constitutive activation of FLT3 receptor leads to aberrant proliferation of myeloid leukemic blasts. FLT3 gene mutations have been reported in 20–30% of AML patients; the most common being the internal tandem duplications (ITD) in juxtamembrane domain and point mutations in tyrosine kinase domain.

Aim: to evaluate the incidence of FLT3-ITD and D835-TKD mutations in a diagnostic cohort of AML patients and to molecularly characterize the ITDs.

Methods: Genomic DNA was extracted from bone marrow samples with PureLink DNA Kit (Invitrogen). A PCR-based assay covering exons 14 and 15 was applied for FLT3-ITDs assessment. For D835 mutation, PCR amplification of TKD followed by EcoRV digestion was performed. Fragment analysis of amplified products was performed by capillary electrophoresis (ABI 3500 Genetic Analyzer, GeneMapper Software). For ITDs sequence characterization Sanger sequencing using BigDye Terminator v3.1 protocol was applied.

Results and discussion: 35 AML patients were evaluated for the presence of FLT3-ITDs and D835 mutations. FLT3-ITDs were detected in 8 (22.85%) patients, while D835 mutation was present in 2 (5.71%) patients. Sanger sequencing was done for 24 samples. ITD mutations showed different position of the insertion point and variable sizes. More than one ITD mutant allele were observed in at least one patient.

Conclusions: FLT3 gene mutations were identified in 28.5% of AML patients, with a high frequency of ITD variants (80% of FLT3 mutations). The data obtained in our patient group are consistent with previously published data and add valuable information regarding less well understood aspects such as insertion point, size and sequence of FLT3-ITDs.

Acknowledgement: Funding: Ministry of Research and Innovation through grants PN18.21.01.03 and PN16.22.05.01.

IMPROVING THE PURIFICATION OF MITOCHONDRIA FOR CRYO-ELECTRON MICROSCOPY

Silvia-Diana Prelipcean, Tudor Emanuel Fertig, Ana-Maria Enciu, Mihaela Gherghiceanu

Victor Babeș National Institute of Pathology, Bucharest

Keywords: mitochondria, organelle purification, cryo-EM

Introduction: Mitochondria are membrane bound organelles, that are essential for cell viability by providing energy through oxidative phosphorylation, contribute to the synthesis of fatty acids, heme and iron-sulfur cluster and are involved in apoptosis. Approximately 1000 proteins are imported inside the mitochondria, but the mitochondrial genome also encodes thirteen polypeptides that are synthesized inside the mitoribosomes, ribosomes that are associated to the mitochondrial inner membrane. A growing number of studies set out to identify and precisely localize proteins within mitochondria, especially the membrane protein complexes involved in the respiratory process. One of the most useful and novel tools for this purpose is cryo-electron microscopy (cryo-EM), which offers unprecedented, nanometer-scale resolution. Because it undergoes quick freezing in vitreous ice, the cryo-EM sample must be free of contaminants (e.g. other organelles, membrane fragments, cellular debris etc.). However, currently available commercial methods of mitochondrial purification from cultured cells lack the required purity for cryo-electron microscopy and cryo-electron tomography analysis. Here we present an improvement on these methods, which offers suitable mitochondrial purity for cryo-EM analysis.

Methods: For cryo-EM, mitochondria were purified from Ba/F3 cells by differential centrifugation, using a commercially available isolation kit (Thermo Fisher Scientific) and/or previously published methods. Cryo-EM was done by rapid plunging in liquid nitrogen cooled liquid ethane using an automated Leica EM GP, then grids were transferred to a 626 Gatan cryo-holder and visualized at -180°C inside a Talos F200C transmission electron microscope. Digital micrographs were recorded using a Ceta 4x4k camera at 6,700x to 22,000x nominal magnifications.

Results and conclusions: Mitochondria were identified as round or ellipsoid organelles, characterized by an outer membrane that enclosed an inner membrane, the inner membrane being folded into lamellar cristae, and a relatively constant intermembrane space. When using standard protocols for density gradient and differential centrifugation isolation sample purity was relatively low, with numerous vesicles and membrane fragments in contact with or in the proximity of mitochondria. This made identification of mitochondria relatively difficult, especially compared to multilayered vesicles (an artefact generated by the hypertonic solution in which the probes are processed). Following the adjusted protocol, sample purity was higher, with clearly discernable mitochondria and less frequent accumulations of other subcellular fragments. This allowed for the acquisition of high resolution cryo-EM images and represent a useful method for future studies involving mitochondria.

