ISSN 2392 – 7232
ISSN-L 2392 – 7232

Vol. 20, Supplement II, April 2015
www.jtmr.ro

JOURNAL OF TRANSLATIONAL MEDICINE AND RESEARCH

Journal of the Romanian Academy of Medical Sciences

CELSIUS PUBLISHING HOUSE

Journal of Translational Medicine and Research

Supplement II / Vol. 20 / 2015

Journal of Translational Medicine and Research is atested and indexed in Elsevier Bibliographic Databases: SCOPUS

Printed in Romania, Celsius Publishing House

UNIUNEA EUROPEANĂ
GUVERNUL ROMÂNIEI
Fondul Social European
POSDRU 2007-2013
Instrumente Structurale
2007-2013

Proiect cofinanțat din Fondul Social European prin Programul Operațional Sectorial
Dezvoltarea Resurselor Umane 2007 – 2013 – „Investește în oameni!"
„Formare și sprijin pentru întreprinderi și angajați pentru promovarea adaptabilității" POSDRU/186/3.2/S/155295
Conferința de Medicină Translațională (finanțat în cadrul POSDRU 186/3.2/S/155295
„DEZVOLTAREA COMPETENȚELOR ÎN TRANSPLANT")

CONTENTS

The supplement also includes papers presented during the Translational Medicine Conference
(financed within POSDRU 186/3.2/S/155295 "Development of Competences in Transplantation").

CO-01

PRELIMINARY RESEARCH ON CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF SOME NATURAL BIOACTIVE COMPOUNDS

Ioana M. Aldea[1], Daniela Ionescu[2], Coralia Bleotu[1], Mihaela Chivu Economescu[1], Lilia Matei[1], Iuliana Crisan[2], Stefan Stangaciu[3], Daniela A. Ion[4,5], Carmen C. Diaconu[1]
[1]*Stefan S. Nicolau Institute of Virology, Bucharest, Romania*
[2]*S.C. Hofigal Export Import S.A., Bucharest, Romania*
[3]*Apitherapy Consulting & Trading International Ltd., Romania*
[4]*Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
[5]*Prof. dr. Matei Balș National Institute of Infectious Diseases, European HIV/AIDS Academy, Bucharest, Romania*

Introduction: We investigated cytoxicity and the antiviral effect of some natural bioactive compounds, extensively studied for their hepatoprotective and stimulant effects, sometimes with contradictory results. Cytotoxicity is the first that should be ruled out when searching for an antiviral entity. Therefore, first we investigated the potential cytotoxicity of these compounds, in order to eliminate this confounder in the analysis of the virucidal effect.
Methods: We have used different methodologies for testing cytotoxicity (microscopy, flowcytometry, 3D cell culture) and a set of European standardized procedures for the virucidal activity testing in human medicine. The standard impose strict testing methods on two non-enveloped viruses, one DNA and one RNA, highly resistant and stable in different conditions (Adenovirus type 5, Poliovirus type 1).
Results: All tested natural bioactive compounds (T gallica, S. marianum, Apicol cocktail I and II)on different 2D or 3D cultured cell lines, proved to be non-cytotoxic at the level that would impose employing other techniques to eliminate cytotoxicity in order to evaluate virucidal effect. One compound (T. gallica) had greater cytotoxicity on HeLa cells, however, the reduction of the residual infectivity titer could be followed over a range of more than 4 lgTCID50 without being necessary to employ other techniques to eliminate cytotoxicity. T gallica extract reduced the poliovirus infectivity to a lgTCID50 of 5.5 after 60 min of treatment and in the presence of a high protein content of the medium, 3g/l BSA, lgTCID50 was 5.2. At the same time, T gallica extract was able to decrease the infectivity of adenovirus from a lgTCID = 9.2 to 5.5 in the presence of low amount of interfering protein (0.3% BSA) while in the presence of high protein concentration (3% BSA) lgTCID decreased from 9.5 to 5.2, which qualify T. gallica as an effective virucid. The other natural bioactive extracts tested showed less cytotoxicity compared to T. gallicabut also less virucidal effect. However, the combination 1:1 of T. gallica and S. marianu-mextract, proved to be more effective as virucid as each individual extract, infectivity of both viruses reaching a lgTCID50 of 5 when treated with both extracts simultaneously.
Conclusion: Virucidal activity demonstrated especially by T. gallica extract or the combination of this with S. marianum extract, sustain our previous results on hepatoprotective effect of a product that has both extracts in its composition.
Acknowledgements: Ioana M. Aldea was financed from the European Social Fund and the Romanian Government under the contract number POSDRU/159/1.5/S/137390. This study was supported by a grant from the National Authority for Scientific Research -PCCA PNII 90/2012.

CO-02

PROTEOMIC PROFILING OF THYROID DIFFERENTIATED CARCINOMA

Felicia Antohe[1], Elena Uyy[1], Viorel-Iulian Suica[1], Raluca Maria Boteanu[1], Dana Manda[2], Maya Simionescu[1], Corin Badiu[2]
[1]*Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania*
[2]*National Institute of Endocrinology "C.I.Parhon", Bucharest, Romania*

Introduction. The significant increase in the number of thyroid carcinoma-diagnosed patients and its aggressiveness prompted us to utilize high resolution mass spectrometry methodology to identify the patient's tumor proteomic profile that could facilitate the understanding of tumor biogenesis and help in prognosis and therapy. The study aimed at the identification of molecular markers of the follicular adenoma or papillary thyroid carcinoma by differential proteomic analysis.
Materials and methods. Thyroid tissues sampled from the operation theatre were collected from two groups of female patients: 1. with follicular adenoma (D) and 2. with papillary thyroid carcinoma (P). Control (CD and CP respectively) tissue samples adjacent to the tumors were also collected and analyzed from each specimen. The tissue homogenates were processed for liquid nano-chromatography mass spectrometric analysis. The label free quantification with SIEVE 2.1 software was used to determine the ratios of D/CD and P/CP protein abundance.
Results. The statistically significant differentially expressed proteins matched with KEGG databases revealed the over-representation of the proteins involved in thyroid hormone synthesis inter-relation map. Bioinformatics gene ontology analysis (Protein Center 3.12 software) of this signaling pathway's proteins evidenced their localization in the endoplasmic reticulum and membrane-bounded vesicles. In addition, they are involved in cellular response to stress and binding chaperone molecules. The relative quantification of these proteins by label free mass spectrometry was validated by immunological assays and correlated with their serum expression levels.
Conclusion. The mass spectrometry analysis revealed significant differences in protein expression in follicular adenoma versus papillary thyroid carcinoma that could help in early prognosis and adequate treatment decision.
This work was supported by the Romanian Academy, Ministry of Education and Research grant CNDI-UEFISCDI PN-II-PCCA-2011-3 no. 135/ 2012 and POSDRU/159/1.5/S/133391-financed by the European Social Fund within the Sectorial Operational Program Human Resources Development 2007-2013.

