Victor Babeș National Institute of Pathology
Romanian Academy of Medical Sciences
Romanian Division of the International Academy of Pathology
COMUNIC Association
SOMS | Scientific Organisation of Medical Students

12th National Pathology Symposium

Annual Scientific Meeting

21 - 23 November, 2019
Bucharest, Romania

ABSTRACT BOOK
(and Meeting Program)

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PROGRAM

THURSDAY, NOVEMBER 21

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)
Victor Babeș National Institute of Pathology – A Short Overview
Mihail Eugen Hinescu, Mihaela Gherghiceanu, Cristiana Tănase, Monica Neagu, Gina Manda, Bogdan Ovidiu Popescu

11:30 – 11:45 COFFEE BREAK

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

Podocyte Injury in Fabry Disease: Old Concepts and New Paradigms

Prof. Behzad Najafian

Department of Pathology, University of Washington, Seattle, USA

13:00 – 14:00 LUNCH BREAK

14:00 – 16:00 **Session 2A: Nephropathology** (Victor Babeș Auditorium)
Chairs: Assoc. Prof. Gener Ismail & Assoc. Prof. Mihaela Gherghiceanu

Scoring System for Renal Pathology in Fabry Disease
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[1]Emergency University Hospital, Bucharest, Romania; [2]Nephrology Department, Fundeni Clinical Institute, Bucharest, Romania; [3]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; [4]Victor Babeș National Institute for Pathology, Bucharest, Romania

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[1]Nephrology Department, Fundeni Clinical Institute, Bucharest; [2]Carol Davila University of Medicine and Pharmacy Bucharest; [3]Fundeni Clinical Institute, Center of Uronephrology and Kidney Transplantation; [4]Victor Babeș National Institute of Pathology, Bucharest, Romania

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[1]Nephrology Department, Fundeni Clinical Institute, Bucharest; [2]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

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[1]University Emergency Hospital Bucharest, Romania; [2]Carol Davila University of Medicine and Pharmacy Bucharest, Romania

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Leila Ali[1], Valentin Moldovan[1], Ondrej Hes[2]
[1]Victor Babeș National Institute of Pathology, Bucharest, Romania; [2]Charles University Hospital and Medical Faculty Plzen, Czech Republic

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

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

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Anca Potecă[1,2,3], Valentin Tiberiu Moldovan[1,2], Maria Victoria Olinca[1,2,3]

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Florin Andrei[1,2], Angela-Stefania Varban[3]

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16:00 – 16:15 COFFEE BREAK

16:15 – 18:00 **Session 3: Neurosciences** (Victor Babeș Auditorium)
Chair: Prof. Bogdan Ovidiu Popescu

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[1]Dept. of Research Methodology, University of Medicine and Pharmacy of Craiova, Romania; [2]Dept. of Pathology, University of Medicine and Pharmacy of Craiova, Romania; [3]Dept. of Physiology, University of Medicine and Pharmacy of Craiova, Romania; [4]Dept. of Histology, University of Medicine and Pharmacy of Craiova, Romania; [5]Dept. of Human Anatomy, University of Medicine and Pharmacy of Craiova, Romania

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Carol Davila University of Medicine and Pharmacy, Department of Clinical Neurosciences, Department of Neurology, University Hospital, Bucharest, Romania

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Dorel Arsene
National Institute of Neurology and Cardiovascular Diseases, Bucharest, Romania; Victor Babeș National Institute of Pathology, Bucharest, Romania

18:00 – 18:30 **Posters mounting**

18:30 – 20:30 WELCOME PARTY

FRIDAY, NOVEMBER 22

09:30 – 11:30 **Session 4A: Immunopathology** (Victor Babeș Auditorium)
Chair: Prof. Monica Neagu

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[1]Department of Biochemistry and Molecular Biology, University of Bucharest; [2]Immunology Department, Victor Babeș National Institute of Pathology, Bucharest; [3]Advanced Polymer Materials Group, University Politehnica of Bucharest, Romania

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[1]Biochemistry and Molecular Biology Department, Faculty of Biology, University of Bucharest; [2]Advanced Polymer Materials Group, University Politehnica of Bucharest, Romania

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[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Prof. N. Paulescu National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest; [3]Colentina Clinical Hospital, Bucharest; [4]Victor Babeș National Institute of Pathology, Bucharest; [5]Faculty of Biology, University of Bucharest, Bucharest, Romania

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Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

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Marinela Bostan[1,2], Carolina Constantin[2], Monica Teodora Neagu[2]
[1]Center of Immunology, Ștefan S. Nicolau Institute of Virology, Bucharest; [2]Department of Immunology, Victor Babeș National Institute of Pathology, Bucharest, Romania

09:30 – 11:30 **Session 4B: Gastrointestinal Pathology** (Ioan Moraru Auditorium)
Chair: Assoc. Prof. Gabriel Becheanu

Advances in Molecular Pathology of Colorectal Carcinoma
Sibel Erdamar
Mehmet Ali Aydinlar University, Istanbul, Turkey

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Bernhard Stamm
University of Medicine, Zürich, Switzerland

Unusual Duodenal Tumor with Liver Metastases in a Young Syndromatic Female Patient: Clinical Aspects, Imaging, Histopathological Diagnosis, Immunohistochemistry and Evolution
Siyana Ivanova[1], Gabriel Becheanu[1,2]
[1]Fundeni Clinical Institute, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest, Romania

11:30 – 11:45 COFFEE BREAK

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

Oxygen Sensing: From HIF Prolyl Hydroxylases to Noncoding RNAs

Prof. Mircea Ivan

Indiana University School of Medicine, Indianapolis, IN, USA

13:00 – 14:00 LUNCH BREAK

14:00 – 16:00 **Session 5: Omics in Pathology** (Victor Babeș Auditorium)
Chair: Prof. Cristiana Tănase

Copy Number Variants in a Group of Romanian Patients with Autism Spectrum Disorders
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Obregia Clinical Hospital of Psychiatry, Bucharest, Romania; [4]Titu Maiorescu University, Faculty of Medicine, Bucharest, Romania

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Gina Manda[1], Maria Dobre[1], Elena Milanesi[1], Ionela Victoria Neagoe[1], Andreea Csolti[1], Ulrich Weber[2], Nicole Averbeck[2]
[1]Victor Babeș National Institute of Pathology, Bucharest, Romania; [2]Horia Hulubei National Institute for Physics and Nuclear Engineering, Magurele, Romania; [3]GSI Helmholtz Centre for Heavy Ion Research, Darmstadt, Germany

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[1]Centre for Genomic Regulation (CRG), The Barcelona Institute for Science and Technology, Barcelona, Spain; [2]Institute of Protein Biochemistry, National Research Council, Naples, Italy; [3]Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

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Gabriela Anton
Ștefan S Nicolau Institute of Virology, Bucharest, Romania

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Alina Butu, Steliana Rodino, Gina Fidler, Bogdan Miu, Adelina Ghincea, Alexandra Dobrovici, Melania Bica-Popi, Marian Butu
National Institute of Research and Development for Biological Sciences, Bucharest, Romania

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[1]Ştefan S Nicolau Institute of Virology, Bucharest; [2]Fundeni Clinical Institute, Bucharest, Romania

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Livia E. Sima[1#], Horacio Cardenas[1], Siqi Chen[2], Yinu Wang[1], Hao Huang[1], Guanyuan Zhao[1], Bin Zhang[2], Daniela Matei[1]
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Advances of Complex Biological Testing of Calcium Phosphate-based Bioceramics with Orthopedic and Dentistry Applications

Cristiana Tănase[1,2], Adrian-Claudiu Popa[3,4], Ana-Maria Enciu[1,5], Lucian Albulescu[1], Maria Dudău[1,5], Ionela Daniela Popescu[1], Simona Mihai[1], Elena Codrici[1], Sevinci Pop[1], Andreea-Roxana Lupu[1,6], George E. Stan[3], Gina Manda[1], Radu Albulescu[1,7]

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16:00 – 16:15 COFFEE BREAK

16:15 – 18:00 **Posters Session**

SATURDAY, NOVEMBER 23

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

Unlimited Gene Expression Meta-Analysis Through Microarray Data Mining

Victor Ștefan Ionescu[1,2], Ioana Maria Lambrescu[1], Alina Mihaela Micu[1,3], Dan Sebastian Soare[1,4], Gisela Găină[1], Valeriu Cișmașiu[1]

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Intestinal Barrier Integrity, Bacterial Endotoxin Exposure and Inflammation Markers in Parkinson's Disease: Intermediary Stage Results of a Case-Control Study

Laura Dumitrescu[1,2], Daciana Marta[3], Emilia Manole[2,3], Adela Dănău[1,2], Antonia Lefter[1,2], Laura Cristina Ceafalan[1,3], Mihaela Gherghiceanu[1,3], Bogdan Ovidiu Popescu[1,2,3]

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Behavioral Modification of Tumour Cells Induced by Essential Fatty Acids

Maria Dudău[1], Victor Ștefan Ionescu[1], Lucian Albulescu[1], Sevinci Pop[1], Cristiana Tănase[1,2]

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Cognitive Evaluation Method in Nrf2-KO Mice Using the Eight-Arm Radial Maze

Cătălina Anca Cucoș[1], Ana-Maria Enciu[1,2], Laurențiu Anghelache[1]

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12:00 – 12:30 COFFEE BREAK

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

THURSDAY, NOVEMBER 21

SESSION 1

TRADITION, CONTINUITY, INNOVATION

VICTOR BABEŞ NATIONAL INSTITUTE OF PATHOLOGY - A SHORT OVERVIEW -

Mihail Eugen Hinescu, Mihaela Gherghiceanu, Cristiana Tănase, Monica Neagu, Gina Manda, Bogdan Ovidiu Popescu

Victor Babeş National Institute of Pathology, Bucharest, Romania

PLENARY LECTURE 1

PODOCYTE INJURY IN FABRY DISEASE: OLD CONCEPTS AND NEW PARADIGMS

Behzad Najafian

Department of Pathology, University of Washington, Seattle, USA

Fabry is a lysosomal storage disease, affecting multiple organs and causing significant morbidity in the patients. Progressive chronic kidney disease is one of the major complications of Fabry disease. There is a growing body of evidence that podocyte injury plays an important part in pathogenesis of Fabry nephropathy. Over ten years ago we documented that podocyte injury starts early in life and is progressive with age in patients with Fabry disease. Since then, our research has been focused on answering questions such as how to assess podocyte injury in a quantitative way? What is the natural history of podocyte injury in Fabry and how is that related to kidney dysfunction? Do podocytes respond to Fabry treatment? Can podocyte loss in the urine be regarded as a non-invasive biomarker of disease progression? I will also share with you how these structural studies led us to develop in-vitro models to test pathomechanistic hypotheses and emergence of novel concepts and paradigms about Fabry disease.

SESSION 2A

NEPHROPATHOLOGY

Chairs: Assoc. Prof. Gener Ismail & Assoc. Prof. Mihaela Gherghiceanu

SCORING SYSTEM FOR RENAL PATHOLOGY IN FABRY DISEASE

George Terinte-Balcan[1,4], Elena Rusu[2], Ismail Gener[2,3], Mihaela Gherghiceanu[3,4]

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Keywords: Fabry disease, kidney biopsy

Introduction. Fabry disease represents a X-linked genetic disorder in which the deficiency of the lysosomal enzyme alpha-galactosidase leads to accumulation glycosphingolipids. The aim of this study is to present the clinical and histological aspects of Fabry nephropathy.

Methods. We conducted a retrospective study of all the patients that were diagnosed with Fabry disease at the Victor Babeș National Institute of Pathology, Ultrastructural Laboratory, between 2016 and 2019. We selected 7 cases (2 of them were male) with complete sets of clinical data. The average age was 47.3 years (range 30 - 61 years). Glomerular filtration rate values were estimated between 49 and 123 ml/min (average 90 ml/min). All cases presented with proteinuria (range 0.1 – 0.8 mg/dl), except for one female patient. Only one male patient had microhematuria. Kidney biopsy was performed for all patients and samples were processed for electron microscopy. The accumulation of lysosomes in the cells was quantified on semi-thin sections from Epon-embedded tissue using the ISGFN score.

Results. The average number of glomeruli examined on semi-thin sections stained with toluidine blue was 6.14 (range 3 – 8). Two of the cases presented with segmental sclerosis and other 2 with global sclerosis. The average value of abnormal lysosome accumulation in the podocytes was 1.5 (range 0.4 – 2.6) according with the scoring system proposed by ISGFN. All cases presented with inclusions in the parietal cells of the Bowman capsule. All patients had inclusions in the distal convoluted tube and in the tunica media, except for one case.

Conclusion. In order to correctly asses the prognosis and clinical outcome of patients suffering from Fabry nephropathy, it is important to accurately quantify the renal involvement of this disease. In our study, the extent of inclusions in the epithelial cells indicated towards a reduced glomerular filtration rate and confirm the efficacy of ISGFN scoring system.

THE ROLE OF KIDNEY BIOPSY IN A PATIENT WITH DIABETES MELLITUS

Cristina Cristache[1], Andreea Andronesi[1,2], Mihaela Gherghiceanu[2,3], Gener Ismail[1,2]

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Keywords: diabetes, hypertension, hyperfiltration, ischemia, kidney biopsy

Case presentation. We report a 61 years-old obese male patient, who is an active smoker (25 pack-year), suffering from grade III arterial hypertension for over 30 years, type 2 insulin-dependent diabetes mellitus for 13 years who presented in our clinic for the evaluation of renal function impairment diagnosed 6 months earlier. The patient presented history of hairy-cell leukemia (treated with cladribine, in remission for over 1 year), class II CCS stable angina, stage IIA peripheral artery disease, 40-50% stenosis of the right internal carotid artery and mixed dyslipidemia. On admission, the patient's arterial pressure was 175/80mmHg, the absence of peripheral pulse in the distal extremity of the lower left limb was noted and his 24 h diuresis comprised 3000ml. Being evaluated twice in six months, worsening of renal dysfunction was identified, even though both arterial pressure and diabetes control were achieved (a drop from 55 ml/min/1.73m2 to 27 ml/min/1.73m2(CKD-EPI) in only 6 months). The patient admitted that he raised his perindopril dose from 10 mg to 20 mg daily. Moreover, the proteinuria increased to nephrotic range (5.6 g/24h). Taking in account those changes and the exclusion of possible secondary etiologies of glomerulopathies (negative viral markers, immunology panel, normal immunogram, normal serum complement level) we decided to perform a kidney biopsy. Even though the renal impairment could be explained in the context of diabetes mellitus and the long history of high arterial pressure, the occurrence of nephrotic range proteinuria, as well as the doubling of serum creatinine may imply the existence or juxtaposition of a primary glomerulopathy over diabetic nephropathy. Seven glomeruli were identified in the biopsy specimen, all of them with morphological changes suggestive for ischemia, the tuft capillaries presented aneurismal enlargement and diffuse hypocellularity was identified. The Bowman space was occupied by an acellular, amorphous material. Marked tubular atrophy was accompanied by focal tubular enlargement and circumferential hyalinization of the arteriolar wall with subsequent luminal shrinkage. Therefore, the renal biopsy indicated global glomerulosclerosis, with ischemic glomerular lesions. This lesion may have appeared secondary to the hyperfiltration in the glomerular tuft generated by hypertension, diabetic hyperosmolarity and obesity. Sustained hyperfiltration impair the tubulo-glomerular feedback causing loss of glomerular autoregulation. Doubling the ACE inhibitor dose may have played a role in the worsening renal function of already suffering kidneys.

Conclusion. This case illustrated the importance of performing renal biopsy in diabetes mellitus patients, more so in the context of accelerated renal function loss and worsening proteinuria (especially if nephrotic syndrome is present). It is not unusual that another nephropathy (other than diabetic nephropathy) to be discovered in this kind of patients.

FAVORABLE EFFECT OF CORTICOSTEORIDS IN DENSE DEPOSIT DISEASE WITH RECURRENCE ON KIDNEY GRAFT: A CASE REPORT

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Keywords: dense deposit disease, kidney transplant, corticosteroids

Introduction. C3 glomerulopathy (C3G) is a rare complement mediated disease caused by dysregulation of the alternative complement pathway, that can occur either as dense deposit disease (DDD) or C3 glomerulonephritis (C3GN), individualized according to the deposits' location in electron microscopy. DDD is associated with a high risk of recurrence on kidney graft and, also, with a high risk of graft loss. There are no controlled randomized studies on which to base therapeutic recommendations for recurrent C3G in kidney transplant (KT). Eculizumab was used with a variable success.

Case presentation. A 30-years old female kidney transplant recipient was admitted for fatigue, edema and important weigh gain. She had a history of membranoproliferative glomerulonephritis type II diagnosed at the age of 16, 4 years later she was put on dialysis and underwent a kidney transplant from a cadaveric donor at the age of 28. One month prior to admission she developed cytomegalovirus (CMV) disease and maintenance immunosuppression reduction was needed. At presentation, creatinine level was 2.7mg/dl compared to the previous value of 1.9 mg/dl, albumin was 1.9 g/dl, C3 level of 32 mg/dl with a normal C4 and proteinuria was 14g/day. Complement work-up revealed a dysregulation of the alternative pathway, decreased activity of C3 component and factor I and a C3 nephritic factor at the upper limit of normal. The kidney biopsy showed a membranoproliferative glomerulonephritis pattern on light microscopy, immunofluorescence showed bright granular staining for C3 in the mesangium and along glomerular capillary walls and electron microscopy showed electron-dense osmiophilic intramembranous deposits in glomerular basement membranes. The final diagnosis was DDD with recurrence on kidney graft associated with severe nephrotic syndrome and kidney graft dysfunction. The patient was treated with i.v. methylprednisolone 500mg/day, 3 days in a row, followed by another pulse 1 month apart and oral prednisone 40mg/day slowly tapered. Patient renal outcome was favorable, proteinuria was 3.4g/day, the albumin level was normal and creatinine level decreased to 1.7g/dl after 6 months.