MUCINOUS ADENOCARCINOMA OF THE COLORECTUM – A SERIES OF EIGHT CASES: CLINICAL CHARACTERISTICS, MORPHOPATHOLOGICAL ASPECTS AND IMMUNOHISTOCHEMICAL REACTION FOR MLH1, PMS2, MSH2, MSH6

Irina Alexandra Ostahi[1], Valentin Enache[1], Florin Andrei[1], Stefania Varban[2], Florina Vasilescu[1], Ioana Ruxandra Pirvu[1], Gabriel Becheanu[1]

[1]Victor Babeș National Institute for Pathology, Bucharest
[2]Emergency University Hospital, Bucharest

Keywords: colorectal carcinoma, microsatellite, instability, MSI

Colorectal cancer is the most frequent digestive tract tumor. Colorectal cancer morphology includes many variants, the most common being adenocarcinoma. Adenocarcinoma of the colon has several subtypes: conventional type, cribriform comedo-type adenocarcinoma, medullary, micropapillary, mucinous, serrated and signet ring cell. The designation of mucinous adenocarcinoma (MA) is used if >50% of the lesion is composed of pools of extracellular mucin containing malignant epithelium. It is thought that many MAs have a high status of microsatellite instability (MSI-H).

We analyzed a series of eight cases of colorectal mucinous carcinomas registered at Victor Babeș National Institute of Pathology, according to clinical characteristics, morphopathological aspects and immunohistochemical reaction for MLH1, PMS2, MSH2, MSH6 (Automatic Ventana Benchmark).

The average patient age was 56.37 years old. 75% of cases were present in male patients, while 25% in female patients, rendering a male:female ratio of 3:1. Regarding the tumor location, 62.5% were located on the left colon, 25% were located in the right colon, and the rest had an unknown exact location. The average tumor dimension was 6.38 cm, ranging from 3.5 to 12 cm. The majority of the tumors (75%) were pT3, invading the subserosa. Angiolymphatic invasion (AI) was observed in three of the cases, while perineural in only one case, which also had AI. 37.5% of the cases had lymph node dissemination, 50% did not have lymph node dissemination, and 12.5% had tumoral deposits. Immunohistochemical analysis for microsatellite instability status revealed that three of the cases were MSI-H, two of them having no lymph node dissemination and one having tumoral deposits. The three cases with MSI-H were located as following: one on the left colon, one the right colon and one with unknown exact location.

Mucinous adenocarcinoma is commonly associated with an unfavorable outcome. However, a MSI-H status confers a better prognosis and outcome, which suggests immunohistochemical analysis with microsatellite markers should be performed for all colorectal mucinous adenocarcinomas.

ROLE OF EPITHELIAL TO MESENCHYMAL TRANSITION IN INVASIVE SQUAMOUS CELL CARCINOMA ARISING IN ACTINIC KERATOSIS

Alexandra Ioana Cioroianu, Patricia-Irina Stîngă, Cristiana Popp, Luciana Nichita, Mirela Cioplea, Liana Sticlaru, Răzvan Andrei, Sabina Zurac