CO-03

IMPACTUL CLINIC AL CARDIOLOGIEI MOLECULARE

Eduard Apetrei
UMF Carol Davila, Institutul de Cardiologie, Fundeni, Bucuresti, Romania

Bolile cardiovasculare rămân principal cauză de morbiditate si mortalitate, fără schimbări importante în ultimii 25 ani. În România rata decesului prin boli cardiovasculare rămâne, cu toate progresele făcute, peste 60 %. Cercetarea moleculară are un rol important în descifrarea mecanismelor unor boli , în introducerea unor noi metode de investigații și tratament si în identificarea subiecților cu risc crescut. Cercetarea moleculară eclipsează cercetarea clinică/fenomenologică și în acelaș timp crește costul asistentei medicale. Mă voi opri numai la 2 domenii importante și anume genetica si imagistica moleculara. Genetica moleculară a intrat progresiv și definitiv în multe domenii ale medicinii. In cardiologie întâlnim cel mai frecvent afecțiuni monogenice și putem vorbi de 3 mari clase de modificări genetice ale proteinelor: modificări structurale, modificări electrice și modificări de reglare. Modificări structurale întâlnite în cardiomiopatii, tezaurismoze, sindromul Marfan etc; modificări ale proteinelor țesutului electric: sindromul QT lung. sindromul Brugada etc Modificări poligenice apar aproape în toată patologia CV: HTA, cardiopatia ischemică, ateroscleroză, dislipidemii. Să luăm un exemplu. Cardiomiopatia hipertrofică este produsă de modificări ale genelor proteinelor sarcometrie, din care 3 sunt mai

importante (cu o heterogenitate intra genică f.mare cu aprox 200 mutații), ce explică peste 90% dintre formele clinice . Determinarea genetică este recomandată la probanți, pentru a afla ce mutație este prezentăşi la rudele de gr.I pentru a afla care dintre acestia vor face boala. La început, în urmă cu 20-30 ani,entuziasmul era foarte mare, vom afla cine produce boala și mai ales care din descendenți va face boala. Ulterior am constatat că nu putem preveni bola şi nici nu putem lua decizii terapeutice dacăștim modificarea genetică. Cu totul altă situație este cu Boala Fabri unde s-a descoperit enzima ce blochează depozitarea de ceramid în lizozomii celulelor miocardice, renale, piele. Astfel impactul clinic al cercetării genetice în cardiologie este mult sub speranțele noastre privind prevenirea şi tratamentul unor boli. Exclud impactul asupra cunoasterii. Imagistica moleculară se adaugă imagisticii structurale. Aduce date noi din interiorul celulelor vii, in timp real, invizibile cu alte metode; implicate in diverse procese patologice. Imaginile moleculare pot detecta: inflamatia – (PET- F-fluorodeoxiglucoza (FDG), tromboza – PET. IRM, vase de neoformatie – (PET, IMR), hemoragia intra placa - (IRM-particole de fier, Apoptoza – PET) . Daca mă refer numai la ateroscleroza: avem multe neinpliniri privnd prevenirea apariției plăcii de aterom, nu ştim prea bine cum,dece şi mai ales când se fisurează placa de aterom. Stăm destul de bine la tratamentul plăcii fisurate(infarctul miocardic) si prevenirea unui nou episode acut. Ne intrebăm dacă imaginile moleculare pot modifica atitudinea medicului şi a bolnavului. Datele publicate până în prezent sunt încurajatore privind rolul efortului fizic,al statinelor si IECA. Ştim bine astazi rolul inflamatiei în afecțiunile cardiovasculare dar nu avem încă un tratament eficient al inflamației,iar spernțele sunt mici. Cercetarea medicală necesită costuri imense. Ex. In SUA se cheltuiesc 30 miliarde anual pentru cercetarea medicala. Ne punem uneori intrebarea daca cercetarea medicală se face pentru ingrijirea mai buna a bolnavilor sau pentru business ? Am aici în vedere tratamentul hipertensiunii arteriale,dar nu numai. Doctorul navei Enterprise punea diagnosticul cu ajutorul mâinilor ce le trecea deasupra bolnavului,deci folosea un "scanner"ce făcea posibil diagnosticul neinvaziv al unei patologii complexe. Tehnologia actuala , totusi departe de scanerul Dr. Mc Coy, în laboratoarele puternice şi chiar in unele clinici din țările bogate au un iz de science fiction Misiunea cardiologiei molecular este "to boldly go where no man has gone before"

CO-04

ACTORS INVOLVED IN B-CATENIN GENE EXPRESSION DEREGULATION IN PANCREATIC CANCER

Anca Botezatu[1], Iulia V. Iancu[1], Adriana Plesa[1], Gabriela Anton[1], Raluca Florea[2], Veronica Ilie[2], Andrei Sorop[2], Nicolae Bacalbasa[3], Valeria Tica[2], Simona Dima[2], Irinel Popescu[2]
[1]*Institutul de Virusologie Stefan Nicolau, Bucuresti, Romania*
[2]*Institutul Clinic Fundeni, Bucuresti, Romania*
[3]*UMF Carol Davila, Bucuresti, Romania*

Currently, pancreatic cancer remains a major challenge for therapy and biomarkers identification. Early detection of pancreatic cancer could decreases the mortality caused by this disease. The main focus is deciphering the molecular biology of this tumor type, in order to identify new factors related to pancreatic oncogenesis. β-catenin gene regulation in cancer and in pancreatic cancer, particullary is controversial. However, nuclear β-catenin protein accumulation has been correlated with late stages of tumor progression and metastasis A major role in genes regulation is epigenetic modification, especially DNA methylation. Identification of genes which undergo cancer-specific methylation changes and correlation of these data with tumor stage, progression, and long-term prognosis are becoming increasingly common. Another key factor for β-catenin gene regulation is ERBB2 which is over expressed in several cancer types. The aim of this study was to identify the factors involved in β-catenin gene expression deregulation in pancreas oncogenesis. For this purpose we evaluated the β-catenin gene expression and promoter methylation status, and ERBB2 gene expression in 60 paired samples (normal/pancreatic adenocarcinoma). Promoter methylation status was quantified in qMS-PCR using bisulphite treated DNA samples (EpiTect Bisulfite Kit – Qiagen) while β-catenin and ERBB2 genes expression was determined in qRT-PCR. We found a decreasing methylation pattern of β-catenin promoter gene related to the disease stage. Significantly lower methylation frequencies ($p<0.05$) was noted in pancreatic adenocarcinomas stage III and IV (0 -20%, median=0.0128%). β-catenin and ERBB2 gene expression was increased in adenocarcinomas advanced stages. Fold change expression for β-catenin was between 0.1219- 5.907, median = 1.8462 and

between 0.07118- 9.651, median = 1.7524 ERBB2 gene in pancreatic adenocarcinomas III-IV stages. These results sustain the increased β-catenin gene expression trough epigenetic alterations (demethylation of gene promoter) and the increasing of ERBB2 gene expression, β-catenin regulatory factor. Therefore, these factors may represent a trigger for pancreatic oncogenesis process. This study was supported by PCCA90/2012.