Discussion. In this case, the most probable cause of DDD recurrence could be immunosuppression reduction due to CMV disease, but, also, infection itself can act as a trigger. Considering the lack of guidelines for the management of DDD, based on KDIGO recommendations and the lack of Eculizumab availability and, also, the variable results with this type of treatment, we opted for corticosteroids.

Conclusion. In conclusion, we presented a case of DDD with recurrence on kidney graft which responded favorably to steroid treatment.

THE EFFICACY OF DIRECT-ACTING ANTIVIRAL AGENTS IN THE MANAGEMENT OF PATIENTS WITH SEVERE HCV-RELATED CRYOGLOBULINEMIC GLOMERULONEPHRITIS

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Keywords: cryoglobulinemic vasculitis, hepatitis C virus, kidney biopsy

Introduction. Cryoglobulinemic glomerulonephritis occurs in approximately 20-30% of patients with HCV-associated cryoglobulinemic vasculitis and is an important negative prognostic factor, the 10 year overall and renal survival being approximately 80%. Newer treatment protocols involving direct-acting antiviral agents (DAAs) have been associated with high rates of sustained virologic response and clinical remission in patients with hepatitis C virus (HCV) associated cryoglobulinemic vasculitis (HCV-CV), but clinical response in those with renal involvement is less clear.

Material and Methods. This is an observational study that included a cohort of 9 of patients with a diagnosis of cryoglobulinemic glomerulonephritis associated with HCV infection treated with DAAs in our department from January 2015 to January 2019. Clinical and immunological response, during pre and post antiviral therapy was followed for each patient.

Results. We describe a cohort of 9 patients with a mean age of 57 years and known duration of chronic HCV infection ranging 3-20 years. All patients received the ritonavir-boosted paritaprevir/ombitasvir/dasabuvir regimen for 12 weeks and achieved sustained virologic response without subsequent viral relapse. Following antiviral treatment completion, 1 patient developed “new-onset” cryoglobulinemic glomerulonephritis, 6 showed either persistent or worsening glomerulonephritis and only 2 patients had a complete clinical response (CCR). Of the 6 patients with either persistent or worsening CV, 67% received additional IS therapy for uncontrolled CV. Of the two patients that had a CCR, one patient received prior IS therapy while the other one improved without any additional intervention.

Conclusion. Newer HCV treatment protocols involving DAA are highly successful in eradication of HCV infection. However, in our experience DAA treatment alone is insufficient in the management of patients with cryoglobulinemic glomerulonephritis and additional immunosuppressive therapies should be considered.

DIFFERENT CLINICAL AND HISTOLOGICAL PATTERN IN PATIENTS WITH HYPOCOMPLEMENTEMIC URTICARIAL VASCULITIS WITH RENAL INVOLVEMENT

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Keywords: vasculitis, C1q complement, diabetes, kidney biopsy

Introduction. Hypocomplementemic urticarial vasculitis (HUV) is a leukocytoclastic vasculitis of the small vessels affecting women in their fourties, with unknown real incidence. Organ damage is seldom severe, but 14-50% patients have renal disease with various presentations.

Case presentation. We report the cases of three women with HUV, evaluated in our clinic for nephrotic syndrome. **First patient** came for oligoanuria, anasarca, facial rash, acute kidney injury with nephrotic syndrome and hematuria. Immunological and viral tests were negative, with positive anti-C1q-antibodies and hypocomplementemia. Kidney biopsy was prohibited because of severe pancytopenia after two pulses with methylprednisolone and cyclophosphamide. Unfortunately, she remained on chronic hemodialysis, wait for kidney transplantation. **The second patient** also presented with anasarca, gross hematuria, preserved diuresis and no skin rash, with severe renal impairment and nephrotic syndrome. Again, only antiC1q antibodies were positive and complement level was low. Kidney biopsy described fibrinoid necrosis and extracapillary proliferation in all glomeruli. She was treated aggressively with plasma exchange and pulses of methylprednisolone/ cyclophosphamide, achieving complete immunological and clinical remission after 4 months of induction therapy**. Our third patient**, with long history of type 1 diabetes mellitus, was evaluated for generalized edema, hypertension and urticarial exantema on the upper limbs. Although nephrotic syndrome was severe, contrary to previous cases, renal dysfunction was mild, but she had the highest antiC1q-antibodies titer. Renal biopsy showed cellular crescents, interstitial inflammation, Kimmelstiel-Wilson nodules in light microscopy and intense Cq1-staining on immunofluorescence. The patient received iv cyclophosphamide and three shots of 100 mg Rituximab over the course of ten months, with persistent immunologic activity, even though she has partial clinical remission and stable renal function.

Conclusion. Anti-C1q vasculitis is a very rare condition (less than 100 cases in literature), with heterogeneous presentation, histology and evolution. No standard induction or maintenance therapy is approved, given the limited experience so far. Reporting cases like these could have a beneficial impact for establishing the best management for these patients.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS - BEYOND PATHOLOGY. SHOULD GENETICS GUIDE OUR INTERVENTION?

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Keywords: podocytopathy, genetic evaluation

Focal segmental glomerulosclerosis (FSGS) is a histologic entity resulting from a variety of pathogenic processes that cause injury to the podocytes. Recently, mutations in more than 60 genes expressed in podocyte or glomerular basement membrane were identified as causing genetic forms of FSGS, the majority of which are characterized by onset in childhood.

The prevalence of adult-onset genetic FSGS is likely to be underestimated and its clinical and histological features have not been clearly described. A small number of studies of adult-onset genetic FSGS showed that there is heterogeneity in clinical and histological findings, with a presentation ranging from sub-nephrotic proteinuria to full nephrotic syndrome. A careful evaluation of adult-onset FSGS that do not have typical features of primary or secondary FSGS (familial cases, resistance to immunosuppression and absence of evident cause of secondary FSGS) should include a genetic evaluation.

Recognizing genetic forms of adult-onset FSGS is of the utmost importance, given that this diagnosis will have major implications on treatment strategies, selecting of living-related kidney donor and renal transplantation success.

SESSION 2B

MOLECULAR APPROACHES IN PATHOLOGY

Chair: Prof. Emil Iancu Pleșea

PREDICTIVE ELEMENTS IN THE LEUKEMIC TRANSFORMATION OF MYELODYSPLASTIC SYNDROMES

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Keywords: myelodysplastic syndromes, leukemia, molecular analysis

Myelodysplastic syndromes represent clonal diseases of hematopoietic stem cells that occur predominantly in elderly people, but myelodysplastic syndromes can affect younger patients as well. Myelodysplastic syndromes are characterized by ineffective hematopoiesis, causing different forms of cytopenia in peripheral blood, contrasting normal or hypercellular bone marrow. Although less known, being inserted neither into current practice nor in prognostic score, molecular analysis could bring a significantly contribution in the early diagnosis process of disease. In this respect, the latest studies (since 2011) show that this type of analysis represents a great importance in terms of the information that could bring regarding to pathogenic mechanisms on the establishment of Myelodysplastic syndromes subtypes with high conversion, as well as regarding the optimal timing of initiation of specific therapy.

NOVEL PCR BASED TOOLS IN MOLECULAR DIAGNOSTICS

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Keywords: RNase H2-dependent PCR, droplet digital PCR

Several technologies for detection and quantitation of sequence variations have been developed for genomics research and molecular diagnostics, namely polymerase chain reaction (PCR), isothermal amplification, hybridization, and next-generation sequencing (NGS). Although NGS is optimal method for multiplexed analysis of many genes and their variants, it has a significant error rate due to signal ambiguity, enzyme infidelity, imperfect deprotection and others, making the method very inefficient in case of the low frequency targets. PCR is more accurate than microarrays or NGS, it has high molecular sensitivity, and ease of use. Most of the FDA approved assays for molecular diagnostics are based on PCR methods. The main limitations of PCR relates to primer malfunction that results in false positives or false negatives, and second, the absolute quantification depends on good standard calibration. These issues are significantly reduced with the new PCR methods. The RNase H2 dependent PCR, the most recent development of this technology, has several advantages: eliminate the primer dimers, allows multiplexing for a high number of targets, improved precision for detection of low abundance targets. Droplet digital PCR (ddPCR), the third generation of PCR, has higher sensitivity and specificity than the standard real-time PCR. It enables absolute quantification of targets without a need for calibration curves. Because ddPCR performs by identifying single partitions as positive or negative, it offers the potential to perform precise quantification of rare mutations. We will show that the new PCR methods are suitable for molecular diagnostics.

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UTILIZATION OF MOLECULAR PATHOLOGY/IHC IN THE MAIN FORMS OF RENAL NEOPLASIA

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Renal tumours include a heterogeneous and diverse spectrum of neoplasms. Recent advances in this field have significantly improved our understanding of the morphological, immunohistochemical, molecular, epidemiological and clinical characteristics of renal tumours.

This review aims to summarise the new information and evidence on utilization of molecular pathology and immunohistochemistry testing in the main forms of renal neoplasia. We include in this review the following entities: clear cell renal cell carcinoma, papillary renal cell carcinoma type 1 and type 2, oncocytic papillary renal cell carcinoma, chromophobe renal cell carcinoma, mucinous tubulary and spindle cell carcinoma, fumarate hydratase-deficient renal cell carcinoma, succinate dehydrogenase-deficient renal cell carcinoma.

Some of these entities, such as succinate dehydrogenase-deficient renal cell carcinoma and fumarate hydratase-deficient renal cell carcinoma have recently been recognised as new entities in the WHO classification.

For common renal cell carcinomas, such as clear cell carcinoma, papillary renal cell carcinoma and chromophobe renal cell carcinoma has been achieved a consensus. However, in the case of newly-described renal cell carcinomas, little is known on the molecular characteristics.

We analyze their clinical pathologic characteristics, discuss their morphologic and immunohistologic features, and summarize molecular and genetic traits. We expect this review would be beneficial for the understanding of renal cell carcinomas, and eventually promote clinical management strategies.

JAK2-POSITIVE HYPERTROMBOCYTOSIS SYNDROMES

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Keywords: JAK2; osteomedullary biopsy; myeloproliferative; essential thrombocytosis

Introduction. In the context of an adult patient, persistent thrombocytosis invokes different causes in the differential, but clinically the main suspect is Essential Thrombocytosis (TE). This condition is a part of the group of negative myeloproliferative syndromes for the Philadephia chromosome (Ph-). Mutations of the JAK2-V617 gene are encountered with variable frequencies in myeloproliferative syndromes (MPNs), reaching a frequency of approximately 90% in polycythemia vera (PV) 40% in ET and MFP. As a result, the clinician requires a reflex molecular diagnosis of Jak2 and exon 12 mutation as part of WHO major criteria for positive diagnosis. A small part of these disorders are triple negative (JAK2, CLAR, MPL & Ph-) in these situations the diagnosis is supported by morphological and clinico-biological aspects. The mutation has long been considered as a driven one, but its role is questioned due to the triple negative cases as well by is presence normal populations without signs of MPN. We analyze morphologically and immunohistochemically in our laboratory cases clinically diagnosed as TE. We emphasize the morphological particularities in the differential diagnosis, the exceptions encountered (masked-PV, metastatic neoplastic syndromes, etc.) and the variable morphology in the evolution of the positively diagnosed cases.

Materials and methods. During the period 2018 – September 2019, thirty-six cases with hypertrombocytosis and clinical diagnosis of TE were addressed to our laboratory. They were examined morphologically (HE, Giemsa and Gömöri) and immunohistochemistry in standard battery with extensions as necessary.

Results and discussion. From a morphological point of view, a number of 26 cases (72%) were confirmed morphologically, of which the positive JAK2 status was known for 4 cases. For seven cases, the initial diagnosis was reviewed into the diagnosis other SMP (PV or MF). Three cases were identified and classified as secondary thrombocytosis due to neoplastic process with bone determinations (4%) and in one case eosinophilic transformation was invoked. TE is a slowly evolving disease in adults that is sporadically associated with other conditions that can lead to erythrocytosis and associated leukocytosis - aspects that make clinical diagnosis difficult. Clinically, patients are asymptomatic mostly being intercepted during routine tests or for other conditions. A fraction of these have nonspecific manifestations of myeloproliferative syndromes such as thrombotic, or hemorrhagic episodes, much less frequently hepato- or splenomegaly. On the other side of the spectrum an increased number of platelets is observed in chronic inflammatory processes, splenectomy, infectious diseases / autoimmune diseases, rapid regeneration after hemorrhage / anemia, to name but a few.

Conclusion. In summary, the osteo-medullary biopsy represents a useful diagnostic procedure that can exclude secondary thrombocytosis, raise diagnostic hypotheses and remain the essential instrument in the assessment of medullar fibrosis.

THE USE OF FRACTAL DIMENSION ANALYSIS IN TUMOR ARCHITECTURE ASSESSMENT IN CORRELATION WITH DIFFERENT GRADING SYSTEMS

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Background. The aim of the study is to evaluate the main components of tumour architecture using the fractal dimension (FD) analysis in correlation with two different grading systems of prostate adenocarcinoma.

Methods. 433 fields with different patterns of prostatic adenocarcinoma according to Gleason and Srigley grading systems were selected and stained on four serial sections with: H&E for grading the tumor areas and guidance for the other slides and Gömöri technique, Goldner's trichrome, and CD34 immunomarker to assess: tumour cells architecture (GO), tumor stroma (TC) and vascular network (VN) respectively. Images coming from virtual slides obtained by scanning of histological specimens with X20 objective were binarized using a color focused approach for Goldner's and CD34 stainings and an intensity focused approach for Gömöri staining. The FD was computed for each binary image using a box-counting algorithm. The three computed values were used for clustering and classification, k-nearest neighbour proving to be a good choice with a classification rate, due to the irregular distribution of cases in different patterns.

Results. The disposition of the tumor cells showed a tendency to stabilize more around an "Area type" model, with a tendency to evolve towards the "Area type" pattern as the degree of differentiation expressed in the Srigley system decreases. The stromal arrangement also had the same orientation trend towards the "Area type" model, but lower than in the tumor cells population and with an evolution trend towards "Area type" model, as the degree of differentiation decreases both in the Gleason and Srigley systems. The vascular network generally showed an arrangement trend towards a "Linear" model, and increased from the well differentiated to the poorly differentiated patterns. The tumor stroma and the intratumoral vascular network adapt to the arrangement of the tumor cells population, following the same arrangement with it, either towards the "Area type" model or towards the "Linear" model.

Conclusion. FD analysis proved on one hand that tumor cell population models and adapts the stromal component and the surrounding vascular component in the same sense in which its architectural disposal evolves and, on the other hand, the classification system of the distribution arrangement of the tumor architecture proposed by Srigley offers a more accurate description of the correlation between the tumor architecture and the degree of differentiation.

TRANSCRIPTOMIC IMPLICATIONS IN INFLAMMATORY BOWEL DISEASES

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Keywords: Ulcerative Colitis, Crohn's Disease, mucosal gene expression

Background. Inflammatory bowel diseases are chronic relapsing remitting diseases which could benefit from genetic and molecular modulation of treatment in their management plan. Current approaches have limitations regarding both diagnosis and primary response to treatment.

Materials and methods. A panel of 84 genes from the inflamed and normal endoscopic mucosa were investigated by RT-qPCR method in patients with a previous diagnosis of Inflammatory Bowel Disease (IBD). The values obtained were compared to a paired control group (patients without colonoscopic endoscopic lesions). We wanted to identify a specific mucosal signature associated with distinct parameters of IBD in order to characterize the pathophysiological mechanisms involved in generating and perpetuating the inflammatory response.

Results. Evaluation of the IBD non-inflamed endoscopic mucosa revealed persistently elevated markers suggesting gene overexpression. On this tissue, 12 genes were significantly over-expressed, HLA-DQA1 and CCL11 being the most important. This finding suggests that molecular inflammation exists beyond the observable aspect at endoscopy. Also, we identified two genes specifically overexpressed in UC non-inflammatory tissue (CCL11, MMP10) and 4 genes in non-inflammatory BC tissue (LCN2, S100A8, C4BPB, IL1RN) compared to the control group. The inflammatory profiles obtained were relatively similar between BC and UC, with 26 genes specifically expressed in UC and only one gene with BC. Patients with unclassified colitis had a profile similar to that of UC / BC and only 2 specific genes overexpressed (SELE, IL1B).

Conclusion. Overall, the level of gene expression was more pronounced in patients with UC compared to those with BC. Persistence of pro-inflammatory upregulated genes in non-inflamed mucosa from IBD patients might raise the problem of molecular remission in these patients.

PLEURAL TB – CLNICAL-MORPHOLOGICAL PROFILE

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Keywords: extrapulmonary tuberculosis, pleura, tuberculosis, morphology

Purpose. The authors performed a morphological evaluation of the pleural tissue fragments from patients admitted to the thoracic surgery section of the County Emergency Hospital in Craiova, Romania, for a period of 26 years, diagnosed with tuberculous lesions in the pathology department of the same hospital.

Materials and methods. The studied material consisted of pleural tissue samples - biopsy or surgical excision - taken from 39 cases out of 841 patients investigated during the above-mentioned period and diagnosed from the histological point of view with tuberculosis (TB). Granuloma cell population was evaluated by immunohistochemical method. To confirm the diagnosis, the Ziehl – Neelsen stain was usually used, but in some cases immunohistochemistry was also used.

Results. Tuberculosis lesions have predominated in men, usually around 50 years. The diagnosis was suspected in almost half of the cases. The right cavity was more affected and the expanded fibrosis was present in a significant number of cases. The inflammatory reaction was reactive, with Langhans giant cell granulomas and acidophilic necrosis, but sometimes with significant infection or fibrous sequelae.

Conclusion. Pleural effusion of tuberculous origin is an increasingly present reality due to the recurrence of pulmonary tuberculosis in recent decades. Their presence should be suspected if there is evidence of a unilateral pleural effusion with free fluid, with a predilection in men of all ages.