Colentina Clinical Hospital, Bucharest

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Background: Actinic keratosis (AK) is the most common precursor of invasive squamous cell carcinoma (iSCC), but many AKs will either persist at the same stage or regress, while only a few will progress into iSCC following two main pathways, classical and differentiated.
Epithelial to mesenchymal transition (EMT) is an intricate process by which epithelial cells loose epithelial characteristics and acquire a mesenchymal-like phenotype, being involved in tumour invasion, dedifferentiation and metastasis. Recently, EMT was found to be involved in the transformation from AK into iSCC (differentiated pathway), whereas a higher proliferative capacity facilitates intraepidermal extension in the classical pathway. Our objective was to determine the expression pattern of E-cadherin, vimentin and beta-catenin in normal skin, AKs, iSCCs arising from AKs, and de novo iSCCs.
Methods: We evaluated by immunohistochemistry (checking for E-cadherin, vimentin and beta-catenin) 20 consecutive cases of iSCC arising from AKs, 10 of AKs without any evidence of iSCC, 10 of de novo iSCCs and 30 samples of normal skin. Levels of membranous or cytoplasmic staining and the presence of aberrant expression were examined.
Results: In 50% of cases, E-cadherin expression was reduced in the same fashion in iSCCs and superjacent AKs. In 40% of cases the expression was progressively reduced in the epidermis of skin, with solar elastosis through solar keratosis to SCC, independent of AK type, differentiation grade, tumor thickness or level of invasion. Reduced expression was also found in cases of AK without evidence of iSCC and more clearly separates atypical areas from normal skin. E-cadherin was better preserved in de novo iSCCs and progressively reduced expression correlated better with tumor thickness and SCC differentiation than in iSCCs arising in AKs. Diffuse pattern of loss of membranous beta-catenin staining correlated better with SCC differentiation than reduced expression in general or aberrant cellular localization of beta-catenin in iSCCs arising in AKs. De novo SCCs presented a decreased expression of beta-catenin from superficial to deeper tumor areas and from better to poorly differentiated areas. Also, a good correlation was observed between beta-catenin and E-cadherin loss/reduced expression in both types of SCC and in AKs. The number of vimentin positive cells correlated with tumor thickness and SCC differentiation and they were usually located at the periphery of the tumor and adjacent to the stroma.
Conclusion: We suggest that cutaneous SCC shows EMT, but E-cadherin, beta-catenin and vimentin are not eligible markers to evaluate the risk of progression from AK into iSCC. It is also possible that the loss of intercellular adhesion is only one of the stages required for the occurrence of progression and transformation.

Acknowledgement: This work was supported by a grant of Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number 61PCCDI/2018 PN-III-P1-1.2-PCCDI-2017-0341, within PNCDI-III.

INDEX

G

H

I

J

K

L

M

N

O

P

R

S

Ș

T

U

V

W

Z

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OMNIVET este o companie cu capital exlusiv privat, infiintata in Romania in anul 2004.Cu o prezenta de peste 12 ani in Romania Omnivet este partener exclusiv al unor companii prestigioase din intreaga lume:

Cu un portofoliu de peste 150 de clienti, (spitale, laboratoare private, centre medicale, laboratoare sanitar-veterinare si institute de cercetare) Omnivet oferta solutii pentru a permite partenerilor nostrii sa obtina rezultatele de care au nevoie in cel mai scurt timp posibil cu cele mai inovatoare tehnologii, in diferite domenii:
Cercetare (prin tehnici PCR, qPCR, secventiere, NGS)
Diagnostic molecular (prin tehnici NAT, qPCR, pirosecventiere, hybrid capture, NGS) pentru diferite tipuri de cancere, boli infectioase virale si bacteriene si serologic(ELISA) pentru tuberculoza latenta.
Identificare umana (determinarea paternitatii si a profilelor umane in cazuri judiciare prin fluxuri de lucru validate)
Diagnostic veterinar, sanatate animala si siguranta alimentelor (prin tehnici ELISA, PCR si qPCR).

Roche este unul dintre liderii mondiali ai industriei farmaceutice și de diagnostic, care se axează pe promovarea științei pentru îmbunătățirea vieții oamenilor. Prin sinergia farmaceuticelor și tehnicilor de diagnostic, Roche a devenit lider în îngrijirea medicală personalizată - o strategie care vizează asigurarea tratamentului adecvat pentru fiecare pacient în cel mai bun mod posibil.
Roche este cea mai mare companie de biotehnologie din lume, cu medicamente diferențiate clinic în domeniile oncologiei, imunologiei, bolilor infecțioase, oftalmologiei și al afecțiunilor sistemului nervos central. Lider mondial în diagnosticul in vitro și în diagnosticul histologic al cancerului, Roche este pioner și în managementul diabetului.
Fondată în 1896, Roche continuă să caute modalități mai bune de prevenire, diagnosticare și tratare a bolilor și de a aduce o contribuție durabilă în societate. Compania își propune să îmbunătățească accesul pacienților la inovațiile medicale, colaborând cu toate părțile relevante interesate.
Treizeci de medicamente dezvoltate de Roche sunt incluse pe Lista Medicamentelor Esențiale elaborată de Organizația Mondială a Sănătății, printre care antibiotice vitale, antimalarice și medicamente împotriva cancerului.
Roche a fost recunoscută pentru nouă ani la rând de către Indicele Dow Jones pentru Durabilitate (DJSI) ca lider de grup în domeniul durabilității în Industria Farmaceutică, Biotehnologie și Științe ale Vieții. Având sediul central la Basel, în Elveția, grupul Roche operează în peste 100 de țări, iar în 2017 numărul angajaților săi la nivel global se ridica la aproximativ 94.000 de persoane.
În 2017, Roche a investit 10,4 miliarde de franci elvețieni în cercetare și dezvoltare și a înregistrat vânzări de 53,3 miliarde de franci elvețieni. Compania Genentech, din Statele Unite, este deținută în totalitate de Grupul Roche. De asemenea, Roche este acționarul majoritar al companiei Chugai Pharmaceutical, Japonia.