CO-05

LIPOSOMII TINTA SENZITIVI TRANSPORTORI DE ANTAGONISTI DE RECEPTORI CHEMOKINICI CCR2 INHIBA METASTAZAREA CELULELOR TUMORALE

Manuela Calin[1], Daniela Stan[1], Marko Robleck[2], Lubor Borsig[2], Maya Simionescu[1]
[1]*Institutul de Biologie si Patologie Celulara "Nicolae Simionescu", Bucuresti, Romania*
[2]*Institutul de Fiziologie, Universitatea din Zürich, Elvetia*

Metastaza celulelor tumorale implica un proces inflamator caracterizat prin exprimarea de catre celulele endoteliale vasculare (CE) a chemokinelor, a receptorilor lor si a moleculelor de adeziune celulara. Urmare a acestui proces, celulele sistemului imunitar (de exemplu, monocitele) sunt atrase şi participa la transmigrarea celulelor tumorale printre CE si la formarea metastazelor. Datorita implicarii chemokinelor în metastazarea cancerelor de diferite origini, intervenția terapeutica asupra sistemului de chemokine deschide noi oportunitati în tratamentul unor tipuri de cancer şi prevenirea metastazarii.
Scopul acestui studiu a fost de a testa daca directionarea liposomilor tinta-senzitivi (TSL) care transporta inhibitori ai receptorilor chemokinici si ii elibereaza specific la situsuri cu endoteliu activat reduc metastazarea celulelor tumorale.
Metode: Am preparat si caracterizat TSL in care am incorporat un antagonist de receptor chemokinic CCR2, Teijin compund 1 (Tj). Directionarea specifica a liposomilor fost realizata prin atasarea la suprafața lor a unui peptid care recunoaste molecula de adeziune VCAM-1 (Vp) exprimată pe suprafata CE activate (Vp-TSL-Tj). Rezultatele au aratat ca in vitro, Vp-TSL-Tj se leagă specific de suprafata CE activate unde elibereaza compusul incorporat; acesta determina reducerea transmigrarii monocitelor si a celulelor tumorale prin endoteliul activat. In vivo, soareci injectati cu celule tumorale murine MC-38GFP, au prezentat o localizare specifica a Vp-TSL marcat fluorescent in plaman, in vecinatatea celulelor tumorale, unde s-a demonstrat anterior o exprimare crescuta a VCAM-1. Interesant si incurajator, doua administrari de liposomi Vp-TSL-Tj in soareci (cu o ora inainte si la 16 ore dupa injectarea celulor tumorale) a determinat o scadere semnificativa a metastazelor in plaman la 28 de zile de la injectarea celulelor tumorale. Aceste rezultate sunt o prima dovada ca nanocarausii care transporta și eliberează inhibitori de chemokine CCR2 la situsurile vasculare cu endoteliu activat reduc metastaza celulelor tumorale.
Studiile au fost finantate prin schema ERA-NET a programului FP7 al Comisiei Europene de catre UEFISCDI, Romania Grant 4-001 si Swiss National Science Foundation, Switzerland Grant 31NM30-136033, acronim proiect: NANODIATER.

CO-06

THE CHANGING EPIDEMIOLOGY OF MEASLES IN ROMANIA

Costin Cernescu
Stefan S Nicolau Institute of Virolog, Bucharest, Romania

Measles is one of the most contagious human diseases, causing many complications and deaths. The disease is preventable by a live attenuated vaccine, that provides lifelong immunity in most recipients. Measles' elimination by 2015 (interruption of indigenous viral transmission) is part of the WHO strategic plan for immunization in the European Region. A national measles vaccination program was implemented in Romania in 1979-an initiative of academician Nicolae Cajal. Vaccination was conducted with an indigenous

monovalent measles-containing vaccine (Schwarz strain) produced by Cantacuzino Institute. Nevertheless, during the last decade,Romania experienced at least two important measles outbreaks. The first one started in December 2004 and lasted until early 2007. More than 9,000 cases were detected, frequently in non-immunised patients belonging to the Roma ethnic group. In the following years, measles' incidence dropped from 163 per million population in 2008, to less than 10 per million population in 2009. The second major epidemic episode occurred during 2010-2012, simultaneously with a rubella epidemic in 2011. More than 90% of cases were in children who were not vaccinated or had unknown vaccine status. In contrast to other vaccine - preventable diseases, most cases of measles reflect a failure to receive vaccination. Failure to introduce appropriate infection-control measures,as well as the potential for and transborder viral transmission are alternative explanations for the extenssion of the transmission chain.

CO-07

REVEALING NEW SIGNALLING PATHWAYS BETWEEN GUT MICROBIOTA AND ENTEROENDOCRINE CELLS

Catalin Chimerel, Fiona Gribble and Frank Reimann
Institute of Metabolic Science and MRC Metabolic Diseases Unit, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

Obesity and type 2 diabetes mellitus (T2DM) represent one of the biggest health and socioeconomic burdens of the 21st century. Enteroendocrine cells in the gut produce hormones that play essential roles in the regulation of food intake, energy expenditure and glucose homeostasis. Analogues of the gut hormone glucagon-like peptide-1 (GLP-1) are widely used for the treatment of T2DM, and are under development for the treatment of obesity. Recent findings, showing that many cases of T2DM resolve after gastric bypass surgery, have triggered a global interest in the gut endocrine system, and whether it could be harnessed for the treatment of diabetes and obesity. A body of evidence supports the idea that gut microbiota influence peripheral metabolism, and it is hypothesised that the underlying pathway includes modulation of the enteroendocrine cells with which they come into contact. The types of enteroendocrine cells vary along the length of the intestinal tract. The colon contains the highest density of enteroendocrine L cells together with most of the gut bacteria. Here, we reveal that indole, a metabolite produced from the dissimilation of tryptophan, is able to modulate the secretion of GLP-1 from immortalized and primary mouse colonic Lcells and we reveal the molecular mechanism behind this modulation [1]. Indole stimulates GLP-1 secretion over short time scales and it reduces the rate of GLP-1 secretion over longer periods. The short term effects are due to the ability of indole to block voltage-gated K(+) channels, increase the temporal width of action potentials fired by L cells, and enhance Ca(2+) entry, thereby acutely stimulating GLP-1 secretion. Over longer periods indole slows ATP production by blocking NADH dehydrogenase, thus leading to a prolonged reduction of GLP-1 secretion. Our results identify indole as a signalling molecule by which gut microbiota communicate with Lcells and influence host metabolism.
Reference: [1] C. Chimerel, E. Emery, D.K. Summers, U. Keyser, F.M. Gribble, F. Reimann, Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells, Cell reports, 9 (2014) 1202-1208.