HPV INDUCED ALTERATIONS IN ENT AND GYN PATHOLOGY

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Keywords: HPV, squamous epithelium, squamous cell carcinoma

Human Papilloma Viruses have been the subject of numerous studies related to cervical cancer. But the tropism of these viruses for the squamous epithelium does not stop at the cervical level. Histologically similar lesions can be identified in other epithelia, including those in the ENT sphere. A clear distinction of the place of origin of the tumor is often difficult to achieve both clinically and by imaging techniques, especially in advanced diffuse lesions, exceeding the limits of anatomical structures. Although HPV-induced squamous cell carcinomas are not as frequent as the cervical ones, when compared to the non HPV induced oral carcinomas, they have different clinical and phenotypic features and have a better prognosis. Also, in these cases the possibility of prevention by vaccination is discussed.

PHENOTYPICAL VARIATIONS OF INTRAUTERINE POLYPOID MASSES

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Keywords: polypoid masses, hyperplastic polyps, malignant

The presence of intrauterine polypoid masses is a frequently encountered gynecologic pathology, usually associated with abnormal bleeding and infertility. These lesions are most often benign, but there are exceptions which raise the necessity of a careful examination of the histological specimen. We investigated a large series of consecutive patients (857) admitted in the "Prof Dr Panait Sarbu" Clinical Hospital of Obstetrics and Gynecology with a preliminary diagnosis of uterine polyp. Although most lesions are benign lesions, the incidence of neoplastic lesions that can manifest macroscopically as polypoid masses increase with age. Endometrial polypoid lesions are a frequent gynecological pathology. Benign lesions are more common in women during fertility period and usually are the result of hormonal imbalances. The incidence of neoplastic lesions that can manifest macroscopically as polypoid masses increase with age. Heterogeneity of histopathological aspects of endometrial polypoid lesions requires a thorough differential diagnosis.

AUTOPHAGY IN COLORECTAL CANCER

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Keywords: colorectal cancer, autophagy

Introduction. Colorectal cancer ranks fourth globally in terms of mortality. Basal autophagy is a catabolic mechanism, characterized by the removal of aged or damaged cell components and toxic residues derived from cellular metabolism, produced by the action of lysosomes. The main aim of this paper is to find a potential new therapeutic targets in colorectal cancer therapy by studying some key genes involved in autophagy: LC3, BECN1 and BCL2.

Materials and methods. The material was collected from patients diagnosed and operated for colorectal cancer, two samples being taken: one from the malignant tumor formation and the other from normal tissue. The samples were evaluated by Immunohistochemistry, Western Blot and Real Time PCR to determine the gene and protein expression of the targets of interest.

Results. Expression of LC3 protein on paraffin sections obtained from normal and tumor tissue revealed a highly significant overexpression of LC3 at tumor tissue level ($p<0.001$) as opposed to normal tissue (which was considered as the reference level for LC3 expression). Quantitative evaluation of LC3 protein by Western blot showed an increased level of expression for the LC3-II isoform (considered as a marker for autophagy) in tumor tissue compared to normal ($p<0.05$). The LC3 gene expression evaluation did not find any statistically significant difference between the mean LC3 gene expression values for normal tissue (0.48) and the mean for tumor tissue (0.77), with a coefficient $p=0.28$. When comparing the expression levels of LC3 and BECN1 genes, an average of 1.16 for LC3 and 0.92 for BECN1 was obtained, resulting in a statistically insignificant difference ($p = 0.53$), which supports the assertion that autophagy is up-regulated at the level of malignant colorectal tissue, here BECN1 being overexpressed compared to normal tissue, as opposed to BCL2 which is overexpressed in normal tissue. When comparing the expression levels between BECN1 and BCL2, an average of 1.047 was obtained for BECN1 and 0.8638 for BCL2, the Student t test being highly significant ($p<0.0001$), which shows a promoter effect of the autophagic process in the malignant tissue. compared to the normal one.

Conclusions. The results obtained in this study show an increase of the autophagic process in the malignant colorectal tissue compared to the normal one. BECN1, the promoter gene of the autophagic process, is overexpressed in tumor tissue compared to BCL2, the inhibitory gene of the autophagic process, the results indicating that the progression of autophagy promotes carcinogenesis. Our results support the fact that the autophagic process may be a new therapeutic target for colorectal cancer.

BIOLOGIC BEHAVIOUR OF TUMOUR CELLS IN PROSTATE ADENOCARCINOMA IN CORRELATION WITH DIFFERENT GRADING SYSTEMS

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Keywords: prostate adenocarcinoma, biological behaviour, Gleason system, Srigley system

Background. The aim of the study is to assess some of the main biological behaviour characteristic features of the tumour cells populations in correlation with two different grading systems of prostate adenocarcinoma (PC).

Materials and methods. A series of 435 fields with different PC patterns of Gleason (GlS) and Srigley (SgS) grading systems were selected and immunomarked on four serial sections with: MMP9, MMP2, ECAD and PTEN in order to assess: the capacity to degrade extracellular matrix (ECM), intercellular adhesion and aggressiveness degree. Images coming from virtual slides obtained by scanning of histological specimens with X40 objective were analyzed through a proprietary computational algorithm. Regions of interest (ROI) were defined as containing only pixels with the value of the "Green" channel not higher than a fixed threshold of 220 and the value of the "Red" channel at least 1.1 times higher than that of the "Blue" channel. All other pixels were marked "0" on all channels. Then the overall colour intensity of previously selected mask was inverted and considered as the value for the intensity of IHC staining, larger values meaning more intense staining.

Results. The degradation capacity of the extracellular matrix was dominated by MMP9 - Gelatinase B. The intensity of MMP9 expression had a general discrete upward trend from the well differentiated (WD) patterns to the moderately (MD) and poorly differentiated (PD) patterns in both grading systems. Evaluation of MMP2 expression showed that Gelatinase A plays a secondary role in ECM degradation in PC evolution, with MMP9/MMP2 ratio values ranging from 1.8 to 2. The ECAD expression showed an obvious downward trend in the intensity values from the WD patterns to the PD patterns, indicating a decrease in intercellular cohesion as the degree of differentiation is reduced. The expression PTEN did not seem to sketch any correlation with the degree of differentiation, although in the patterns of the Gleason system it outlined a slightly upward trend that, however, flattened out when assessing the mean values of the expression in the patterns of the Srigley system. In general, the trends of the evolution of the analysed parameters were more clearly outlined in the patterns of the system designed by Srigley.

Conclusions. The behavioral characteristics of the evaluated tumor cell populations showed trends of synchronous evolution. Of all, however, only the decrease in intercellular adhesion was correlated with the decrease in the degree of cell differentiation.

TISSULAR TESTING FOR BRAF MUTATIONS AND MELANOMA TREATMENT DECISIONS

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BRAF mutation can be detected in 40%-60% of patients with advanced melanomas (unresectable or metastatic), curently being the only marker that can predict the therapeutic response.

BRAF-mutated melanomas tend to behave more aggressive: are diagnosed at younger patients, in anatomical regions without chronic sun damage, with a higher incidence of brain metastasis and shorter survival.

BRAF mutation can be detected using DNA based tests or antibody based tests. DNA-based tests include using of PCR to amplify the mutant *BRAF* gene, while antibody-based tests use the VE1 monoclonal antibody to reveal the presence/absence of the gene.

DNA-based tests are qualitative tests, which indicate whether an abnormal protein has been sintetised or not, while antibody based tests can also give quantitative informations, even if these are not very accurate.

Testing for *BRAF* mutations has become a standard for determining the therapeutic options in patients with advanced melanoma.

BRAF inhibitors have improved the overall survival for patients with metastatic disease.

In this presentation we review the testing strategy, compare available current methods and identify the most important steps of BRAF status testing in melanoma tumoral tissue.

SESSION 3

NEUROSCIENCES

Chair: Prof. Bogdan Ovidiu Popescu

AQUAPORIN 4 IN ISCHEMIC STROKE AND ALZHEIMER'S DISEASE

Daniel Pirici[1], Claudiu Mărgăritescu[2], Bogdan Cătălin[3], Laurențiu Mogoanta[4], Ionica Pirici[5]

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Keywords: Aquaporin 4, ischemic stroke, Alzheimer's Disease

Aquaporins (AQP) are a water channel protein family which regulate water homeostasis through the body. AQP4 is essential for water diffusion in the brain in normal and pathological conditions. AQP4 is primarily located in astrocytes' end-feets, and it has been demonstrated on MRI that its inhibition can reduce brain water content in models of cytotoxic oedema. Localization at the level of the astrocytes end-feets also postulated that AQP4 might control fluid drainage from the neuropil, at the level of the newly described glymphatic system of the brain, controlling not only water dynamics but also clearing different solutes like amyloid Aβ peptides.

In our group, after characterizing the expression patterns of AQP4 on human ischemic stroke pathology, we have assessed the morphological changes induced by the use of a selective AQP4 inhibitor (N-1,3,4-Thiadiazol-2-yl-3-pyridinecarboxamide, TGN-020) on a mouse model of ischemic stroke (with permanent occlusion of the medial cerebral artery). Immunohistochemistry helped us to assess the vascular density and extravasation of endogenous murine albumin, as well as to characterize the vascular basement membranes. A second set of experiments aimed to evaluate the modulation induced by the TGN-020 inhibitor onto the drainage of Aβ from within the brain parenchyma, and to these extent mice have been injected with fluorescently-labelled Aβ40 into the cerebral cortices (with and without simultaneous AQP4 inhibition), and followed up under a two-photon laser-scanning microscope.

Our results first showed that AQP4 expression extends throughout the astrocyte membranes in both human stroke pathology and the ischemic animal model. On the animal model, the use of TGN-020 increased vascular densities (as a surrogate for decreasing oedema), reduced apoptosis, and, very interestingly, seemed to result in trapping serum albumin in the thickness of the basement membranes, probably as a result of blocking water trafficking at this level. The in-vivo experiments with the soluble Aβ40 showed first of all that within 40 minutes Aβ40 diffuses through the parenchyma and is cleared along the blood vessels. For both treated/untreated animals Aβ40 was localized around large pial and penetrating arteries, but for the treated animals Aβ40 was localized in more numerous deeper small vessels, where it persisted longer compared to control animals.

Overall, our work suggests that AQP4 inhibition reduces vasogenic oedema in ischemic stroke, but vascular protection and AQP4 integrity might be protective against the deposition of Aβ without overt overproduction, as in sporadic Alzheimer's Disease.

PRENATAL AND PERINATAL MODULATION OF BRAIN RESPONSE TO BIRTH ASPHYXIA

Ana-Maria Zăgrean[1], Sebastian Isac[1], Anca Maria Panaitescu[1,2], Bogdan Pavel[1], Alexandru Cătălin Pâslaru[1], Mihai Moldovan[1,3], Leon Zăgrean[1]

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Keywords: maternal diet, oxytocin, epigenetic, neurodevelopment, neuroprotection

Parturition is accompanied by a transient period of asphyxia (hypoxia and hypercapnia) in the neonate as part of a birth stress, during the shift from maternal-fetal umbilical respiratory gas exchange towards fetal lungs activation. This non-pathologic condition can turn to a prolonged birth asphyxia in birth complications like prolonged labor or umbilical occlusion, with deleterious long-term consequences, mostly on the highly hypoxia-sensitive immature brain. Usual prenatal factors, like maternal dietary intake, and perinatal factors, like oxytocin, are currently discussed for their influence on neurodevelopment and brain response to birth asphyxia.

This presentation will focus on the effects of prenatal (trans-resveratrol, citicoline and high-fat maternal dietary supplementation) and perinatal (oxytocin) factors on brain response to perinatal asphyxia, in an experimental study in rat. The early (24-hour) post-asphyxia outcome was evaluated by the assessment of seizure burden (cumulative loss of righting reflex/2 h), neuroinflammation (IL-1β and TNF-α) and injury (S100B protein) markers, and related epigenetic factors (non-coding microRNAs miR-15a, miR-34a, miR-124, miR-132, miR-134 and miR146) from hippocampus. The late neurological outcome was assessed at maturity by electrocorticogram and behavioural tests (open field test, novel object recognition test, T-Maze and forced swimming test).

Our results indicate that maternal diet influences the immature brain vulnerability to asphyxia by epigenetic mechanisms, with early changes in the seizure burden, neuronal injury and inflammation, but also with late impact on electrophysiological and behavioural outcome. Trans-resveratrol and citicoline supplemented maternal diets have shown protective effects on offspring, while high-fat diet triggered an early increase in neuronal injury and inflammation. Oxytocin administered before asphyxia decreases the hippocampal inflammation and injury, decrease the post-asphyxia seizure burden, and improved the electrocortical activity in adult offspring.

Our data propose a novel perspective on the influence of maternal supplemented diet as potential epigenetic modulator of the offspring brain response to birth asphyxia. Also, our results recommend oxytocin as a neuroprotective mechanism accompanying parturition, that make the clinical use of atosiban, the specific oxytocin receptor antagonist, to be regarded cautiously. In conclusion, regular prenatal and perinatal factors should be carefully considered for their potential impact on offspring brain development and vulnerability to injury.

BIOMARKERS IN STROKE

Cristina Tiu

Carol Davila University of Medicine and Pharmacy, Department of Clinical Neurosciences, Department of Neurology, University Hospital, Bucharest, Romania

According to the definition formulated by NIH, everything can be considered a biomarker, from NIHSS score or imagistic aspects, to molecules produced during the acute phases of an ischemic or hemorrhagic stroke. Which can be the utility of a biomarker in the management of an acute stroke? Practically, every aspect has been approached, from diagnosis to treatment and potential complications of the natural evolution of stroke or of different therapies. Predictive scores for the risk to develop secondary epilepsy, the risk to develop dysphagia, or a predictive score for the functional prognostic of the patient, are examples of biomarkers that can be useful in the daily clinical practice, although usually do not succeed to be generally accepted and used and do not have anything spectacular (maybe creating mobile phone application can help). The scientific research in the last years have led to the identification of several biomarkers which can differentiate ischemic from hemorrhagic stroke, to differentiate etiological subtypes of stroke or to contribute to the guidance of selecting between reperfusion therapies. The idea of testing a number of biomarkers on a POC device in the ambulance, which can allow the safe identification of an ischemic stroke and the administration of a thrombolytic substance in the absence of an imagistic examination seems more plausible now. Twenty years ago, nobody could predict the huge progress obtained regarding the treatment of stroke, so that, while trying to trespass day by day the obstacles we meet, we can trustfully believe in a better future.

CERVICO-CEREBRAL ATHEROSCLEROSIS - A PATHOLOGICAL APPROACH -

Dorel Arsene

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Victor Babeș National Institute of Pathology, Bucharest, Romania

Keywords: atherosclerosis; cerebrovascular arteries; stroke; histopathology; immunohistochemistry

Atherosclerosis is a major health problem today. Two territories are mostly affected – the coronary and cerebrovascular ones. If coronary artery disease and myocardial infarct are still leading causes of death and severe disability, stroke – the consequence of cerebral arteries obstruction – is even more devastating. This is due especially to the large number of cases with severe chronical illness that need palliative care. That is added to the huge number of deaths worldwide caused by the same pathology. Two main causes of stroke are present in practice: hemorrhage and ischemia. If hemorrhagic stroke is only a small part of all stroke cases, the ischemic one is by far more frequent. In the latter category, two more subdivisions are described: embolic and atherothrombotic. Embolic cases are in relationship with atrial fibrillation. The thrombotic ones are generated by atheroma plaques and their complications.

The present material makes an essential update, from a pathologist's point of view, about the mechanisms driving the apparition, evolution and complications of atherosclerosis located in the cerebrovascular territory. Pathogenesis, diagnostic tools, as well as practical tips are presented. Future directions are suggested. Improving diagnosis in atheroma could lead to better primary and secondary prevention, therapy and a major outcome improvement in this field of medical practice.

FRIDAY, NOVEMBER 22

SESSION 4A

IMMUNOPATHOLOGY

Chair: Prof. Monica Neagu

MELANOMA RESISTANCE TO ANTI-PD-1 IMMUNOTHERAPY VIA JAK / STAT SIGNAL PATHWAY

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Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Keywords: immunotherapy, anti-PD-1, melanoma

In recent years the outcome of melanoma patients was greatly improved, benefiting from a novel immunotherapy that target programmed cell death protein 1 (PD-1) and/or its ligand, programmed cell death ligand 1 (PD-L1).

But clinical observations demonstrated that only about 40% of treated patients had had a good response to single anti-PD-1 therapy. This response correlates with an intact tumor response to type-II interferon (i.e. IFN-γ). Alterations in the JAK/STAT pathway, which lies downstream of the interferon gamma receptor (IFNGR1/2) can be the cause of de novo or acquired resistance to anti-PD-1 immunotherapy.

This paper aims to investigate the possible types of alterations in the JAK/STAT pathway and their relation to the drug-resistance observed in the immunotherapy management of the melanoma patients.

NANOCELLULOSE-BASED SMART MATERIALS MODULATE TUMOR MICROENVIRONMENT DURING BREAST RECONSTRUCTION APPLICATIONS

Liliana-Roxana Balahura[1,2], Sorina Dinescu[1], Alexandra Cernencu[3], Adriana Lungu[3], Marieta Costache[1]

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Keywords: breast cancer, nanocellulose, 5-fluorouracil, inflammasome, NFkβ

Introduction. Breast cancer is the most common type of cancer with unpredictable evolution, a heterogeneous disease which present multiple subtypes. For mammalian reconstruction, biocompatible materials based on nanocellulose and pectin (CNP) and nanocellulose and alginate (CNA) have been tested. 5-fluorouracil (5FU), an anti-tumoral agent was added in the composite materials at different concentrations, respectively 5% and 10%, with the purpose to decrease the proliferation rate of cancer cells. Inflammasomes represent protein complexes composed by Nod-like receptor (NLR), the adapter protein ASC and caspase-1, involved in inflammation and inflammatory cell death or pyroptosis. Another indicator of stress stimuli is NFkβ, involved in cancer, inflammatory diseases or infections. The purpose of this experiment was to evaluate the anti-tumoral effect of CNA-5FU and CNP-5FU composites in vitro against a mammary carcinoma line compared to the effect on normal cells and to observe the inflammatory profile of the cells exposed to 5FU treatment.