The organizers would like to express their gratitude to the
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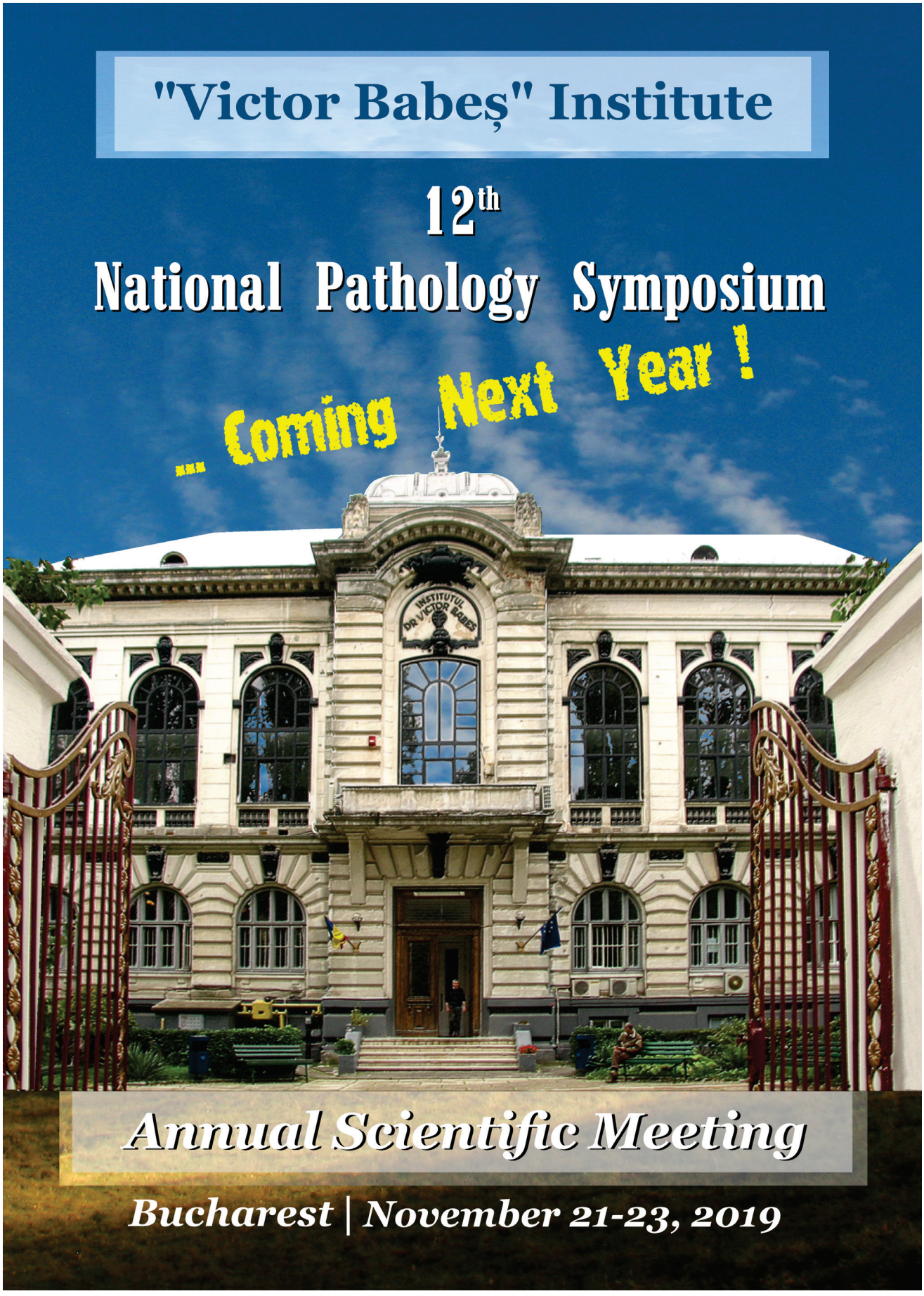
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