CO-08

MOTILITY OR MORBIDITY IN NEUROSURGER

AV. Ciurea[1,2], H. Moisa[1]
[1]*Department of Neurosurgery, Carol Davila University School of Medicine, Bucharest, Romania*
[2]*Department of Neurosurgery, Sanador Medical Center Hospital, Bucharest, Romania*

Motto: „I move, therefore I am" (Haruki Murakami, Japanese Writer)
Background: The lack of motion is a basic process in the birth of diseases.

Materials and methods: Classical physics describes motion to be the change in position of an object with respect to time and its refference point. Motion in the universe is described using two apparently contradictory sets of laws. The motion of familliar objects – including cells – are described by classic mechanics while the movement of atoms and subatomic particles are described by quantum mechanics. Biological motility is the capacity of an organism to move spontaneously and voluntary at the expense of energy and represents one of the crucial traits (if not the most important trait) of all living matter. Motility is a key process present in unicellular organisms as well as multicellular organisms and is the basis of embryonic development, healing, immune response, tumor formation, metastasis, cell migration, digestion, circulation, respiration and reasoning and various other critical processes including biological evolution itself. As creatures evolved, various types of motility were developed – these include chemotaxis (motility along a chemical gradient), thermotaxis (motility along a temperature gradient), phototaxis (motility along a light gradient), magnetotaxis (motility along a magnetic field), galvanotaxis (motility along an electrical field), gravitaxis (motility along the direction of gravitational force), durotaxis (motility along a rigidity gradient), haptotaxis (motility along a gradient of cell adhesion sites) and many others. Nowadays researchers only begin to understand the crucial part motility played in the evolution of species. All surgical interventions (cheiron+ergon = gesture done by hand) represent – in broad lines – small controlled traumas with the sole purpose of removing tumors (surgical oncology), removing foreign bodies and restoring organ integrity (trauma surgery), restoring vascular flow to the body (cardiac surgery and vascular surgery), decompressing the brain (neurosurgery) and so on. In all areas of surgery cell and tissue motility is singlehandedly the key to healing and succes. The constant motility of developing organisms (from trilobites to human beings) has undoubtedly shaped evolution by constantly improving the functions of the skeletal system and other internal organs such as the digestive tract, liver, kidneys etc. The human central nervous system makes no exception from this rule. The development of the human nervous system takes place by employing chemotaxis, galvanotaxis, magnetotaxis and haptotaxis. The human heart is constantly moving blood throughout the body ensuring the correct metabolism of living tissues. Oxygenated red blood cells come from the lungs to the tissue filled with life-giving oxygen while red cells filled with carbon dyoxide are moved from the tissue to the lungs for the cycle of respiration. Blood moves through larger veins and arteries at speeds of about 0.3 m/s, though considerable variation exists. Smooth muscles of the body are also constantly moving. The most familiar motion to scientists is peristalsis which is how ingested food is moved through the digestive tract for further processing – the average speed of food through the intestines is about 2m/h. The ear is another organ functioning based on motion, besides the vibration of the eardrum generating the sense of hear the inner ear is able to record motions of the head equal to the diameter of a single hair. The human lymphatic system is constantly moving excess fluids, lipids and immune system-related products around the body. Intracellular cytoplasmatic streaming is responsible for the transport of key mollecules in living cells. Motor proteins such as kynesin move along microtubules and transport vesicles with various content while other motor proteins serve as biological engines for cells – this is the case of sperm cells which move propelled by a spiral-shaped protein which acts exactly as a boat's propeller. The bipedal posture of humans enabled prehistoric humans to hunt, go fishing and even grow crops and it allowed for a continuous development of the central nervous system which was mandatory for survival, self defense and preservation of the species. The basic process for thought is neural networking which is the motion of information through neurons. This can be done chemically or electrically. Chemical synapses use chemical mollecules to transport information while electrical synapses use massive ionic influx curents for signalling. The evolution from prehistoric human beings to modern men went through several steps during which human motility represented once more a crucial element. Once industrial food production appeared – with the overproduction we are familiarized with, human motility became a protection factor against obesity which is one of the most problematic medical conditions of our time. According to WHO data, in 2008 there were more than 1.4 billion adults aged 20 or more who were overweight. More than 500 million people are obese while more than 40 million children suffer of this ailment. The cure ? Physical activity ! Swimming, Tennis, Cycling and limiting of hypercaloric food intake are the symplest and most effective ways to get rid of obesity and improve the individual quality of life and treatment for all medical patients. As soon as „fast track" surgery began being implemented in hospitals, neurosurgeons took the concept and adapted it to neurosurgery. Besides the critical concern for the patient's motility expressed through diffuse tensor immaging (DTI) fiber tracking, doctors began to understand that the key to a fast neurosurgical recovery is a fast treatment. The patient should firstly be able to move spontaneously and independantly and secondly he should be able to do that as soon as possible after surgery. Pulmonary thrombembolism is therefore avoided, intestinal transit is ensured, csf transit is ensured, complete body motions are ensured and the nervous system is stress-free.

Provided the fact that any lesions of the brain have a tremendous impact of the motility of the patient the authors consider that the motility of the patient has a tremendous impact on the healing of the patient. The authors perform a comprehensive literature review about the effects of industrial food production and the vicious circle that is born between consumerism, reckless alimentation, a bad lifestyle and lack of movement. At the same time the authors present their own experience concerning the importance of motility in neurosurgical patients and how it can dramatically change the outcome of patients. To conclude – we believe that motility is the key to limiting and improving worldwide morbidity while also limiting the effects of other diseases and increasing the healing rates of other pathologies while also preventing thrombosis. Neurosurgical and neurovascular pathologies are prime targets for early motility of patients. Under these circumstances a modern medicine without early postoperative mobilization of the patient is no longer concievable, a fact which is in strict accordance with the old saying in the bible „Take up your bed and walk"(John 5:8). Key Words: Neurosurgery, Mobility, Morbidity, Evolution, Outcome.