Materials and methods. To accomplish this study, mesenchymal stem cells (hASC) and breast cancer cells from the ZR 75-1 line were seeded in CNA and CNP composite materials enriched with different concentrations of 5FU, resulting three-dimensional systems. Cytotoxicity assays were performed at two and seven days after cells seeding. Cell viability was quantitatively determined by MTT assay but also qualitatively via the Live-Dead test, while the cytotoxicity of the material was assessed by the LDH assay. The Caspase-1 activity from inflammasome complex was measured directly from culture medium. For immunofluorescence (IF) assay, the 3D systems were fixed, permeabilized and incubated with rabbit polyclonal NFkβ antibody and respectively with goat anti-rabbit secondary antibody Alexa Fluor 546.

Results. A high percentage of normal cell viability was observed on all types of materials, especially those based on CNP. Cell viability was also conditioned by the anti-tumoral effect of 5FU on breast cancer cells after contact with the materials. The presence of 5FU induced decreased normal cell viability, with significant decrease in cancer cell viability. The highest levels of casapase-1 were observed then the breast cancer cells were put in contact with materials with a higher concentration of 5FU. IF staining revealed the presence of NFkβ in the cells exposed to 5FU, especially tumor cells.

Conclusion. The CNP composite material sustained a higher rate of cell proliferation compared to CNA. The presence of the anti-tumoral agent confers to the materials a certain degree of cytotoxicity that is observed both on cancer and control cells. Cancer cells showed the lowest percentage of viability at the contact with materials enriched with the highest concentration of 5FU. Also, cancer cells exposed to 5FU acquired a specific inflammatory profile, expressing NFkβ, in comparison with normal cells which were affected by the anti-tumoral treatment but in small percentage.

BIOCOMPATIBILITY ANALYSIS OF HEMA/AMPS/LDH 3D BIOCONSTRUCTS DESIGNED FOR SOFT TISSUE RECONSTRUCTION

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Keywords: soft tissue engineering, human stem stells derived from adipose tissue, nanomaterials

Introduction. Soft tissue engineering involves carefully selecting the type of cells and scaffold's material to achieve the best outcome. In regard of choosing the biomaterial, copolymerization of two different monomers usually brings more advantages to the final material. In our study, we used (2-hydroxyethyl methacrylate) (HEMA/H) and 2-Acrylamido-2-methylpropanoesulfonic acid (AMPSA/A). During recent years, some nanomaterials also gained interest for tissue engineering applications such as layered double hydroxides (LDH/L). The aim of this study was to evaluate the biocompatibility and the potential to sustain adipogenesis of HEMA/AMPSA/LDH materials and select the ones with the greatest potential for future soft tissue engineering applications.

Materials and methods. Human stem cells isolated from adipose tissue (hASCs) were seeded in tridimensional materials with different concentrations of HEMA (95/97% wt.), AMPSA (3/5% wt.) and LDH (1/2% wt.). Their biocompatibility was evaluated by quantitative and qualitative tests after 3 and 6 days. Cell viability and proliferation rate were evaluated by MTT test. LDH assay showed the cytotoxicity induced by the materials. The LiveDead assay allowed simultaneous visualization of dead and live cells by fluorescence microscopy. The potential of the materials to sustain adipogenic differentiation was evaluated at gene and protein expression levels (perilipin marker) by qPCR and confocal microscopy, over 21 days of differentiation.

Results. The MTT test showed the highest cell viability on the 95% H 5% A 2% L material after 3 days post-seeding hASCs in the materials. After 6 days, the same material showed the best results, suggesting a good copolymerization of HEMA with AMPS in 95% - 5% concentrations. The LDH assay showed that 97% H 3% A 1% L material induced the highest cytotoxicity. The Live/Dead assay confirmed the results from the quantitative tests and showed less live cells in the material with 97% H 3% A than in the materials with 95% H 5% A. In the differentiation study, perilipin expression showed an increasing profile over 21 days for all materials tested. On the 95% H 5% A 2% L material was registered the highest gene expression level for perilipin, showing a faster evolution of the adipogenic differentiation. At 21 days, on all the materials, the perilipin expression was increased confirming the initiation and development of adipogenesis, but on the 95% H 5% A 2% L material, perilipin expression was statistically significantly higher compared to the other materials. The gene expression results were also confirmed at protein level by immunofluorescence staining.

Conclusion. All of the materials evaluated proved to be biocompatible. The 95% H 5% A 2% L material showed the best results that recommend it for future soft tissue engineering applications.

SKIN INFLAMMATION UNDER THE MAGNIFYING GLASS

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Keywords: skin inflammation, reflectance confocal microscopy

The process of skin inflammation is activated in different physiological and pathophysiological conditions and investigation of its complex mechanisms is in focus for both clinical and fundamental research.

Using reflectance confocal microscopy, we are able to perform a high-resolution evaluation of the skin structures, in vivo and in real time. Moreover, the same skin area can be investigated at different time-frames allowing us to follow-up various skin lesions. Thus, this modern skin imaging technique can be very useful in the diagnosis and therapeutic monitoring of skin inflammatory conditions.

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DOPACHROMETAUTOMERASE IS A CANDIDATE REGULATOR OF INTEGRATED STRESS RESPONSE IN MELANOMA

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Keywords: melanoma, integrated stress response, amino acid response

Melanoma is a type of skin cancer that develops from melanocytes, the pigment-containing cells located in the basal layer of the skin, the uvea, the inner ear, meninges, vaginal epithelium bones and heart. Cutaneous melanoma (CM) occurs on the skin, due to convergence of environmental and genetic factors, the sun burns caused by excessive UV radiation being often the culprit for oncogenic transformation of melanocytes. CM is the most aggressive type of skin cancer with rising incidence in the last 50 years, especially amongst people under 40 years old and the second type of cancer diagnosed in young people aged 15-19. Dopachrome tautomerase (DCT) is a member of the Tyrosinase Related Protein (TRP) family which acts in the distal steps of melanogenesis thus contributing to melanin formation in melanocytes and their malignant counterparts, the melanoma cells. Apart from the role in melanin biosynthesis, DCT is involved in cellular processes that confer melanoma cells resistance to different stress factors such as UV radiation that induce DNA degradation and apoptosis, chemotherapeutic agents that crosslink DNA and interfere with cell division (cisplatinum and carboplatin), hypoxic stress, and oxidative stress. Although DCT involvement in these processes is well-acknowledged, the molecular mechanisms of how this melanoma antigen acts are not yet well understood. In response to diverse stress stimuli (AAR-aminoacid deprivation), viral infections, heme deprivation and endoplasmic reticulum stress (ER stress), eukaryotic cells activate the integrated stress response (ISR), to restore cellular homeostasis. ISR activates a gene expression program that optimizes the cellular response to stress and is dependent on the cellular context, as well as on the nature and intensity of the stress stimuli. Although the ISR is primarily a pro-survival, homeostatic program, exposure to severe stress can drive signaling toward cell death. All the stress signals converge to the phosphorylation of eukariotic translation initiation factor 2 alpha (eiF2α) by one of the 4 specific kinases namely GCN2 in case of AAR and PERK in case of ER stress. We present here, for the first time, experimental evidence that DCT is a new player involved in modulation of AAR and possibly ER stress in melanoma. A well known regulator of cell stress mechanisms in tumor biology, acting in GCN2 branch of AAR has been identified as target of DCT in a human melanoma cell line. Additional data indicate that DCT is involved also in regulating ER stress mechanisms by altering expression of a chaperone needed for protein folding, including essential components of ECM. Importantly, ECM integrity is required for tumor migration and invasion processes. Further studies aim to dissect DCT impact on other participants of both AAR- and ER-stress pathways. These new data introduce DCT as a novel, specific regulator of stress mechanisms in melanoma and a potential therapeutic target.

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MODULATION OF THE CHEMOTHERAPY RESPONSE IN ENT CANCERS

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The extracellular signal-regulated kinases (ERKs) are key transducers of the extracellular signals into intracellular responses and represent major molecular players in tumorigenesis. The aim of this study was to determine how curcumin (CRM) used as an adjuvant supports the apoptotic process induced by a mono chemical agent treatment (cisplatin-CisPT) on two head and neck squamous cell carcinoma cell lines (FaDu and PE/CA-PJ49) and the involvement of ERK1/2 and/or p53 activation in this process. Data have shown that the CisPt effect is potentiated by CRM. CRM induced an increase of p53 protein phosphorylation in both cell lines. CisPt decreased p53 protein phosphorylation in FaDu cells, but increased it in PE/CA-PJ49 cells. We found that the constitutively expression of activated ERK1/2 protein-kinase was different in the two analyzed tumor cell lines. ERK1/2 activation status was essential for both cell processes, proliferation and apoptosis induced by CisPt and/or CRM treatment on squamous cell carcinoma cells. Our data suggest that p53 phosphorylation in the apoptotic process induced by CRM treatment might require the involvement of ERK1/2. In this regard the CisPt treatment suggested that p53 phosphorylation is ERK1/2 independent in FaDu cells having a p53 gene deletion and ERK1/2 dependent in PE/CA-PJ49 cells having a p53 gene amplification. Moreover, in both tumor cell lines our results support the involvement of p53 phosphorilation-ERK1/2 activation-dependent in the apoptosis induced by combined treatments (CisPt and CRM). The use of CRM as adjuvant could increase the efficiency of chemotherapy by modulating cellular activation processes of ERK1/2 signaling pathways. In conclusion, the particular mode of intervention by which ERK1/2 might influence cell proliferation and/or apoptosis processes depends on the type of therapeutic agent, the cells' particularities, and the activation status of the ERK1/2.

SESSION 4B

GASTROINTESTINAL PATHOLOGY

Chair: Assoc. Prof. Gabriel Becheanu

ADVANCES IN MOLECULAR PATHOLOGY OF COLORECTAL CARCINOMA

Sibel Erdamar

Mehmet Ali Aydinlar University, Istanbul, Turkey

ANAL PATHOLOGY IN ROUTINE BIOPSY PRACTICE

Bernhard Stamm

University of Medicine, Zürich, Switzerland

In our laboratory we receive about 2 anal biopsies per 100 colon biopsies what means that they are rather rare. What we receive are mostly biopsies or only small surgical specimens referred in almost 90% by Gastroenterologists. All specimens are totally embedded, cut at three levels and routinely examined with at least an HE and a connective tissue stain. Patients are exclusively adults, most of them between 30 and 70 years old and slightly more women than men.

The precise localization of the sample is seldem mentioned and specimens labeled "anal" frequently include also the perianal region or even the lower rectum. This problem is further compounded by the fact that there are numerous anatomical terms regarding this area, often with controversial definitions or hardly applicable in biopsry material. Best approach in biopsy practice is to remember that normally there are from proximal to distal four different types of epithelium following each other: (1) rectal, (2) transitional (inconsistent) both above the dentate line, and (3) squamous-nonkeratinizing (pecten) below the dentate line and (4) the perianal skin with appendages.

One third of our specimens are anal tags and hemorrhoids. Anal tags are a frequent incidental finding especially in the elderly, most of them unexplained skin tags, rarely corresponding to hypertrophic chronically inflamed anal papillae.

In about one fifth of our patients' inflammation, itching and/or ulceration (or fissures) are the presenting symptom and biopsies are made primarily to rule out tumor or otherwise to find any specific forms of inflammation. The common fissures consist of a tear within the perianal skin, most likely due to trauma and ischemia during defecation and typically in a posterior location. They have a tendency to chronification, then often accompanied by a so-called sentinental tag. There are no specific histological signs.

In the solitary-rectal-ulcer or mucosal-prolapse syndrome, due to repeated prolapse of rectal/anal mucosa, the histology shows various pictures depending on the actually prevailing pathogenetic mechanism: Ulceration (usually anterior, may be absent) and reparative mucosal hyperplasia with muscular obliteration of the lamina propria, forming the so-called inflammatory cloacogenic polyp, that can be covered by rectal or anal epithelium. Misplaced glands and reactive changes should not be confused with neoplasia.

Another frequent reason for (peri)anal biopsies is chronic inflammation with anal itching as the predominant symptom. Biopsies frequently show only nonspecific inflammatory changes but occasionnally can lead to a more useful diagnosis as eg eczematous dermatis, lichen sclerosus or in the case of a granulomatous lymphocyte-rich inflammation to the suspicion of Crohn's disease.
To diagnose a specific anal infectious disease (other than HPV) is a rare event in our experience and often only possible on condition that the particular clinical context is pointed out to the pathologist.
The most significant change in the field of anal biopsy in recent years is however the increase in human papillary virus (HPV)-related lesions, currently more than 20% of our biopsies, partly because of screening programs in high risk patients.
Condyloma acuminatum is the most often seen morphological manifestation of infection with HPV. Not infrequently one sees also keratotic lesions resembling but not completely fulfilling the histological criteria for condylomas ("condyloma-like" lesions). Genetic testing may be recommended in these cases.
Of more consequences is the diagnosis of dysplasia (anal intraepithelial neoplasia, AIN), which can occur in condylomas or in flat mucosa. Grading and nomenclature of anal (and perianal) intraepithelial neoplasia has been adapted to the nomenclature of cervical dysplasia with which it shares many similarities.
Two special clinical forms of AIN are anal Bowen's disease and bowenoid papulosis. The latter occurring in younger patients with multiple, smaller and rapidly growing warts frequently also present on the external genitals. The histological picture of both is practically indistinguishable from ordinary high grade dysplasia. The correct assignment depends heavily on the clinical context.
Anal squamous cell carcinoma (ASCC) accounts for more than 80% of all anal cancers and is in more than 90% the result of a chronic infection with HPV. Histologically it shows in varying proportions basaloid, large cell and keratinizing patterns. Since moreover the diagnosis is usually based on biopsy only the nomenclature has been simplified to ASCC and the older terminology (basaloid, transitional, cloacogenic) was abandoned. Histological grading in biopsies is also discouraged because of no proven prognostic value.
Anal carcinoma is by definition in contact with the anal canal and the distinction from perianal carcinoma must be done clinically. The therapeutic approach may be different, especially in case of a basal cell carcinoma of the perianal skin, which may histologically resemble a basaloid ASCC.
Care should be given not to misdiagnose an anal carcinoma infiltrating the lower rectum as a poorly differentiated rectal adenocarcinoma.

UNUSUAL DUODENAL TUMOR WITH LIVER METASTASES IN A YOUNG SYNDROMATIC FEMALE PATIENT: CLINICAL ASPECTS, IMAGING, HISTOPATHOLOGICAL DIAGNOSIS, IMMUNOHISTOCHEMISTRY AND EVOLUTION

Siyana Ivanova[1], Gabriel Becheanu[1,2]

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PLENARY LECTURE 2

OXYGEN SENSING: FROM HIF PROLYL HYDROXYLASES TO NONCODING RNAs

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Keywords: oxygen, HIF prolyl hydroxylases, noncoding RNA

All metazoan cells monitor changes in environmental O2 availability via prolyl hydroxylase enzymes that control the abundance of HIF-a subunits, thus functioning as gatekeepers of an adaptive transcriptional program. While the molecular core of O2 sensing is highly conserved, increasingly complex regulatory mechanisms have emerged during evolution in higher organisms. Among these, circuits involving noncoding transcripts have elicited particular interest in recent years. In 2007 our group was the first to expand the hypoxic response to noncoding transcripts, with the identification of several microRNAs that accumulate in O2- starved cells. During the past decade, miR-210 has become generally recognized as the "hypoxia-miR", being induced in HIF1 dependent fashion in most mammalian cells tested. Functionally, multiple studies have reported fine-tuning effect of miR-210 on mitochondrial activity. Our ongoing work expands the O2-sensitive transcriptome with a previously uncharacterized lncRNA generated by MIR193BHG locus and provides the first evidence for its importance in steroid hormone biology.

SESSION 5

OMICS IN PATHOLOGY

Chair: Prof. Cristiana Tănase

COPY NUMBER VARIANTS IN A GROUP OF ROMANIAN PATIENTS WITH AUTISM SPECTRUM DISORDERS

Aurora Arghir[1,2], Sorina Mihaela Papuc[1], Alina Erbescu[1], Raluca Colesniuc[1], Catrinel Iliescu[2,3], Ioana Minciu[2,3], Bogdan Budișteanu[3], Ina Ofelia Focșa[1,2], Andreea-Cristina Țuțulan-Cuniță[1], Iuliana Dobrescu[2,3], Magdalena Budișteanu[1,3,4]

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

Keywords: neurodevelopmental disorders, autism, copy number imbalances

Background. Autism spectrum disorders (ASD) are complex neurodevelopmental conditions with a heterogeneous genetic architecture; a wide range of anomalies, from point mutations to large chromosomal defects, has been reported as causal or risk factor for ASDs, each of them accounting for only a small percentage of patients. Microarray-based comparative genomic hybridization (array-CGH) has become a first tier genetic test in ASDs contributing to detailed genome-wide characterization and leading to discovery of new autism genes.

Aim. We report on the results of chromosomal microarray investigation in a group of ASDs patients.

Material and methods. 55 patients were referred to our laboratory for genetic testing with ASDs, intellectual disability and other clinical features (facial and or limb dysmorphism, congenital malformations, various behavior problems, speech delay, etc). The phenotypic evaluation included a general clinical examination completed with neurologic, dysmorphologic, psychiatric, and psychologic evaluation with specific ASDs tests (ADOS, ADI-R). Genetic investigations included array-CGH, completed with classical karyotyping, FISH and qPCR tests; parental studies were performed when necessary.

Results and discussion. Pathological CNVs were detected in 11 patients. Besides well-described syndromic regions associated with ASDs (e.g. 22q13.3 deletion, 22q11.2 deletion, Xq28 duplication), other genomic regions, rarely reported in patients with ASDs, were either deleted or duplicated in our cohort: deletion of 8p11.2p21.2, 9q34.1, 9p13. Some of these regions have been rarely associated with autism and thus have the potential to reveal new genes contributing to ASDs. Ongoing studies will enable us to enlarge the ASDs patient group and to establish an internal database of controls, relevant for our geographic region.