CO-09

SIGNALING CROSS-TALK BETWEEN TYPE I INTERFERON AND THROMBOPOIETIN SIGNALING: RELEVANCE FOR CYTOKINE THERAPIES AND MYELOID NEOPLASMS

Stefan N. Constantinescu[1], Jean-Philippe Defour[1], Thomas Michiels[2], Jean Luc Villeval[3], Isabelle Plo[3], William Vainchenker[3], Judith Staerk[4] and Christian Pecquet[1]

[1]*Ludwig Institute for Cancer Resarch and Université catholique de Louvain, de Duve Institute, Brussels*
[2]*VIRO Unit, Duve Institute, Université catholique de Louvain, de Duve Institute, Brussels*
[3]*INSERM U1009, Institut Gustave Roussy, Villejuif, France*
[4]*EMBL Nordic Partnership, University of Oslo, Center for Molecular Medicine, Norway*

Thrombopoietin (Tpo) is a cytokine that regulates platelet production and homeostasis of hematopoietic stem cells (HSCs). Signaling downstream Tpo involves the specific cell surface receptor (TpoR, c-MPL), the kinase JAK2, Signal Transducer and Activators of Transcription (STATs) as well as the MAP-kinase and PI-3'-kinase pathways. Mutants of TpoR where W515 is replaced by Lys, Arg, Ala, Leu signal constitutively, as W515 is unique in maintaining the receptor inactive in the absence of Tpo, by preventing transmembrane domain dimerization (Defour et al, 2013). In megakaryoblasts and early myeloid progenitors, Tpo induces survival, proliferation and differentiation by activation of tyrosine phosphorylation of STAT5, STAT3 and STAT1. While STAT5 promotes proliferation and survival, STAT1 promotes bipotent megakaryocyte-erythroid progenitors to engage along the megakaryocytic pathway, while STAT3 appears to antagonize STAT5. We have discovered that in cells expressing high levels of JAK2 or TYK2, like late megakaryocyte progenitors, or UT7-MPL cells, Tpo induces an anti-proliferative effect (Besancenot et al, 2010; Pecquet et al, 2012). Gene expression profiling shows that in such cells Tpo induces a set of genes that overlaps with those induced by type I interferon (Constantinescu et al, 2014). The molecular basis of this effect is represented by stabilization by JAK2 of one particular dimeric conformation of TpoR (Staerk et al, 2011) that favors activation of ISGF3 complexes, initiated by activation of STAT2. Using several genetically modified mouse lines we show that the activation by Tpo of the ISGF3 complex is direct, does not involve type I IFN receptors and that STAT2 activation requires two distal tyrosines of the receptor. Importantly, Tpo induces ISGF3 complex formation in vivo in platelets and presumably in late megakaryocytes of patients treated with Tpo agonists, Elthrombopag and Romiplostim (Constantinescu et al, 2014). These data indicate that as a function of JAK2 levels, Tpo can switch its function from a proliferative to an antiproliferative cytokine, becoming functionally an interferon for subpopulations of megakaryocytes. This has implications for patients treated with Tpo for thrombocythopenia and for a potential interferon-like antiviral effect of Tpo on cells expressing TpoR and high JAK2 levels.

References: Besancenot R, Chaligne R, Tonetti C, Pasquier F, Marty C, Lecluse Y, Vainchenker W, Constantinescu SN, Giraudier S (2010) A senescence-like cell-cycle arrest occurs during megakaryocytic maturation: implications for physiological and pathological megakaryocytic proliferation. PLoS biology8: e1000476 Constantinescu SN, Defour JP, Mouton C, Plo I, Vainchenker W, Villeval JL, Michiels T, Staerk J,

Pecquet C (2014) Thrombopoietin Activates STAT2 Inducing Type I Interferon Effects and Gene Expression: Implications for in Vivo Tpo Treatment and for Myeloproliferative Neoplasms Blood (ASH Annual Meeting Abstracts)124: Abstract 820
Defour JP, Itaya M, Gryshkova V, Brett IC, Pecquet C, Sato T, Smith SO, Constantinescu SN (2013) Tryptophan at the transmembrane-cytosolic junction modulates thrombopoietin receptor dimerization and activation. Proceedings of the National Academy of Sciences of the United States of America110: 2540-2545 Pecquet C, Diaconu CC, Staerk J, Girardot M, Marty C, Royer Y, Defour JP, Dusa A, Besancenot R, Giraudier S, Villeval JL, Knoops L, Courtoy PJ, Vainchenker W, Constantinescu SN (2012) Thrombopoietin receptor down-modulation by JAK2 V617F: restoration of receptor levels by inhibitors of pathologic JAK2 signaling and of proteasomes. Blood119: 4625-4635 Staerk J, Defour JP, Pecquet C, Leroy E, Antoine-Poirel H, Brett I, Itaya M, Smith SO, Vainchenker W, Constantinescu SN (2011) Orientation-specific signalling by thrombopoietin receptor dimers. The EMBO Journal30: 4398-4413

CO-10

REMODELING CYTOKINE RECEPTOR DIMERS BY TARGETING JUXTAMEMBRANE DOMAINS AND ASSEMBLING COMPLEXES USING NOVEL LIGANDS

Stefan N. Constantinescu[1], Vitalina Gryshkova[1], Christian Pecquet[1], Jean-Philippe Defour[1], Emilie Leroy[1], Steven O. Smith[2], Ignacio Morega[3], K. Christopher Garcia[3]
[1]*Ludwig Institute for Cancer Research and Université catholique de Louvain, de Duve Institute, Brussels, Belgium*
[2]*Department of Biochemistry, SUNY Stony Brook University, Long Island, NY, USA*
[3]*Department of Biochemistry and Structural biology, Stanford University, CA, USA*