Conclusion. The genetic factors are known contributors to human neurodevelopment and behavior. Thus, revealing the underlying genetic causes of ASDs is of great importance both in research and in clinical practice. Our data illustrates the utility of array-CGH in the investigation of patients with ASDs, specifically in the context of complex phenotypes.

Acknowledgement. The research leading to these results has received partial funding from the EEA Grant 2014-2021, under the project contract No 6/2019, and Core Programme – Projects PN 92.033.02.03 and PN 16.22.05.01.

MOLECULAR NETWORKS IN HUMAN MONOCYTES EXPOSED TO SPACE-RELEVANT RADIATION

Gina Manda[1], Maria Dobre[1], Elena Milanesi[1], Ionela Victoria Neagoe[1], Andreea Csolti[1], Ulrich Weber[2], Nicole Averbeck[2]

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Keywords: monocytes, space-relevant radiation

Introduction. Experimental evidence has highlighted that low-dose ionizing radiation can disturb cell homeostasis at multiple levels due to the inflicted oxidative, inflammatory and genotoxic stresses. Therefore, it is of utmost importance to investigate the biological impact of radiation exposure from a "systems biology" perspective, addressing the complex molecular networks that are underlining either the radiation-inflicted injuries or the sophisticated mechanisms through which cells tend to restore homeostasis.

Aim. To identify particular molecular networks activated in human monocytes exposed in vitro to space-relevant radiation, and to develop a "proof of concept" study on the therapeutic potential of NRF2 activators for counteracting radiation effects.

Results. The human monocytic cell line CRL-9855 (ATCC) was exposed to ^{56}Fe beams at GSI (Darmstadt, Germany). Cells were investigated at various time points post-irradiation regarding their response to the radiation-induced stress by addressing a network of 84 relevant genes (Qiagen, Stress and Toxicity Pathway Finder). Preliminary results obtained in monocytes exposed to 1 Gy ^{56}Fe beams revealed important gene expression changes at 48-72 hrs post-irradiation: **1)** profound down-regulation of the pro-apoptotic gene *BBC3* which was only partially due to the down-regulation of the tumor suppressor gene *TP53*; **2)** moderate up-regulation of genes involved in cell cycle arrest: *CDKN1A* and *GADD45A*, complemented by a significant down-regulation of *HUS1*; **3)** up-regulation of some target genes of the NRF2 transcription factor, such as the *HMOX1*, *AKR1B1*, *GSTP1* and *TXNRD1* genes, which may confer radioprotection to the exposed cells, albeit other NRF2 target genes were found to be down-regulated (*GCLC*, *GCLM* and *GSR*). Treatment of monocytes with dimethyl fumarate (activator of the NRF2 system) triggered the up-regulation of critical genes involved in DNA repair (*GADD45A*, *RAD9A*, *RAD51* and *TP53*), in addition to the over-expression of conventional cytoprotective NRF2 targets (*HMOX1*, *NQO1*, *GSR*, *GSTP1*).

Conclusion. Analysis of the expression profile of stress responsive genes in human monocytes exposed in vitro to 56Fe beams highlighted the main molecular networks underlining the cytotoxic effects of irradiation as well as the adaptive cellular responses. Accordingly, pharmacologic stimulation of the NRF2 pathway as master regulator of more than 250 cytoprotective genes is a promising therapeutic strategy to reinforce the cytoprotective tools of normal cells in the stressful spaceflight environment.

Acknowledgement. Work was supported by the Romanian Research and Innovation Ministry through the grant AO-2017-IBER_003/2018 financed by the European Space Agency, and through the grant PCCDI 64/2018 financed by the Romanian Ministry.

GRASP55 AND IRE1α/XBP-1 SIGNALING CONTROL INTERLEUKIN-1β SECRETION AND AGGREGATION FROM ACTIVATED MACROPHAGES

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Keywords: Interleukin-1β; GRASP; unconventional protein secretion; unfolded protein response; inflammation

Interleukin (IL)-1β is the most potent pro-inflammatory cytokine, produced as response to tissue damage and infections by cells of the immune system. The cytokine is synthesized as inactive form in the cytoplasm (*pro*IL-1β-37 kDa) and is cleaved by caspase-1 to its bioactive form: mature *m*IL-1β (17 kDa) as result of the activation of several signaling pathways. *m*IL-1β is secreted to the extracellular space without entering the classical endoplasmic reticulum-Golgi pathway by a process generally termed unconventional protein secretion. Therefore, an important question in the field is how macrophages and cells of the immune system control the amount of secreted IL-1β? We report that primary macrophages isolated form GRASP55 knockout mice have impaired *m*IL-1β secretion. Under these conditions the reduced secretion is correlated with its retention as intracellular amyloid-like aggregates. Furthermore, proteolytic processing and transport across the plasma membrane of endogenous IL-1β from murine macrophages are processes sensitive to inhibitors of the unfolded protein response (UPR). Inhibitors targeting the PERK-mediated pathway of the UPR affect caspase-1 proteolytic activity thereby controlling the amount of *m*IL-1β generated intracellularly. However, inhibitors targeting of IRE1α RNase activity impair *m*IL-1β secretion, which assembles into SDS-resistant aggregates intracellularly. We found GRASP55 knockout cells have a deficient IRE1α mediated *XBP-1 m*RNA splicing and are insensitive to IRE1α inhibitors; however, deletion of GRASP55 does not affect the PERK pathway. Therefore, we propose GRASP55 as novel regulator of IRE1α/XBP-1 signaling pathway, which controls the secretion and aggregation of IL-1β in primary murine macrophages.

EPIGENETIC MARKERS ASSOCIATED WITH PRECURSOR LESIONS AND CERVICAL CANCER

Gabriela Anton

Ștefan S Nicolau Institute of Virology, Bucharest, Romania

Keywords: cervical cancer, epigenetic markers

Cervical cancer, the fourth most common cancer in women worldwide, is virtually caused by the persistent infections with high-risk HPV (hrHPV). The progression of viral infected epithelial cells to cervical carcinoma takes place through intermediate stages, characterized by the accumulation of genetic and epigenetic changes which are induced by the expression of viral oncogenes.

The slow progression from precursor lesions to cancer (between 15-30 years) allows early detection of the disease. Currently used cervical screening programs are based on cytology (PAP) and/or hrHPV testing but they have several limitations due to low sensitivity and specificity respectively. Therefore, new-triage approaches for HPV-positive women are needed. These are based on epigenetic changes that occur early in the HPV – induced oncogenesis. The new epigenetic markers are selected on the basis of the molecular events that control the physiological and pathological aspects in this disease. Currently, DNA methylation biomarkers, which are highly reproducible and easy to measure are being exploited, and several tests have been developed. The performances of these tests are based on the direct link between the level of methylation, the degree of CIN lesions and the duration of the disease, the HPV genotype. These methylation biomarkers can be used as triage methods since provide information on both diagnosis and prognosis.

Apart of host gene methylation, viral methylation for screening and triage is under investigation. Regarding the prognostic markers, studies have been carried out on some histone markers associated with the expression of viral oncogenes, but also miRNAs and long-noncoding RNAs.

PLANT EXTRACT-MEDIATED SYNTHESIS OF NANOPARTICLES AND EMERGING APPLICATIONS IN MEDICINE

Alina Butu, Steliana Rodino, Gina Fidler, Bogdan Miu, Adelina Ghincea, Alexandra Dobrovici, Melania Bica-Popi, Marian Butu

National Institute of Research and Development for Biological Sciences, Bucharest, Romania

The rising need for safe and cost-effective methods to obtain nanoparticles resulted in an increased attention towards green synthesis. The scientific research studies have shown that plant extracts can act as a potential mediator for the synthesis of nanoparticles. The plant extracts are efficiently used in the synthesis of ecological metallic nanoparticles such as silver, gold, zinc, copper, palladium, platinum, cobalt, titanium. Biologically synthesized nanoparticles tend to have higher biological activity compared to traditionally synthesized nanoparticles. Superior biological activity is considered to be the result of the action of synergistic proteins involved in the capture and stabilization of nanoparticles. Nanoparticle production is considered to be the result of a combination of several factors. One of the factors is the presence of organic functional groups at cell wall level, and the second depends on the corresponding environmental parameters such as pH and temperature. The size, shape and composition of a nanoparticle can be significantly influenced by pH and temperature. For example, particle size is an important factor because physico-chemical properties are more pronounced at smaller sizes. Therefore, it is necessary to optimize the synthesis parameters during the formation of nanoparticles in order to increase the general properties of the particles and to influence the efficiency of them. The objective of our research was to study nanoparticles obtained through synthesis mediated with plant extracts and to evaluate their biological activity. Various concentrations of nanoparticles - plant extracts solutions were investigated for their biological activity. The synthesis of nanoparticles was characterized by the color change of mixture. Our study demonstrated the potential of biogenic nanoparticles and the synergistic effect of plant extract and nanoparticles in biomedical applications.

Acknowledgement. This work was supported by a grant of the Ministry of Research and Innovation through Program 1 - Development of the National R&D System, Subprogram 1.2 - Institutional Performance - Projects for Excellence Financing in RDI, Contract no. 22PFE / 2018 and Program NUCLEU 25N-19270104/2019.

NEW MOLECULAR CLASSIFICATION OF GASTRIC CANCER OPENS NEW AVENUES TO NOVEL THERAPEUTIC STRATEGIES

Mihaela Chivu-Economescu[1], Laura Georgiana Necula[1], Denisa Dragu[1], Lilia Matei[1], Ana I. Neagu[1], Coralia Bleotu[1], M. Sârbu[2], Simona Dima[2], Carmen Cristina Diaconu[1], Irinel Popescu[2]

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Keywords: gastric cancer, molecular classification, targeted therapy, immunotherapy

Gastric cancer (GC) is one of the most lethal and aggressive cancers, being the third cause of cancer related death worldwide. This is due to its high biological heterogeneity based on the interaction between multiple factors, from genomic to environmental factors, diet or infections with various pathogens. Therefore, understanding the molecular characteristics at a genomic level is critical to developing new treatment strategies.

Recent advances stratify GC into Epstein-Barr virus positive tumors, microsatellite-instable tumors (MSI), genomic stable (GS) tumors showing epithelial to mesenchymal transition (EMT) with intact TP53, and tumors with chromosomal instability (CIN) and functional loss of TP53.

The implementation of the novel system, in which GC patients can be classified in molecular subtypes, represents a substantial advance for therapy. The first two subtypes, EBV positive and MSI subtypes, presents tumour infiltrating patterns with overexpression of PD-1, PD-L1, PDL-2, therefore a high response rate to immunotherapy was noted. Other molecular features that can be therapeutically exploited are JAK2 overexpression and non-silent mutations in PIK3CA, by combining emerging immunotherapies with molecularly targeted drugs.

For the third subtype, genomic stable or MSS/EMT subtype, which includes the diffuse type of GC, currently effective therapies for are lacking. However, mutations in CDH1 and RHOA genes or CLDN18-ARHGAP6/26 fusions may be exploited as therapeutic targets. The only targeted therapies approved so far are for CIN and MSS/TP53- subtype of GC, and are currently limited to Trastuzumab and Ramucirumab (HER2 and VEGFR2 inhibitors). This classification can be translated in clinical practice based on RNA and protein expression assays: EBER in situ hybridization for EBV detection, and immunohistochemistry (IHC) for mismatch repair proteins (MLH1, PMS2, MSH2, and MSH6), PD-L1, E-cadherin and p53. Our group has implemented this protein expression method to stratify 122 GC patients into five subtypes: Gp1 comprised of EBV positive samples (29.51%), subtype Gp 2 – MSI high (36.07%); subtype Gp 3 characterised by aberrant expression of Ecadherin (13.93); subtype Gp 4 with a high number of TP53 mutations (18.01%); and subtype Gp5 – TP53 negative samples (2.46%). The application of new classification methods provides the unique opportunity to improve CG clinical therapy by allowing a better stratification of patients in GC subtypes based on biomarkers. These findings could have important translational relevance in the field of GC therapy, improving clinical care, the survival, and quality of life of patients with GC.

Acknowledgement. This work was supported by the UEFISCDI project PN-III-P4-ID-PCCF-2016-0158.

TISSUE TRANSGLUTAMINASE IN ANTI-TUMOR IMMUNE RESPONSE

Livia E. Sima[1#], Horacio Cardenas[1], Siqi Chen[2], Yinu Wang[1], Hao Huang[1], Guanyuan Zhao[1], Bin Zhang[2], Daniela Matei[1]

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Keywords: ovarian cancer, anti-tumor immunity, ID8 cells, tumor microenvironment

Tissue transglutaminase (TG2) is a multifunctional protein that can perform enzymatic (transglutaminase, isopeptidase, protein disulfide isomerase, GTPase) and non-enzymatic activities (extracellular fibronectin binding). TG2 is overexpressed in cancer and is involved in metastasis, resistance to chemotherapy, and cancer stem cell signaling to the tumor niche. The functions of TG2 in cancer cell lines have been already described for several types of solid tumors. However, little is known about the role of TG2 in the host. We hypothesized that by knocking-out TG2 in the host ($TG2^{-/-}$) the OC tumor progression will be modified. Using the Roby syngeneic mouse model, we injected ID8 ovarian cancer cells intraperitoneally to investigate tumor progression and anti-tumor immune response in wild-type (WT) and $TG2^{-/-}$ mice. We observed a significant decrease in tumor burden in $TG2^{-/-}$ vs. WT mice, as evidenced by less ascites accumulation, and decreased number of cancer cells in ascites, which consequently led to increased median survival. This phenotype was accompanied by significant changes in anti-tumor immunity, as revealed by multicolor flow cytometry examination of major immune cell subsets in spleens and ascites from tumor-bearing animals. $CD8^+$ cells recovered from ascites or spleens of $TG2^{-/-}$ mice expressed higher levels of PD-1. Ex vivo stimulation of T cells from ascites revealed a significant increase in responsive IFNγ-secreting $CD8^+$ and $CD4^+$ cells in $TG2^{-/-}$ compared with WT mice. Interestingly, $TG2^{-/-}$ mice ascites contained an increased number of effector/memory $CD8^+$ T cells. At a molecular level, we found STAT3 activation dampened in ascites derived $TG2^{-/-}$ cytotoxic lymphocytes or after treatment of spleen derived WT $CD8^+$ T cells with TG2 inhibitors. Hence, TG2 deficiency tips the balance of STAT1/STAT3 regulated immune response to augment anti-tumor $CD8^+$ T cell immunity. Expression of PD-L1 was decreased on all myeloid subsets as well as on $EpCAM^+$ cells in $TG2^{-/-}$ ascites. RNA sequencing revealed an upregulation of the antigen presentation pathway in $TG2^{-/-}$ ascites tumor cells enriched fractions while top enriched pathways in $TG2^{-/-}$ vs. WT ascites-treated ID8 cells being related to cholesterol biosynthesis and immune response. Collectively, our data suggest decreased tumor burden concurrently with increased activation and effector functions of T cells, and loss of immunosuppressive signals in the peritoneal tumor microenvironment resulting in development of an anti-tumorigenic phenotype in $TG2^{-/-}$ mice.

ADVANCES OF COMPLEX BIOLOGICAL TESTING OF CALCIUM PHOSPHATE-BASED BIOCERAMICS WITH ORTHOPEDIC AND DENTISTRY APPLICATIONS

Cristiana Tănase[1,2], Adrian-Claudiu Popa[3,4], Ana-Maria Enciu[1,5], Lucian Albulescu[1], Maria Dudău[1,5], Ionela Daniela Popescu[1], Simona Mihai[1], Elena Codrici[1], Sevinci Pop[1], Andreea-Roxana Lupu[1,6], George E. Stan[3], Gina Manda[1], Radu Albulescu[1,7]

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Nowadays, a large spectrum of biomaterials has emerged, with emphasis on various pure, blended or doped calcium phosphates (CaPs). Although basic cytocompatibility testing protocols are referred by ISO 10993 (parts 1 to 22), rigorous in vitro testings using cutting edge technologies should be carried out in order to fully understand the behavior of various biomaterials (irrespective if in bulk or low dimensional object form) and to better gauge their outcome when implanted. In this review current molecular techniques are assessed for the in-depth characterization of angiogenic potential, osteogenic capability, modulation of oxidative stress and inflammation properties of CaPs and their cation and/or anion substituted derivatives. Using such techniques, mechanisms of action of these compounds can be deciphered, highlighting the signaling pathways activation, cross-talk and modulation by microRNAs expression, which in turn can safely pave the road towards a better filtering of the truly-functional, application-ready innovative therapeutic bioceramic-based solutions.

Acknowledgement. Project PN-III-P1-1.2-PCCDI-2017-0062 (contract no. 58)/component project no. 2; Program 1—The Improvement of the National System of Research and Development, Subprogram 1.2—Institutional Excellence—Projects of Excellence Funding in RDI, Contract No. 7PFE/16.10.2018; Core Program PN 19.29.01.04; grant COP A 1.2.3., ID: P_40_197/2016 (Romanian Ministry of Research and Innovation.

SATURDAY, NOVEMBER 23

SESSION 6

VARIA

Chair: Lecturer Ana-Maria Enciu

UNLIMITED GENE EXPRESSION META-ANALYSIS THROUGH MICROARRAY DATA MINING

Victor Ștefan Ionescu[1,2], Ioana Maria Lambrescu[1], Alina Mihaela Micu[1,3], Dan Sebastian Soare[1,4], Gisela Găină[1], Valeriu Cișmașiu[1]

[1]Victor Babeș National Institute of Pathology, Bucharest, Romania; [2]Clinical Emergency Hospital, Bucharest, Romania; [3]Aarhus Business Academy, Aarhus, Denmark; [4]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Keywords: chimerism, bioinformatics, polymorphism

Introduction. Open databases can become a powerful tool in the research process if harnessed to their fullest. We aim to employ this approach in choosing RNA targets carrying polymorphisms useful for the detection of post-hematopoietic stem cell transplant chimerism.