Erythropoietin is a helical cytokine that is absolutely required for red blood cell formation, while thrombopoietin (Tpo) is a cytokine that regulates platelet production and homeostasis of hematopoietic stem cells (HSCs). G-CSF critically controls granulocyte formation and its administration results in liberation of HSCs from the marrow into peripheral blood. All three cytokines are protein drugs with thousand of patients treated worldwide every week. Cytokine addition induces the full spectrum of biologic activities, with desired and undesired effects. We asked what were the key switch residues turning on, off or partially activating these receptors, and which dimeric conformations can induce full, partial or no activity. We will present several approaches whereby we impose specific dimeric orientations using coiled-coils (Seubert et al, 2003; Staerk et al, 2011), determine the NMR structure of the juxtamembrane and transmembrane regions (Defour et al, 2013; Kubatzky et al, 2005; Staerk et al, 2006) and use modified cytokines or more recently diabodies targeting the extracellular domain of EpoR, and which were crystallized by the Garcia laboratory in complex with the extracellular domain of EpoR (Moraga et al, 2015). We show that as a function of receptor subunit rotation, or as a function of distance between receptor monomeric subunits a range of activities can be induced, allowing fine-tuning of signaling events and down-stream biologic effects. An example of a novel manner to prevent close receptor apposition using will be presented, such dimers being very weakly activated for the wild type EpoR-JAK2 complexes and inhibited for EpoR JAK2 V617F complexes. We discuss how these approaches can lead to novel therapeutic avenues.References Defour JP, Itaya M, Gryshkova V, Brett IC, Pecquet C, Sato T, Smith SO, Constantinescu SN (2013) Tryptophan at the transmembrane-cytosolic junction modulates thrombopoietin receptor dimerization and activation. Proceedings of the National Academy of Sciences of the United States of America 110: 2540-2545 Kubatzky KF, Liu W, Goldgraben K, Simmerling C, Smith SO, Constantinescu SN (2005) Structural requirements of the extracellular to transmembrane domain junction for erythropoietin receptor function. The Journal of biological chemistry 280: 14844-14854 Moraga I, Wernig G, Wilmes S, Gryshkova V, Richter CP, Hong WJ, Sinha R, Guo F, Fabionar H, Wehrman TS, Krutzik P, Demharter S, Plo I, Weissman IL, Minary P, Majeti R, Constantinescu SN, Piehler J, Garcia KC (2015) Tuning Cytokine Receptor Signaling by Re¬orienting Dimer Geometry with Surrogate Ligands. Cell, In Press, doi: 10.1016/j.cell.2015.02.011. Seubert N, Royer Y, Staerk J, Kubatzky KF, Moucadel V, Krishnakumar S, Smith SO, Constantinescu SN (2003) Active and inactive orientations of the trans-membrane and cytosolic domains of the erythropoietin receptor dimer. Mol Cell 12: 1239-1250 Staerk J, Defour JP, Pecquet C, Leroy E, Antoine-Poirel H, Brett I, Itaya M, Smith SO, Vainchenker W, Constantinescu SN (2011)

Orientation-specific signalling by thrombopoietin receptor dimers. The EMBO journal 30: 4398-4413 Staerk J, Lacout C, Sato T, Smith SO, Vainchenker W, Constantinescu SN (2006) An amphipathic motif at the transmembrane-cytoplasmic junction prevents autonomous activation of the thrombopoietin receptor. Blood 107: 1864-1871

CO-11

QUALIFYING BLOOD-BASED MOLECULAR ASSAYS FOR TARGETED THERAPIES IN PROSTATE CANCER: FROM CLINIC TO BENCH AND BACK TO CLINIC.

Daniel Danila
Memorial Sloan-Kettering Cancer Center

Medicina oncologica de precizie necesită dezvoltarea de biomarkeri specifici tumorali care sa permita optimizarea selecției terapiilor specifice și evaluarea eficienta a răspunsul la terapie. Eforturile actuale din oncologie au aratat ca pacienții care au in sange celulele tumorale circulante (CTC) au un prognostic relativ inferior comparative cu pacientii care nu au CTC detectabile, iar eliminarea sau reducerea CTC in urma tratamentului sunt asociate cu rezultatelor clinice favorabile. Progresele tehnologice în detectarea, izolarea, și caracterizarea CTC obtinute la flebotomie obisnuita în practica de rutina au permis evaluarea a unor biomarkeri moleculari predictivi ai sensibilitatii tumorale la o anumita modalitatea terapeutică, precum și înțelegerea mecanismelir de rezistenta la tratament. În prezent, nu există o definiție unica a CTC și nu exita un singur CTC "biomarker." Mai degrabă mai multe teste sunt in curs de dezvoltare pentru a defini biomarkeri in CTC. Cu toate acestea, înainte ca orice biomarker poate fi studiat in rolul de a influenta deciziile medicale este esențial ca testele utilizate pentru a măsura biomarkerii sa fie validate analitic într-o succesiune de studii pentru a justifica utilizarea biomarker în contextul clinic indicat. Studii clinice prospective, create în jurul biomarkerului în sine și in contextul clinic specific pentru care se aplică, sunt necesare pentru a evaluea în continuare rolul acestor markeri noi in practica de rutina din clinica. Dezvoltarea de biomarkeri robusti in CTC depinde de standardizarea măsurilor critice necesare pentru a califica teste specifice in CTC pentru practica medicala si pentru cercetarea clinica de noi terapii bazate pe biologia tumorala. Potențialul pentru teste clinice la punctul de ingrijire este clar.

Precision cancer medicine requires the development of tumor-specific biomarkers to optimize selection of targeted therapies and to better assess response to therapy. Current efforts in several tumor types have shown that patients in whom circulating tumor cells (CTC) are detected have an inferior prognosis relative to those in whom CTC are not detected, and that the elimination or decrease of CTC following treatment is associated with improved clinical outcomes. Technological advances in the detection, capture, and characterization of CTC from phlebotomy samples obtained in a routine clinical practice setting have enabled the evaluation of different molecular biomarkers predictive of tumor sensitivity to a therapeutic modality, and to understand mechanisms of treatment resistance. At present, there is no single definition of a CTC and no single CTC “biomarker.” Rather, multiple assays are in development for CTC biomarkers. However, before the role of any biomarker in medical decision-making can be determined, it is essential that the assays used to measure the biomarker are analytically validated in a sequence of trials to generate the evidence to support the biomarker’s use in the given context of use. Prospective studies, designed around the biomarker itself and the specific clinical context for which it is applied, are needed to further assess the role of these novel markers in clinical practice. Unmet needs in developing reliable CTC biomarker assays are defining and standardizing the necessary steps to qualify specific CTC tests for medical decision-making in clinical practice or drug development. The potential for point-of-care tests is clear.

CO-12

HIV SUBTYPES INNEWLYDIAGNOSED PATIENTSIN ROMANIA

Claudia Dita[1], Aura Temereanca[1,2], Cristiana Oprea[3], Camelia Sultana[1,2], Adelina Rosca[1], Camelia Grancea[2], Ana-Maria Schweitzer[4], Simona Ruta[1,2]
[1]*Stefan S. Nicolau Virology Institute, Bucharest, Romania*
[2]*Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
[3]*Victor Babes Hospital for Infectious and Tropical Diseases, Bucharest, Romania*
[4]*Baylor Black Sea Foundation Constanta, Romania*

Objectives: In the last 3 years, the main epidemiological HIV-1 changes in Romania are related to the constant increase of heterosexually infected adults, the gradual increase in the percentage of new infections associated with homosexual transmission and, in particular, the explosive growth in the number of cases associated with intravenous drug use. In this context, we aimed to evaluate the prevalence of HIV-1 circulating subtypes and their association with the route of transmission.
Methods: Samples from 77 patients newly diagnosed with HIV-1 infection between 2008-2014were analyzed for viral load by quantitative RT-PCR method (CobasTaqMan HIV-1 Test Roche Molecular Systems, USA). Pol gene sequencing was performed on samples with viral load> 1000 copies of HIV RNA/mlusing the ViroSeq HIV-1 Genotyping System (Abbott Laboratories, USA), and HIV-1 subtype was determined using the REGA database. Statistical analysis of data was performed with IBM SPSS Statistics (V21).
Results: 35.2% of the study patients were intravenous drug users, the rest were infected either by hetero or homosexual route. Most of the patients (76.9%) were young men, median age 31 years (17-59), not married (37.2%). HIV/HCV-coinfection was present in 47, 4% of the cases, only 7.8% were HBsAgchronic carriers. The median CD4 cell count was 417 cells/ml, only 19.2%of the infected patients had CD4 <200 cells/ml. HIV-1 subtype F remains predominant in newly diagnosed cases (73.1%), but other subtypes are frequently detected (G subtype 11.5%, B subtype 7.7%, B/G and G/F recombinant 3.8%). Over the past three years the percentage of seropositive patients involving non- F subtypes increased from 5% in 2008¬2011 to 50% in 2012-2014. In intravenous drug users there is a significant correlation between the degree of immunosuppression and the infecting subtype: 33.3% of the patients with non-F HIV subtype exhibit severe immunosuppression (CD4 <200), compared with only 8% of patients with F subtype (p = 0.05). In thesubgroup of patients with heterosexual transmission route, infection with non- F subtype is correlated with the presence of other STDs (p = 0.04).
Conclusion: We identified an increasing diversification ofHIV-1 circulating subtypes in newly diagnosed patients, especially among intravenous drug userswhocan represent a high risk group for HIV transmission toward the general population. Key words: injecting drug users, HIV, late-presenters.

CO-13

RATIONALLY EXPLOITING ANTI-ANGIOGENESIS FOR IMPROVING HEPATOCELLULAR CARCINOMA THERAPY

Dan G. Duda
Massachusetts General Hospital& Harvard Medical School, Boston, USA

The incidence of hepatocellular carcinoma (HCC) is on the rise, and the therapeutic options remain limited. In fact, sorafenib—an anti-angiogenic drug with broad anti-tumor activity—is the only approved systemic therapy for advanced HCC patients. Moreover, the efficacy of sorafenib in HCC is limited. Furthermore, other anti-angiogenic approaches—while active— have failed so far to match or surpass the efficacy of sorafenib (1). Thus, a better understanding of the effects of anti-angiogenics in HCC is warranted in order to identify biomarkers of response and novel targets for therapy. Chronic liver inflammation and replacement of functional liver tissue by fibrotic scar tissue (cirrhosis) are typically associated with the development of HCCs, and fuel the growth and treatmentrefractoriness of these lethal cancers. These factors do so by creating an environment characterized by low oxygen (hypoxia) and suppression of the immune system's ability to fight the tumor. We have explored

mechanisms of action and potential biomarkers in multidisciplinary studies (2-5). We have also developed murine models of liver disease that recapitulate many features of human disease. In these preclinical models, we showed that sorafenib treatment only delays tumor growth, as seen in HCC patients. Eventually, sorafenib treatment increases intratumoral hypoxia due to vascular rarefaction, which promotes treatment resistance (6-7). This was in part due to the hypoxia-induced increase in expression of an inflammatory factor called stromal cell-derived factor 1-alpha (SDF1α) and its cellular receptor CXCR4 inside the tumors.We discovered that SDF1α promotes intratumoral infiltration of inflammatory and immunosuppressive cells (immune cell types such as certain monocyte/macrophages and regulatory T lymphocytes) and also increases tumor-associated fibrosis in HCC. Thus, through its pleiotropic effects, SDF1α/CXCR4 pathway acted as a master regulator of stroma polarization toward an immunosuppressive, tumor-promoting microenvironment. Indeed, CXCR4 inhibition in combination with sorafenib prevented this polarization and reduced the number of infiltrating immunosuppressive cells. These results also provide strong rationale for combining sorafenib with novel drugs that boost the anti-tumor immune responses involving effector T lymphocytes. Novel immune therapies have shown unprecedented efficacy in other malignancies, such as advanced melanomas. Unfortunately, experimental data for the effects of these therapies in HCC are lacking. We have recently demonstrated that anti-PD-1 immunotherapy can improve tumor response to sorafenib in HCC models (7). The availability of FDA approved drugs (sorafenib and plerixafor) and promising immunotherapies (anti-PD-1 antibodies) make these strategies rapidly translatable into clinical studies. Supported by NIH grant P01-CA80124. Furtherreading 1) Zhu AX, et al. Nature Reviews Clinical Oncology 2011; 8: 292-301. 2) Zhu AX, et al. Journal of Clinical Oncology 2009; 27: 3027-35. 3) Zhu AX, et al. Clinical Cancer Research 2011; 17: 918-27. 4) Zhu AX, et al. Clinical Cancer Research 2013; 19: 1157-66. 5) Zhu AX, et al. Clinical Cancer Research 2013; 19: 6614-23. 6) Chen Y, et al. Hepatology 2014; 59: 1435-1447. 7) Chen Y, et al. Hepatology 2015; ePub as doi: 10.1002/hep.27665 on December 20, 2014.

CO-14

DIFERENȚE TRANSCULTURALE ÎN INTESTINUL IRRITABIL. SUNT ROMÂNII DIFERIȚI?

Dan L Dumitraşcu
UMF Iuliu Haţieganu, Cluj-Napoca, Romania

Introducere şi scop. Sindromul intestinului iritabil (SII) este cea mai frecventă tulburare funcțională digestivă. Există mari deosebiri geografice în raportarea SII. Acestea ar putea fi explicate prin factori psihosociali divergenți. Am studiat diferențe interculturale ai factorilor psihosociali în SII, la români, comparativ cu alte țări.
Metode. Prezentăm aici o sinteză a datelor grupului nostru din studii transculturale şi comparative asupra factorilor psihosociali în SII (studiul Gershon, studiul Bari-Cluj etc).
Rezultate. Spre surpriza noastră, majoritatea studiilor arată scoruri mari pentru catastrofizare şi anxietate la români cu SII comparativ cu alte țări. Pe de altă parte, raportarea abuzurilor este mult mai rară comparativ cu țările vestice.
Concluzii. Se pare că românii cu SII prezintă scoruri mai ridicate pentru unii factori psihosociali, comparativ cu alte populații studiate, dar stresul produs de abuz este mai rar.