Materials and methods. The first step was to find strongly expressed genes in granulocytes. We searched the NCBI Gene Expression Omnibus (GEO) experiment repository for microarray studies which include that cell type and we selected the relevant samples. We mapped each probe to its corresponding expression values and to its targets, then, for each target, listed the expression values of all the probes linked to it. We filtered the targets by median expression, coefficient of variation and number of probes. The second step was to search for polymorphisms in these targets and to verify whether the overlapping transcripts are expressed in blood. The program was written in R 3.6.1 and run on the Amazon Elastic Compute Cloud for enhanced multi-processor performance.

Results and discussion. Sorted by median, the strongest expressed genes code for biomolecules associated with housekeeping functions (RNA28SN5, RN7SL1, RPS10P3, MT-ND3) and pathways likely involved in granulocyte physiology (OAZ1, S100A9, TYROBP, IFITM2, COX2). HLA family proteins (B, E, C) and certain genes typically used as qPCR reference (ACTB, B2M) are also found to be heavily expressed. A panel of 12 deletion/insertion polymorphisms (DIPs) has been selected for chimerism assay development. However, we have designed our program to be capable of meta-analyzing data from any number of unrelated GEO microarray datasets and samples - it is only limited by the available computing power. We plan to extend this capability to RNA-Seq experiments as well.

Conclusion. Using a data-driven approach, we have assembled a panel of targets for post-transplant chimerism detection (DIPs in RNA transcripts). Data mining has provided a valid starting point for our use case, pending experimental confirmation. The wealth of already available experimental data can and should be exploited just like any other scientific resource.

INTESTINAL BARRIER INTEGRITY, BACTERIAL ENDOTOXIN EXPOSURE AND INFLAMMATION MARKERS IN PARKINSON'S DISEASE: A CASE-CONTROL STUDY

Laura Dumitrescu[*1,2], Daciana Marta[*3], Emilia Manole[4,5], Adela Dănău[1,2], Antonia Lefter[1,2], Laura Cristina Ceafalan[3,6], Mihaela Gherghiceanu[3,6], Bogdan Ovidiu Popescu[1,2,3]

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Keywords: beta2 microglobulin, calprotectin, gut microbiota, LBP, zonulin

Parkinson's disease (PD) is a common neurodegenerative disorder with high socio-economic burden. Available evidence suggests that the neuropathology starts in the gut and spreads to the central nervous system via the vagus nerve by a prion-like mechanism. Alpha-synuclein misfolding with subsequent intraneuronal aggregate formation is thought to play an important role in triggering neuronal death, but the events leading to the conformational changes are incompletely known. Amyloidogenic products of the gut microbiota, such as lypopolisaccharide (LPS), a bacterial endotoxin that causes PD neuropathology in animal models, are environmental factors with a putative role in the etiopathogenesis of PD. We conducted a stage analysis within an ongoing case-control study to evaluate the association between inflammatory markers (calprotectin, serum beta2 microglobulin), intestinal barrier integrity markers (zonulin), bacterial endotoxin exposure markers (serum LPS binding protein, LBP) and PD. The analysis included 29 subjects, 18 with PD, 11 without (of which 3 with other neurodegenerative conditions). We found that calprotectin and serum beta2 microglobulin levels above the upper reference limit are associated with the risk of PD (serum calprotectin: OR 29.75, 95%CI 2.80-315.56, p=0.001; fecal calprotectin: OR 12.83, 95%CI 1.69-97.19, p=0.01; beta2 microglobulin: OR 8, 95%CI 0.83-76.36, p=0.09). Interestingly, fecal calprotectin levels above the cut off used for the diagnosis of inflammatory bowel diseases were identified exclusively in PD – considering that Crohn's disease and ulcerative colitis were recently reported as risk factors for PD, these PD patients may deserve additional investigations. Fecal zonulin was also significantly higher in PD versus controls (p=0.002), none of the controls having levels above the upper reference limit. Serum LBP levels were lower in PD (p=0.3), suggesting increased exposure to LPS. Our findings are in agreement with the few data available in the literature and support the hypothesis that intestinal barrier dysfunction and local inflammatory changes are associated with PD. To the best of our knowledge the association between serum beta2 microglobulin and PD was not reported to date. The current analysis is underpowered, but we will further assess our results on a larger sample size.

Acknowledgement. ANCSI PN 19.20.02.01/ 2019

BEHAVIORAL MODIFICATION OF TUMOUR CELLS INDUCED BY ESSENTIAL FATTY ACIDS

Maria Dudău[1], Victor Ștefan Ionescu[1], Lucian Albulescu[1], Sevinci Pop[1], Cristiana Tănase[1,2]

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Background. Fatty acids (FA), especially unsaturated omega-3 FA, have been considered beneficial for human health, and their consumption has been encouraged in disfavour of saturated FA.
Aim. To evaluate the impact of both saturated and non-saturated FA on cell proliferation and migration of both normal and tumour cell lines.
Materials and methods. Cell lines: normal human fibroblasts and melanoma cells. Cytotoxicity profile of purified fatty acids on cells was assessed by end-point assays (MTS, LDH). The anti-proliferative effect of fatty acid treatment on tumor fibroblast cell line was real-time monitored by measuring the cellular impedance (xCellingence platform). Cell migration was assessed by scratch-wound assay, using time-lapse.
Results. At comparable concentrations, saturated FA exhibit cytotoxic effects. At non-toxic concentrations, opposed to non-saturated FA, saturated FA exhibited anti-tumoral effects, quantifiable as both anti-proliferative and anti-migratory properties.
Conclusion. Saturated FA exhibit anti-tumoral effects that can be further exploited in conjunction with selected food supplements

Acknowledgement. This work was partially funded by Ministry of Research and Innovation in Romania, under Program 1 – The Improvement of the National System of Research and Development, Subprogram 1.2 – Institutional Excellence – Projects of Excellence Funding in RDI, Contract No. 7PFE/16.10.2018; by the grant COP A 1.2.3., ID: P_40_197/2016, and by Core Program, implemented with the help of MRI, Project No. PN 19.29.01.04.

COGNITIVE EVALUATION METHOD IN Nrf2-KO MICE USING THE EIGHT-ARM RADIAL MAZE

Cătălina Anca Cucoș[1], Ana-Maria Enciu[1,2], Laurențiu Anghelache[1]

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[2]*Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

Keywords: memory, mice, NRF2, radial arm-maze

Background. Biological aging is associated with a chronic background of oxidative stress and inflammation (inflammaging) that is believed to underline various age-related disorders, including cognitive impairment. The transcription factor NRF2 plays a key role in controlling the endogenous antioxidant system and may be a promising therapeutic target for age-related diseases. Animal models that mimic human disease and reliable cognition tests are still required for clinical translation of basic research. For memory impairment testing in mice models, the dry-land 8-arm radial maze (8RAM) based on food reward represents a better choice for mice than the conventional forced-swim test which is more relevant for rats.

Aim. To establish an 8RAM protocol for memory testing in aged Nrf2-/- mice as compared to "wild type" controls.

Materials and methods. The study was performed on aged (70-82 weeks), female Nrf2-/- mice on C57BL/6J background (n=10) and "wild type" controls (n=11). The cognitive function was evaluated using an 8RAM system (Panlab) and data were processed using the MAZESOFT-8 and the SMART video tracking software. The following cognition-relevant parameters were evaluated: working and reference memory, memory index, performance percentage and the total time to complete the maze. Several variables were investigated for customizing the test such as duration of the habituation phase, odor control and intermittent door opening during training.

Results. After testing several variables, a protocol comprising all three test phases (habituation, training and testing) was optimized as follows. Habituation: 10 minute-sessions per mouse and per day, during 5 days. Training: one 5 minutes-session per day, 20 learning sessions. Testing: one 5 minutes-session per day for three days. During the customized protocol experimental mice acclimated well (ate the food rewards and became accustomed to the experimenter). In the learning phase, the number of errors gradually decreased, as well as the total time to complete the maze. Using this 8RAM protocol, we found that Nrf2-/- mice were more active than "wild type" mice, completed the tasks in shorter time and with fewer errors, hence proving a better reference and working memory than controls.

Conclusion. The protocol allowed the assessment of various types of memory and behaviour in aged Nrf2-/- mice versus "wild type" controls. NRF2 knocking-out in aged mice did not trigger cognitive impairment, possibly due to compensatory mechanisms that protect mice against the increased oxidative stress evidenced in aging. These intriguing results will be investigated in future studies.

Acknowledgement. This study was funded by the European Regional Development Fund, Competitiveness Operational Program 2014-2020, through the grant P_37_732/2016 REDBRAIN.

POSTERS

MOUNTING: THURSDAY 18:00 - 18:30

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

EFFECTS OF CANNABIDIOL ADMINISTRATION ON THE HIPPOCAMPAL MORPHOLOGY AND NEUROSECRETION IN RATS EXPOSED TO REPETEATED RESTRAINT STRESS

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Keywords: neurogenesis, neurosecretion, restraint stress, neuroglobin, neuron morphology

Introduction. In rats, restraint stress is linked to immunosuppression and neuromorphofunctional dissorders. Cannabidiol could be a new therapeutic approach to protect the brain cells by enhancing the neuroglobin expression and the neurosecretions.

Materials and methods. Female Wistar rats were restrained in cylindrical holders for 15 days (4 hours/day). CBD was administered daily by enteral route in 2 different doses, as follows: 60 mg/kg and 120 mg/kg b.w. respectively. Brain samples were dissected out and prepared for Nissl and Golgi-Cox staining, western blotting, CAT activity assay and neuroglobin and BrdU immunohistochemistry.

Results. Cannabidiol administration enhances neurosecretions at 120 mg/kg b.w. At maximum dose an increase in the number of dendritic spines and size of the neuronal extensions associated with the shrinking of neuronal cell bodies were observed in the dentate gyrus and the frontal cortex. CBD reduces the activity of the NF-kB pathway and prevented c-Jun N-terminal kinase pathway activation. After treatment CAT activity recovered to its normal value and Ngb and BrdU expression was increased.

Conclusion. CBD administration is an effective way to induce neurogenesis and to protect the neurons from RS effects by modulating the neuroglobin expression and the neurosecretions.

Acknowledgement. Scientific Performance Scholarship from Babeș-Bolyai University

CHL1 GENE DUPLICATION IN A PATIENT WITH EPILEPSY, DEVELOPMENTAL DELAY AND DYSMORPHIC FACIAL FEATURES

Alina Erbescu[1], Magdalena Budișteanu[1,2,3], Aurora Arghir[1,4], Sorina Mihaela Papuc[1]

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Keywords: chromosomal microarray, duplication, epilepsy, neurodevelopment

Background. Epilepsy is a heterogeneous and complex group of disorders. Many studies have highlighted the importance of detecting recurrent copy number variations (CNVs) that ultimately lead to epilepsy genes discovery. The cell adhesion molecule L1 like (CHL1) gene is located in 3p26.3. The protein encoded by this gene is a neural recognition molecule that is considered to be involved in brain development and functioning, axonal migration, synaptic formation and plasticity. Deletions of CHL1 have been described in patients with neurodevelopmental delay and seizures. The potential effect of CHL1 overexpression is less understood, and the duplications of CHL1 have been rarely detected.

Aim. To analyze the genomic profiles of patients with epileptic seizures, using a customized chromosomal microarray platform, with focus on CHL1 gene structural variation.

Methods. A customized design for an array-CGH platform, that includes a large panel of epilepsy-related genomic regions and genes covered at an exon-level resolution, was used for genomic profiling of a group of 48 patients with epileptic seizures (Agilent Technologies, 4x180k array format).

Results and discussion. We present a 15 years-old girl with epilepsy, global developmental delay and facial dysmorphism with a 3p26.3-p26.2 duplication, including the CHL1 gene. The size of the duplication was 3.74 Mb and, besides CHL1, 5 OMIM genes and 6 RefSeq genes were included in the duplicated region. Duplications of CHL1 are rare events; our patient brings new data on the association of CHL1 with epilepsy, not only by haploinsufficiency but also by copy number gain.

Conclusion. The use of high resolution microarray brings significant progress in understanding the genetic profile of epileptic disorders, and allows for an accurate molecular characterization of structural genomic variations. Duplications of CHL1 have been rarely identified in neurodevelopmental disorders and our patient supports the role of CHL1 in the pathogenesis of these conditions, epilepsy included.

Acknowledgement. Funding: UEFISCDI 249PED/2017; Ministry of Research and Innovation grant PN 16.22.05.01.

DELETION 13q14.3 IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Keywords: del(13q14.3), CLL, prognostic

Del(13q14.3) is the commonest cytogenetic abnormality detected by FISH in B cells in more than 50% of Chronic Lymphocytic Leukemia (CLL) patients and is considered to be an early event in CLL evolution. Del(13q14.3) occurs in hemizygous state in approximately 75-80% of cases positive to this anomaly, and in the homozygous state in the remaining 20-25%. Del(13q14.3) as the sole cytogenetic abnormality has been associated with a better prognosis than del(17p) or del(11q). However, the studies suggest that the clinical course of CLL patients carrying higher percentage of 13q-deleted nuclei (cut-off point range from 65-90%) is accelerated related to shorter time to first treatment (TTFT) and overall survival. In the same time, clonal evolution is frequent in CLL, and the prognostic impact of unique alterations can be changed by additional molecular and/or genetic abnormalities. We focus on the prevalence of del(13q14.3) in a group of 40 CLL patients, evaluating by fluorescence in situ hybridization (FISH) techniques the recurrent CLL chromosomal anomalies, and on the opportunity to better analyze the correlations of cytogenetic lesions with clinicobiological features. We applied interphase-FISH analysis on peripheral blood samples from 40 CLL patients, using Vysis-Abbott Molecular probes for: 13q14.3 (encompassing D13S319 and DLEU1 loci) and 13q34 (LAMP1) region, 11q22.3 (ATM) locus, 17p13.1 (TP53) locus, 6q23 (MYB) locus, and alpha satellite, centromeric region of chromosome 12 (CEP12). A total of 100 interphase nuclei were analyzed for each probe. Del(13q14.3) was found in 6 out of 40 CLL patients, 4 of them bearing del(13q14.3) as a sole aberration, one patient presenting del(13q14.3) co-occurrent with del(11q22.3) and another del(13q14.3) positive patient presenting also del(17p13.1) and del(6q23). The percentage of positive nuclei was 83% for one patient and below 60% for the other 5 patients. Four patients showed heterozygous del(13q14.3), the other 2 patients presented both heterozygous and homozygous del(13q14.3). FISH analysis of del(13q14.3) prevalence and load in CLL-patients showed a percentage of positivity of 15%. Although this value is smaller than previously reported – due to the small patient group - the clinical correlations for del(13q14.3) positive patients are in concordance with literature data. Three out of 6 patients have stable disease and did not reached time for treatment; the patient with 3 co-occurring chromosomal anomalies presented 24 months TTFT. This justifies the need for routine identification of 13q14 and other additional anomalies and for periodic genetic testing during CLL evolution in order to optimize the clinical management.

BLENDING THE PRECISION OF RNASE H-DEPENDENT PCR WITH THE ACCURACY OF DIGITAL DROPLET PCR - THE GOOD, THE BAD AND THE "RAIN"-

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Keywords: RNase H-dependent PCR, droplet digital PCR, single nucleotide polymorphism

Introduction. One of the most pressing issues while performing a PCR reaction is the formation of primer-dimers and off-target amplification. Therefore, the use of 3' end-blocked primers that are cleaved after hybridization by the enzyme RNase H2-(rhPCR) is a promising technique, especially in terms of high specificity. The array of molecular techniques available for the detection of single-nucleotide polymorphisms (SNP) includes a novel method-digital droplet PCR (ddPCR)-based on water oil emulsion. We hypothesized that the merger of rhPCR with ddPCR would increase both sensitivity and specificity for the detection of polymorphisms.

Materials and methods. We devised our experiment as two consecutive reactions. For the first step, we employed the rhAmp SNP Genotyping kit (IDT USA) without Reporter mix, for detecting 2 chosen polymorphisms (rs2230054 and rs857870). Consequently, the amplified product was quantified in the second reaction following manufacturer specifications-Bio-Rad ddPCR Supermix for Probes (no dUTP). In order to reach a λ coefficient of 1.6, we estimated the appropriate number of cycles (4), template quantity (20.000 copies/reaction) and dilution factor (1:5) for the first step. Since the RNase-H2 was inactive in the ddPCR Supermix, an equivalent rhPCR primer (PSII) but without the 3' end-blocker was added to the second step of the reaction. In our attempt to reduce the "rain" phenomena we varied the concentration of PSII (5, 1 and 2.5 μM). The first step cycling conditions were enzyme activation at 95°C (10 minutes), followed by annealing-extension steps (60-68°C for 30-20 seconds). For the second step the Bio-Rad cycling conditions were those stated by the manufacturer.

Results and discussion. Though a very promising technique, rhPCR is not effective in the water-oil emulsion droplet technology due to lack of activation of RNase-H2. Therefore, we separated the two reactions in order to benefit from both techniques. Although positive and negative droplets cluster separately, a large proportion of droplets with intermediate fluorescence were spread between the clusters – a process known as "rain". The "rain" is characterized by inadequate separation of negative and positive droplets, which could affect absolute quantification. Despite varying PSII concentrations, significant amounts of droplets with intermediate fluorescence were generated for both homo and heterozygous samples. The best demarcation between positive and negative droplets was attained with 20.000 copies/reaction, 4 cycles (step 1) and 5 μM of PSII.

Conclusion. The limitation of "rain" phenomena renders accuracy and credibility for ddPCR results. Even after adjusting PSII concentration, the rain pattern does not show signs of improvement, probably due to the existence of both wild type and mutant assays in the same reaction.

Acknowledgement. Ministry of Research and Innovation grants PN19.29.01.03 and Project of Excellence Funding in RDI, Contract No.7PFE/16.10.2018.

PATTERNS OF RENAL INJURY IN ANCA GLOMERULONEPHRITIS: A MORPHOLOGICAL STUDY OF 4 CASES

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Keywords: kidney nephritis biopsy histopathologically aspects

Introduction. The predominant renal injury in ANCA positive patients is crescentic glomerulonephritis. The formation of crescents is a response to glomerular capillary rupture followed by fibrin exudation and accumulation of neutrophils with an extracapillary circumferential or non-circumferential epithelial response. ANCA crescentic glomerulonephritis is the most common cause of crescentic glomerulonephritis and most frequent in older patients.

Objective. The aim of this study was to present a series of 4 cases of ANCA glomerulonephritis diagnosed at Fundeni Clinical Institute between 2018 and 2019, with their distinctive morphological characteristics.

Material and methods. Specimen samples were biopsied at the Nephrology department at Fundeni Clinical Institute, processed by conventional histopathological methods with Hematoxylin – Eosin staining and also special stains were performed (Periodic acid Schiff – PAS and Masson trichrome).

Results. Out of 4 patients diagnosed with ANCA associated glomerulonephritis in our pathology department, three were male patients with a median age of 52.33 years (the youngest being 40 years old and the oldest 71 years old) and one female patient of 69 years of age. Two male patients were p-ANCA positive (89U/ml and 11.5U/ml) and one male patient was c-ANCA positive (>200U/ml). The renal biopsies in pANCA patients revealed a mixed proliferative glomerulonephritis (mesangial and extracapillary) with cellular and fibrous crescents (2/10 glomeruli and 3/17 glomeruli) with focal fibrinoid necrosis, rare intracapillary neutrophils and focal segmental and global glomerulosclerosis (4/10 glomeruli and 3/17 glomeruli). The renal biopsy in cANCA patient revealed a mixed proliferative glomerulonephritis (mesangial and extracapillary) with cellular non-circumferential crescents (1/13 glomeruli), focal segmental glomerulosclerosis (3/13 glomeruli) and intraluminal capillary thrombosis. The female pANCA positive patient (58U/ml) was diagnosed with diffuse crescentic glomerulonephritis with focal fibrinoid necrosis, intraluminal capillary neutrophils and chronic vasculitis.

Conclusion. Crescentic glomerulonephritis is a pattern of injury leading to severe and rapidly progressing nephritis and renal chronic injury if left untreated with immunosuppressive treatment. The extracapillary proliferation, focal or diffuse, cellular or fibrous, of varying degrees with fibrinoid necrosis and subsequent segmental and global sclerosis is an evolutive process in ANCA positive patients with or without chronic associated vasculitis.

A STEP-BY-STEP APPROACH IN A SUSPECTED RARE CASE OF GASTRIC ADENOCARCINOMA AND PROXIMAL POLYPOSIS OF THE STOMACH

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Keywords: GAPPS, digestive polyposis syndromes

Introduction. Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) is a recently described rare autosomal dominant syndrome, characterized by pathognomonic involvement of the gastric region and a unique genetic signature. Making a definitive diagnosis requires clinical-pathologic correlation, exclusion of other digestive polyposis syndromes and genetic testing. The case we present highlights the importance of the differential diagnosis.

Methods. A 61-year-old male patient with dyspeptic syndrome and no relevant history underwent gastroscopy and colonoscopy at our clinic. Hundreds of polyps carpeted the body and fundus of the stomach with total antrum sparing. There were no colonic polyps. Biopsy examination revealed non-dysplastic hyperplastic and fundic gland polyps with H. pylori infection. Eradication treatment and annual follow-up were recommended. Before re-evaluation, the patient presented with hematemesis and melena. Gastrectomy was performed and pathologic examination revealed an increase in polyp number and size, with no signs of infection.

Results. Particular clinical and pathologic findings led to high suspicion index for GAPPS syndrome. We therefore reviewed and checked off the current diagnostic criteria: there were more than one hundred hyperplastic and fundic gland gastric polyps with antral sparing, no intestinal polyps and no history of prolonged proton pump inhibitor intake. These specific features led to exclusion of MUTYH-Associated Polyposis (hallmark – duodenal adenomatous polyps), Generalized Juvenile Polyposis, Peutz-Jeghers, Cronkhite-Canada and Cowden Syndromes (hamartomatous polyps). The challenge was differentiating GAPPS from Familial Adenomatous Polyposis (FAP), due to occasional overlap inbetween. Unlike FAP, GAPPS syndrome increases the lifetime risk of gastric adenocarcinoma, hence the importance of distinguishing the two. FAP usually presents with numerous adenomatous colonic polyps; the stomach may show diffuse fundic gland polyps with no antral sparing. The same gene (Adenomatous Polyposis Coli, APC) is mutated in both. Whereas FAP is defined by biallelic APC inactivation, GAPPS is associated with methylation of IB promoter. The last step towards the definitive diagnosis is genetic testing. In our case, the results are still pending, but all the other features are consistent with GAPPS.

Conclusion. Clinical and pathologic peculiarities in extensive gastric polyposis cases (i.e. antrum and colon sparing) should raise the suspicion of GAPPS syndrome. Numerous studies postulate that gastrectomy is superior to periodic surveillance in preventing gastric adenocarcinoma. The differential diagnosis is hence vital and the families of the patients should undergo genetic testing to determine the individual risk.

THE EFFECTS OF ANAKINETIC STRESS ON THE BIOCHEMICAL BLOOD PARAMETERS AFTER 2 AND 6 DAYS OF CONSTRAINING

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Keywords: anakinetic stress, Wistar rats, biochemical blood parameters

Under various stressful agents, the restraint stress or prolonged anakinetic stress induce the formation of reactive oxygen species (ROS) which causes peroxidation of the lipids, especially to the cell membrane level, with destructive effects on the entire tissue. Prolonged anakinetic stress also induces high levels of corticosterone according to our previously research accompanied by the increases of serum cholesterol and lipoproteins - active risk factors in the pathology of cardiovascular diseases and diabetes.

Our study depicts the biochemical changes induced by anakinetic stress at 2 and 6 days of exposure. The experiment was performed on three female Wistar rats' groups of 6 animals each, as follows: Control (C), Restraint Stress for 2 days (S2) and Restraint Stress for 6 days (S6). Restraint stress was induced by rat's immobilization in plastic holder, 3 hours/day. Animals were obtained from the biobasis of „Iuliu Hatieganu" Medicine and Pharmacy University, Cluj-Napoca and kept under standardized zoohigienical conditions: in accordance to the European Communities Council Directive 2010/63/UE Directive of European Parliament. At the end of the experiment, animals were killed by decapitation and blood and organ were collected and prepared for biochemical and histological assays. As concern biochemical analysis repeated stress induced increases in IgG production in S2 group (177,97%) and 91,24% in S6 group vs C group. Total Proteins increase with 45,98% and cholesterol with 39,31% in S2 group vs C group. Also, glucose is increased at 2 days (27,49%) and 6 days (30,43%). ASAT increase with 48,67% in S2 group and with 60,44% in S6 group. Biochemical modifications are correlated with histochemical modifications and illustrate a reactivity of the body to the stress status.

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TUMOUR-PRIMED MURINE NATURAL KILLER CELLS EXHIBIT DISTINCT PHENOTYPE

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Keywords: NK cells, B16F10 and YAC-1 tumour cell lines

Introduction. Cancer immunotherapy represents a new milestone for cancer therapy, with the development of multiple immune cells as therapeutic tools. A promising cellular immunotherapy for cancer is based on the cytolytic activity of natural killer (NK) cells. Although in vitro activation, or "priming," of NK cells by exposure to cytokines, such as interleukin IL-2, IL-12, IL-15 or IL-18, has been extensively studied, the biological consequences of NK cell activation in response to target cell interactions have not been thoroughly characterized. We investigated the consequences of co-incubation with B16F10 and YAC-1 tumour cell lines on the phenotype of murine NK cells.

Materials and methods. Spleen were harvested from healthy C57BL/6 mice, 8-10 weeks old. Freshly-isolated immune cells from spleen were maintained in complete growth medium (RPMI 1640 supplemented with 10% v/v fetal bovine serum and 2mM l-glutamine). NK cell stimulation was achieved by co-incubating immune cells (3x106) with untreated B16F10 cells, or YAC-1 cells (1x106) for 4 or 24 hours at 37°C, 5% v/v CO2 in complete growth medium. After incubation period the immune cells were washed and immediately incubated with fluorochrome-conjugated mAbs and used for assessing the flow cytometry analyses of NK cells. Stained cells were analysed with a FACSCanto II flow cytometer using DIVA software.

Results and discussion. We observe the downregulation of activation receptor NKp46 and other maturation molecules like CD49b, CD122 following tumour-priming. Although this NK cell phenotype is typically associated with NK cell dysfunction in cancer, we reveal the upregulation of NK cell activation markers, such as CD69, CD132 and CD25, associated with enhanced NK cell cytotoxicity and immunomodulatory functions. Thus, it appears that tumour-mediated ligation of receptors on NK cells may induce a primed state which may or may not lead to full triggering of the lytic or cytokine secreting machinery.

Conclusion. These findings help define the phenotypic of NK cells following their encounters with tumour cells, independent of cytokine stimulation, and provide insight into tumour-specific NK cell responses to inform the transition toward harnessing the therapeutic potential of NK cells in cancer.

Acknowledgement. This work was partially supported by Core Program, implemented with the support NASR, projects nos. 19.29.02.03 and 7PFE/16.10.2018.

TOWARDS ULTRASTRUCTURAL, MOLECULAR AND BEHAVIORAL FEATURES OF AUTISM-LIKE DISORDERS (ASD) INDUCED BY VALPROATE EXPOSURE AND THE PROPHYLACTIC EFFECT OF ALLICIN - A PROBABLE MECHANISM FOR ASD -

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Keywords: myelin, valproate, allicin, autism

Introduction. In rats, in utero exposure to VPA (single high dose, 600 mg/kg, i.p.) at gestational day, 12.5 leads to autism-like disorders (ASD). Allicin could be a therapeutical candidate for ASD by its interaction with GSH and allicin-GSH complex formation.

Materials and methods. The valproate was administered in a single dose (500 mg/kg) in E12.5 in pregnant rat females. The allicin was administered during fetal development. The brain samples (E15) were prepared for TEM investigation, western-blotting, and RT-PCR.

Results and discussion. Valproate exposure induces myelin vacuolation, membrane-synaptic changes and decreased the number and morphology of the MVB associated with EV. Alongside, after valproate administration and allicin treatment, HDAC4, Nrf2, MeCP2 are at normalized expression compared to Control and myelin structure was normalized as well as postnatal behavior (social interaction and anxiety).

Conclusion. ASD induced after VPA exposure seems to be a MVB/EV as well as a myelin-function associated pathology. Allicin normalized the expression of several key proteins which are involved in ASD molecular mechanism and also restored the myelin structure.

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PLACENTAL SENESCENCE IN PRETERM BIRTH

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Keywords: aging, development, embryology

The **aim** of this work is to study placental senescence as a normal/pathological phenomenon in terms of natural selection at the cellular level.

Material and methods: from 78 cases in study, with gestational age between 16 to 40 weeks, 51 were by cesarean operation.

Results and discussion: suggestive lesions for placental senescence were syncytial knots in all 68 cases, fibrinoid necrosis in 62 cases, corangiosis in 38 cases and calcification in 29. In the control of placental growth, there are many agents thought to be of importance as cytokines, growth factors, oncogenes, prostaglandins and leucotrienes. The focal thinning of the villous syncytiotrophoblast, with syncytial knots presence, has often been cited as evidence of syncytial senescence. The decreased fetal perfusion of the placental villi is responsible for fibrinoid necrosis, and possible mechanisms of tissue calcification involved by ischemia-related processes.

In **conclusion,** placenta does not undergo a true senescence change during normal pregnancy. How placenta, as a fetal organ, should age while the other fetal organs do not? The belief in placental aging has been based on a confusion between morphological maturation and differentiation versus aging, i.e. a failure to appreciate the functional resources of the organ.

INFLAMMATION-RELATED PATTERNS IN THE CLINICAL STAGING ASSESSMENT OF CHRONIC KIDNEY DISEASE

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Introduction. Persistent, low-grade inflammation is now considered a hallmark feature of chronic kidney disease (CKD), being involved in the development of all-cause mortality of these patients. An imbalance in the kidney-vascular-bone axis, a multifaceted active process, is induced by mineral metabolism disorders and also by local inflammation; nevertheless, the most extensive mineral disorders are experienced by patients suffering from CKD. The discovery of Wnt inhibitors, among them Dickkopf-related protein 1 (Dkk-1), released during renal repair as crucial components of mineral bone disorder (MBD) pathogenesis, suggests that additional pathogenic factors need to be explored. In this scenario, our study focused on novel CKD biomarker patterns and their impact on staging and severity of the disease.

Materials and methods. The relative expression levels of 105 proteins were assessed by Proteome Profiler Cytokine Array Kit for pooled CKD stages 2–4 serum samples. Among the molecules that displayed significant dysregulation in CKD stages, we have further explored the involvement of Dkk-1, a recognized inhibitor of the Wnt signalling pathway, and its crosstalk with calcitriol as new players in renal bone and vascular disease. Their serum level was quantified by ELISA (on 76 samples) and the results revealed decreasing circulating levels of Dkk-1 and calcitriol in advanced CKD stages, with their circulating expression showing a downward trend as CKD develops. In the next step we analyzed the inflammation and MBD biomarkers' expression in CKD (by Luminex xMAP array). Our results showed that the molecules involved in orchestrating the inflammatory response, IL-6 and TNFα, as well as the mineral biomarkers osteoprotegerin, osteocalcin, osteopontin and fibroblast growth factor 23, were correlated with Dkk-1 and calcitriol, raising as potential useful candidate biomarkers.

Conclusions. Our results revealed the impact of different biomarker patterns in CKD staging and severity, thus opening up novel approaches to be explored in CKD clinical management. Nevertheless, further studies are needed to clearly unravel the complex networking between Dkk-1, calcitriol, the mediators of inflammation, and MBD markers to design promising biomarker signatures for CKD, starting with its early stages.

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PROTEOMIC ALTERATIONS REVEALED IN THE CROSSTALK OF POLYMORPHONUCLEAR NEUTROPHILS AND MACROPHAGES USING DYNAMIC SILAC METHODOLOGY

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Keywords: neutrophil, macrophage, crosstalk, proteomics, SILAC

Introduction. A finely-tuned balance of inflammation and repair orchestrates the efficient cardiac recovery following a myocardial infarction. An instrumental role in mediating these two processes is played by polymorphonuclear neutrophils (PMNs), which recruit and regulate the local differentiation of monocytes towards an inflammatory and\or reparatory macrophage (MAC) phenotype.
The **aim** was to investigate the dynamic crosstalk between PMNs and MACs, identifying the alterations induced by the PMNs' secretome in the MACs proteome.
Methods. Mice bone merrow-derived PMNs were stimulated with either LPS (1000ng\ml) and IFNγ (20ng\ml) or IL4 (20ng\ml) to induce an inflammatory (N1) or reparatory (N2) phenotype. The cells` secretome was collected after a 2h stimulation and transferred on naïve MACs isolated from murine spleen monocytes. Dynamic stable isotope labeling of amino acids in cell culture (dSILAC) was applied in two steps: labeled lysine (for the first 3 days, concomitant with the stimulation with cell secretome) and labeled arginine-containing media (for the next 3 days). MACs with unstimulated PMN secretome (N0) were used for normalization. The cells were solubilized and prepared for peptide nano-chromatographic separation and mass spectrometric analysis. Proteome Discoverer 2.1 and Protein Center 3.16 (Thermo Scientific) were used for the bioinformatic analysis.
Results. Proteins incorporating either one tag or the other were de novo synthesized as a result of a direct activation with N1 or N2 secretome, or following the removal of the stimuli. The global proteomic analysis demonstrated a significantly higher number (>25%) of identified MACs` proteins as a result of direct N2 secretome stimulation ($p<0,005$ when compared with either N0 or N1 activated MACs). An average of 209 and 304 proteins carried the lysine tag the first 3 days, suggesting a differential synthesis rate under N1 and N2 stimulation, respectively, as opposed to the initial state (day 0). After stimuli removal and media change, the arginine tag was identified in 114 and 152 proteins in the two groups of MACs, which continued to differentially synthesize proteins even after N1 and N2 secretome samples were discarded. The bioinformatic analysis demonstrated that the 275 proteins that were uniquely differentially abundant in the first 3 days as a result of the direct stimulation with N2 secretome were implicated in multiple signaling networks, such as phagosome, gap junctions or ECM-receptor interaction pathways. Furthermore, after N2 secretome removal, the proteins that uniquely carried the arginine tag were found to be associated with altered glycolysis/gluconeogenesis or biosynthesis of amino acids processes.
Conclusions. Protein turnover was assessed using dSILAC and mass spectrometry. Novel protein mediators and functional networks have been identified with potential in the better understanding of the dynamics of PMNs and MACs crosstalk.

Acknowledgement. The work was supported by The Romanian Academy and the Ministry of Research and Innovation UEFISCDI grant PN-III-P4-ID-PCCF-2016-0172.

PHARMACOLOGICAL MODULATION OF CHLORIDE MEMBRANE TRANSPORT IN MATURE HIPPOCAMPAL CULTURES SUBJECTED TO OXYGEN-GLUCOSE DEPRIVATION AND REOXYGENATION

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Keywords: hippocampal neurons, NKCC1, KCC2, GABAA receptor, stroke

Ischemic stroke is a severe medical condition for which current standardized therapeutic protocols provide unsatisfying results. The disruption of the excitability-inhibition balance and alterations in ionic gradients are key pathophysiological mechanisms involved in the subsequent neuronal death. Intracellular chloride level shifts, changing the neuronal response to GABAA receptor activation from hyperpolarising to depolarizing in mature cells, impacting on cellular viability. These changes are dependent on the chloride co-transporters (NKCC1 and KCC2) expression and activity. In the present study we examined the effect of NKCC1-antagonist bumetanide, KCC2-antagonist DIOA, GABAA-antagonist gabazine or GABAA-agonist isoguvacine on the response of mature hippocampal neurons to oxygen-glucose deprivation (OGD) and reoxygenation in culture, by measuring cell viability.

Primary hippocampal cultures were obtained from Wistar rats on postnatal day 0. After 7 days in vitro, cultures were exposed to 2h OGD or control conditions (normoxia in glucose-containing medium). Evaluation of cellular viability was performed using resazurin assay during a 3h-reoxygenation in a medium containing glucose. Treatments were performed either during OGD or reoxygenation.

Our results show that the decrease in cellular viability triggered by OGD exposure ($p<0.05$) was further accentuated by DIOA treatment during OGD and gabazine treatment during reoxygenation ($p<0.05$). Bumetanide treatment during OGD increased cellular viability when compared to nontreated OGD-exposed cultures ($p<0.05$).

Our findings underline the potential positive effect that pharmacological modulation of intracellular chloride concentrations may have on post ischemic neuronal viability. We also demonstrated the paramount importance of properly timing the treatment application in relation to the moment of the ischemic insult.

CD36 IN THE AGING NEUROVASCULAR UNIT

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Keywords: CD36, ultrastructural study, aging

The ultrastructural and molecular changes at the level the neurovascular unit (NVU), especially of the blood-brain barrier (BBB) represent one of the prerequisites of the cognitive impairment due to consequent vascular dysfunction. Cognitive impairment is a common finding in both aging and neurodegenerative diseases. Despite the constant progress of neurosciences, the therapeutic approach is still limited. Therefore, presently, one of the major aims is to extend the view on the underlying pathophysiologic mechanisms by discovering new molecular players that could be targeted and modulated by more specific drug classes.

CD36, the scavenger receptor class B member 1 (SRB1) or fatty acid translocase (FAT) is an integral membrane glycoprotein with affinity for different ligands related to various cellular processes but especially for long chain fatty acids translocation. In the normal brain, CD36 works as a regulator of neuronal fatty acid sensing and energy homeostasis. CD36 is expressed by several types of cells, such as endothelial cells, pericytes, astrocytes and microglia, which build up a complex network providing the structural and functional connection between neurons and the blood, the NVU. CD36 was recently declared an essential player for the cerebrovascular oxidative stress and post- ischemic stroke inflammation, however, vascular dysfunction associated with age and metabolic dysregulation are also key factors involved in neurodegeneration.

Our ultrastructural study of the BBB in ageing mice model revealed its increased thickness and the build-up of lipid inclusions within the basement membrane (BM) as well as in perivascular cells, such as microglia and macrophages along with their much higher frequency in the NVU. Immunolabelling highlighted the expression of CD36 in perivascular cells, most probably macrophages, which accumulate in the perivascular space of the old-mice BBB. Our preliminary data suggests a role for CD36 in the build-up of lipid droplets in the NVU that may lead to impaired neurovascular coupling and BBB dysfunction in aging, favoring the accumulation of the altered proteins in the brain and subsequent neurodegeneration.

Acknowledgement. This work was supported by Minister of Research and Innovation under Grant number PN 19.29.01.02/2019.

TOXIC EFFECTS OF NON-ACUTE LEAD EXPOSURE ON RAT MODEL

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Keywords: rat model, the effects of lead exposure

Introduction. Lead (Pb), a heavy metal with not apparently biological function, is widespread and non-biodegradable pollutant of great concern to human health. Lead poisoning can affect many organs and tissues, being associated with a number of morphological, biochemical, hematological, physiological, and behavioral changes. The critical target for lead intoxication is the hematopoietic system, but lead toxicity is linked to toxic effects on the nervous system also. In this study, we investigated the toxicity of lead in a rat model of non-acute exposure.

Materials and methods. We use adult male Wistar rats from Animal Husbandry of "Victor Babeș" National Institute of Pathology. Healthy male rats were divided into six groups of 6 animals each. Intoxication of experimental animals was done with lead acetate dissolved in distilled water, one group received Pb in drinking water and the other five groups of animals received Pb by an oral gavage, as follows: group A control, treated with water only; group B intoxicated 1% lead acetate in drinking water for 6 weeks, group C intoxicated 400mg/kg body weight/day for 4 weeks; group D intoxicated 200mg/kg body weight/day for 4 weeks; group E intoxicated 200mg/kg body weight/day for 6 weeks; group F intoxicated 100mg/kg body weight/day for 4 weeks. Toxic effects of Pb were investigated on haematological and biochemical parameters, concentration of δ-aminolevulinic acid dehydratase (ALAD) in serum, accumulation of lead in tissues, redox status in serum and behaviour of treated rats.

Results and discussion. Lead toxic effects on haematological parameters seem to be leukopenia lead treatment, and leukocytosis for 6 weeks treatment. All experimental groups present thrombocytosis. Assessment of animal behaviour suggests that lead exposure may possibly induce the increasing of anxiety. The accumulation of Pb in tissues is accompanied the disturbances of both hematological and biochemical parameters. It was observed that the level of Pb in tissues had a different distribution pattern after dose and period of exposure.

Conclusion. Our results showed that non-acute exposure to lead induced toxic effects in the blood, and central nervous system of adult Wistar rats. Comprehensive observations suggest that exposure to higher Pb dose for a long period produces more pronounced effects compared to the response observed after exposure to lower dose.

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IN VITRO AND *IN VIVO* EVALUATION OF THE COLLAGEN - INORGANIC NANOPARTICLES COMPOSITES FOR WOUND HEALING APPLICATIONS -

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Keywords: chronic wounds, collagen sponges, inorganic nanoparticles

Introduction. Chronic wounds affect a large number of people and it has been estimated that, in developed countries, 1-2% of the population may suffer a chronic wound during a lifetime. This has a significant impact on the health and quality of a patient's life and, also on the healthcare system. Successful wound management relies on understanding the healing process combined with the development of dressing materials that maintain optimal moisture and aeration parameters as well as ensure antibacterial protection. The aim of our study was to investigate, the biocompatibility and the efficacy of nanocomposite collagen sponges embedded with ZnO and SiO2@ZnO inorganic nanoparticles in wound healing applications.

Materials and methods. *In vitro* cytotoxicity test was assessed by using MG-63 cell line and mesenchymal stem cells (MSC). These cells were co-cultured with collagen sponges embedded with different concentrations of ZnO and SiO2@ZnO inorganic nanoparticles, and toxicity was determined using Trypan Blue microscopy test and the CellTox™ Green Cytotoxicity Assay. The influence of both collagen sponges and ZnO-incorporated nanocomposites on cell cycle progression was evaluated by flow cytometry using propidium iodide staining. For in vivo experiments CD1 mice were used in order to determine the biocompatibility of nanocomposite collagen sponges, along with their influence on the wound healing process. Tissue regeneration and inflammatory responses were evaluated by immunohistochemical analysis and RT-PCR.

Results and discussion. *In vitro* evaluation indicated low cytotoxicity induced by nanocomposite materials with high SiO2@ZnO percent concentrations. The cell cycle analysis showed an increase in the percentage of cells in the G1 phase when collagen ZnO 3% was used. Our *in vivo* results (photographic, immunohistochemical evaluation and tissue cytokine profile) indicate a slow acceleration of the wound healing process in the presence of ZnO in combination with SiO2.

Conclusion. Our *in vitro* and *in vivo* results suggest that the collagen functionalized with ZnO and SiO2@ZnO inorganic nanoparticles exhibits good biocompatibility and sustain initiation of the healing process.

Acknowledgement. This work was supported by CNCSIS-UEFISCSU project PN-III-P2-2.1-52PTE/2016 and structural funds POS CCE O2.2.1. 433/2012.

ALARMINS EXPRESSION IN ATHEROSCLEROTIC LESIONS UNDER SEVERE ANTI-HYPERLIPIDEMIC TREATMENT; A MASS SPECTROMETRY BASED PROTEOMIC APPROACH

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Keywords: alarmins, atherosclerosis, mass spectrometry

Introduction. Atherosclerosis is considered a multifactorial disease with risk factors ranging from high-fat diet, hypertension, smoking, diabetes, to genetic susceptibility and other factors. Classic statin treatment has led to significant reduction of clinical events, but considerable residual risk of cardiovascular related mortality still remains. Alarmins, the host biomolecules that can initiate and perpetuate a noninfectious inflammatory response, are critical players of atherosclerotic plaques progression.

Objective. The main objective of the project is to explore alarmin expression in the different stages of atherosclerotic plaque development, from early to late stages by high performance mass spectrometry-based proteomic analysis, in order to identify biomarkers with predictive potential.

Materials and methods. 12 healthy male 12 weeks-old New Zeeland white (NZW) rabbits were randomized into 4 groups: C (control standard diet); Ae (athero diet followed by standard diet); As (athero diet followed by standard diet and treatment 1 month); Au (athero diet 3 months). Blood samples were drawn from the auricular artery of animals and collected in standard tubes at week 0, 4, 8 and 12 of the experiment. Atherosclerotic plaques developed in aortic and coronary arteries in NZW rabbits were evidenced in semi-thin frozen sections stained with Hematoxilin and Oil Red O. The aortic homogenates were suitably processed for mass spectrometric analysis.

Progress/ Intermediary results. The thoracic aorta homogenate comparative shotgun proteomic experiments revealed a high plethora of proteins (7894 proteins). Thus, 5976 proteins were identified in the control tissue samples (C), 4994 proteins in the early atherosclerotic group samples (Ae), 4928 proteins in the stabilized atherosclerotic group samples (As) and 5124 proteins in the vulnerable atherosclerotic group samples (Au). Label-free relative quantification procedure revealed a total of 24 differentially expressed alarmins in homogenates obtained from atherosclerotic groups (Ae, As and Au) versus healthy tissue (C).

Discussion. After 12 weeks, the tissue fragments harvested from all animal groups demonstrated that atherosclerotic advanced unstable plaques were developed in the lesion prone-areas of all animals fed high fat diet and the applied treatment significantly reduced the plaques dimension and also the histological aspects of the lesion. Using high performance technologies, we revealed the differentially abundance of 24 alarmins, which discriminate between the different stages of aortic atherosclerotic plaque evolution.

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ELECTRON MICROSCOPY FOR SCREENING OF EXTRACELLULAR VESICLES

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Keywords: TEM, cryo-EM, extracellular vesicles

Extracellular vesicles (EVs) are cell-derived particles that have key roles in intercellular communication. In both normal and pathological conditions, EVs transport specific cargo such as DNA, mRNA, proteins and lipids that modulate signaling pathways in recipient cells. EV populations are notoriously heterogeneous and hard to distinguish from spherical lipoproteins when using conventional methods. Therefore, validating the presence of EVs in patient samples through imaging methods, prior to other downstream studies, is crucial. Here we show the use of two electron microscopy (EM) techniques for the validation of EVs from the blood of hepatocarcinoma patients, isolated through two different methods. In addition to negative stain (NS), which is well established, we use cryo-electron microscopy (cryo-EM), which allows for more precise identification and morphological characterization of EVs.

EVs from plasma of patients with hepatocarcinoma were isolated using the ultracentrifugation method and a commercially available kit (Total Exosome Isolation Kit, Invitrogen). NS was employed to analyze the distribution of particles and to select an optimal dilution for cryo-EM. Samples were embedded in a thin layer of vitreous ice by rapid plunging in liquid nitrogen (LN2)-cooled ethane, then viewed under liquid LN2 on a 200 kV Talos F200C TEM.

Ultracentrifugation and commercial kits are common isolation methods for EVs, however our TEM results showed a far lower concentration of contaminants for samples obtained using the kit. As compared to negative stain, cryo-EM is a more powerful tool for differentiating membrane-derived vesicles from other lipid contaminants, stemming from substantial advances in contrast enhancement, detector technology and data processing. Because high levels of contaminants may interfere with downstream applications, we suggest cryo-EM as indispensible for correct sample screening in EV studies.

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PARTIAL UNILATERAL URETERAL OBSTRUCTION IN RAT AS AN EXPERIMENTAL MODEL

Alexandru Cristian Popescu[1,2], Cătălin Gabriel Manole[1,2], Daciana Marta[2], Antoanela Țâncă[1,2], Bogdan Marinescu[2], Mihaela Gherghiceanu[1,2]

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Keywords: rat model, unilateral ureteral obstruction, hydronephrosis

Introduction. In humans, the presence of a partial or total obstruction of the ureter prevents the normal flowing of the urine to the bladder, causing increased upstream pressure with renal pelvicalyceal dilatation and hydronephrosis. Untreated, the unilateral ureteral obstruction (UUO) leads to irreversible injury of the ipsilateral kidney followed by atrophy of the renal parenchyma and kidney failure. The obstruction may be unilateral or bilateral, congenital, acquired, with intrinsic or extrinsic causes, partial or total, acute or chronic, with complicating factors or not. The treatment depends on many factors and is mainly surgical. Experimental animal models of partial ureteral obstruction are increasingly used to investigate the pathophysiology of hydronephrosis and kidney diseases. However, little information is available about the use of similar models to investigate changes on the ureter and renal pelvis as a result of the obstruction.

Materials and methods. We use adult, 16 female Wistar rats provided by animal facility of the INCD "Victor Babeș". The animals were divided into groups (control n=3, sham operation n=3 and study n=10). The left ureter was surgically discovered after substernal incision of the abdomen. Unilateral partial upper ureteral obstruction was created using a Polypropylene 5-0 suture ligature of the ureter together with a metallic guide that is removed after ligation, resulting partial reduction of the ureter's diameter. The abdomen is closed and the animals return to nursery. After different time intervals (postoperative 7, 14, 30 days) and the humane killing of the rats, we harvest both kidneys with pelvis and upper ureters. Samples of normal upper ureter, ligature area and pelvis are examined in light and electron microscope for both left and right kidneys.

Results. After 30 days of ligature, the left kidneys were hydronephrotic and the pelvis and ligatured upper ureters were also dilated compared with normal right kidneys and ureters. The samples stained with H&E were examined under light microscope. Preliminary findings showed that the ureter lamina propria is thinner and the muscle cells are hypertrophic in dilated ureter. By using different time intervals and thickness of metal guides, the degree of ureteral obstruction can vary from mild to severe.

Conclusion. This rat model of partial unilateral ureteral obstruction is reproducible and can be used in structural and ultrastructural studies on ureter.

A RETROSPECTIVE STUDY OF MALIGNANT SALIVARY GLAND TUMORS – CLINICAL AND MORPHOLOGICAL CORRELATIONS

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Objectives. Salivary glands malignant tumors are rare, representing 0,5 % of all cancers and 3-5% of head and neck tumors, with a highest frequency in the elderly. Approximately 80% arise in the parotid gland, 10-15% in the submandibular glands and the rest in the minor and sublingual salivary glands.

Methods. We present a study made on salivary glands tumors during a period of four years at the "Victor Babeş" Institute from Bucharest, Romania, which diagnoses were made using histological and immunohistological metods.IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, Illinois, USA) was used for data analysis.

Results. We studied 22 cases, 13 males and 9 females, with age variations from 22 to 80 years old. The most frequent affected regions were the parotid gland (8), submandibular gland (6), minor salivary glands (8). Malignant tumors were salivary duct carcinoma (5), mucoepidermoid carcinoma (5), adenoid cystic carcinoma (4), acinic cell carcinoma (4), carcinoma ex pleomorphic adenoma (3) and basal cell adenocarcinoma (1). Statistical analysis revealed an indirect correlation between age and tumor location at women.

Conclusion. Among major salivary gland, parotid was the main site and the salivary duct carcinoma was the most frequent tumor; also, males aged between 68 and 80 were the most affected; the palate was the predominant site for malignant tumors of minor salivary gland tumors with a higher incidence of mucoepidermoid carcinoma in young women.

NANOPARTICLES - RELATED EFFECTS ON PROLIFERATION, CYTOTOXICITY AND APOPTOTIC PROCESSES

Elena Codrici[1], Alexandra Cătălina Vîlceanu[2], Ionela Daniela Popescu[1], Simona Mihai[1], Ana-Maria Enciu[1,2], Lucian Albulescu[1], Radu Albulescu[1,3], Eleonora Codorean[1], Mircea Leabu[1], Alina Butu[4], Cristina Mihaela Luntraru[5], Cristiana Tănase[1,3]

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Introduction. Green synthesis of nanoparticles (NP) represents an environmentally-friendly and efficient method based on various plant extracts in order to generate metal NP.

Materials and methods. Several cell types: monocytes (CRL9855), primary epidermal keratinocytes (HEKs), dysplastic oral keratinocyte (DOKs) and aggressive tumor cells-glioblastoma cell line (U87) were used to evaluate the effects of NP obtained by green synthesis (Ag, Au, Pd, Se metals and plant extracts from *Levisticum officinale, Origanum vulgare*). The methods used were MTS assays for viability measurements, LDH assay for cytotoxicity, xCELLigence - electrical impedance measurements and Luminex xMAP array for apoptosis evaluation in cell lysates.

Results and discussion. The results of MTS and LDH assays, on monocytes cell lines, showed that metal salts alone express higher cytotoxicity than green NP, due to the fact that toxicity was reduced by plant extracts; the vegetal extracts alone had proliferative effects. xCELLigence measurements showed a dose-dependent proliferative effect for some of the studied NP. Luminex xMAP array revealed typical effects for apoptosis in tumoral cells: activation of caspase-9 during treatment with Ag-based NP, correlated with the results for p53, suggesting that Au and Ag-based NP induced p53-mediated apoptosis in tumor cells by DNA fragmentation, correlating with decreased Bcl-2 expression.

Conclusion. Our results confirmed that these NP could induce apoptosis in tumoral cells through caspase activation. Green synthesis represents a sustainable technology, being a nontoxic and safe option for biomedical applications, thus improving NP in terms of biodegradability, functionalization, and biocompatibility.

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