TRANSCULTURAL DIFFERENCES IN THE IRRITABLE BOWEL SYNDROME. ARE ROMANIANS DIFFERENT?

Background and aim: the irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder. There are important geographical differences in the reporting of IBS. These could be caused by diverging psychosocial factors. We looked for intercultural differences in pychosocial factors in IBS, in Romanians compared to other countries.
Methods. We present the data from several transcultural studies on IBS and of comparative studies under-

taken in recent years by our group (Gershon study, Bari-Cluj study etc).
Results. To our surprise, most studies show high scores for catastrophizing and anxiety in Romanian patienst with IBS compared to other countries. On the other hand, the reporting of abuse is much less frequently presented than in Western countries.
Conclusions. It seems that Romanians with IBS present higher scores for some psychosocial factrors, compared to other populations studied, but stress caused by abuse is less frequent.

CO-15

DISJUNCTION OF DOPACHROME TAUTOMERASE AND TYROSINASE EXPRESSION IN MELANOCYTIC LESIONS - MOLECULAR EVENTS AND PATTERNS WITH POSSIBLE IMPLICATIONS IN MELANOMA PROGRESSION

Anca Filimon[1], Sabina Zurac[2], Adina-Luminita Milac[3], Livia Elena Sima[1], Stefana Petrescu[1], Gabriela Negroiu[1]
[1]Department of Molecular Cell Biology, Institute of Biochemistry, Romanian Academy, Bucharest, Romania
[2]Department of Pathology, Colentina University Hospital, Bucharest, Romania
[3]Department of Bioinformatics and Structural Biochemistry, Institute of Biochemistry, Romanian Academy, Bucharest, Romania

The present study investigated the usefulness of Dopachrome tautomerase (DCT) or Tyrosinase Related Protein 2 (TRP2), regulatory enzyme of melanin synthesis, in the assessment of melanocytic lesions. TRP2/DCT was comparatively analyzed with Tyrosinase (Tyr), the well-acknowledged melanoma diagnostic marker, in melanoma cell lines and 166 nevi and malignant melanomas, by Real Time qRT-PCR, Western blot, N-glycan analysis and immunohistofluorescence microscopy. In all cell lines, regardless of staging and pigmentation, unlike Tyr, TRP2 was well-expressed as a mature, fully-processed protein. The simultaneous detection of TRP2 and Tyr showed that 81% of nevi were TRP2+/Tyr+ and 19% were TRP2-/Tyr+, whereas 52% of melanomas were TRP2+/Tyr+ and 19% were TRP2-/Tyr+. The TRP2+/Tyr- category was exclusively detected in acral lentiginous and in of achromic melanomas. A number of around 35% of all investigated specimens with exception of junctional nevi, demonstrated a particular molecular architecture of TRP2 - Tyr dissociation, with large areas of TRP2+/Tyr- cells invading dermal tissue. TRP2+ cell populations co–expressed different prognostic/progression markers along tumor components. TRP2 and Tyr expressions in melanoma cell lines and cell populations of melanoma specimens are regulated by distinct mechanisms. TRP2 represents a valuable addition to the cutaneous malignant melanoma antigen panel, particularly for acral lentiginous and achromic melanomas. Given the recently demonstrated anti-apoptotic function of TRP2, the perpetuation of TRP2+ cell populations in the hostile environment of the innermost dermis indicates an aggressive or resistant tumor phenotype. TRP2 expression in distinct tumor cell populations is a significant molecular event of the selection process of melanoma cell phenotypes during malignant progression. Grant Application No. 156, Exploratory Research Projects – PN-II-ID-PCE-2011-3-0492-1, funded by the Ministry of Education and Research Postdoctoral Program POSDRU/89/1.5/S/6074 Partial funding Romanian Ministry of Research and Education, through the Romanian Academy Project 1/2011 of the Institute of Biochemistry

---

CO-16

STUDII GENETICE ÎN PATOLOGIA TUMORALĂ PANCREATICĂ

Dan Ionuț Gheonea, Adrian Săftoiu, Tudorel Ciurea
Centrul de Cercetare în Gastroenterologie și Hepatologie Universitatea de Medicină și Farmacie din Craiova

Introducere/Obiective: Cancerul pancreatic este caracterizat printr-o varietate de modificări la nivel molecular. Mismatch Excision Repair (MMR) este un sistem de reparare a ADN-ului care, prin excizia bazelor împerecheate greşit joacă un rol important în menținerea integrității genomice. Factorul de creștere endotelială vasculară

(VEGF), secretat de celulele tumorale, este implicat în creșterea tumorală și potențialul metastatic. Scopului studiului a fost aprofundarea rolului genelor MMR și compararea expresiei VEGF-A și VEGF-B în formațiunile focale pancreatice benigne și maligne, prin analiza qRT-PCR a probleor obținute prin puncție fină aspirativă ghidată ecoendoscopic (PFA-EUS).
Metodă: Studiul prospectiv a inclus pacienți cu cancer pancreatic și pancreatită cronică pseudotumorală. PFA-EUS a fost efectuată la toți pacienții. Analizele genetice au fost inclus extracția ARNm și determinarea expresiei genelor MMR (MLH1, MLH3, MSH6). Ulterior a fost analizat profilul expresiei genelor VEGF-A, VEGF-A121, VEGF-A169 și VEGF-B.
Rezultate: ARN total a fost izolat la toți pacienții din materialul biologic obținut prin PFA-EUS. Am utilizat curbe ROC pentru obținerea valorilor cut-off semnificative pentru determinarea expresiei genelor MMR în cele două patologii. De asemenea, la pacienții cu cancer pancreatic am constatat o neregularitate a expresiei genelor angiogenezei prin comparație cu probele provenite de la pacienții cu pancreatită cronică. Expresiea izoformelor VEGF a fost polimorfă atât în cancerul pancreatic cât și în pancreatita cronică.
Concluzii: Calitatea și cantitatea materialului biologic obținut prin PFA-EUS a permis extragerea unei cantități suficiente de ARN pentru analiza qRT-PCR și diferențierea pancreatitiei cronice și cancerului pancreatic.

GENETIC STUDIES IN TUMORAL PANCREATIC PATHOLOGY

Background/Aims: Pancreatic cancer is characterized by a variety of molecular alterations. Mismatch Excision Repair (MMR) is a DNA repair system that eliminates mismatched base pairs and it plays an important role in the maintaining of genomic integrity. Vascular endothelial growth factor (VEGF), secreted by tumor cells, is involved in primary tumor development and metastatic potential. The aim of the study was to assess the role of several MMR genes and to compare the gene expression patterns of VEGF-A and VEGF-B in malignant and benign pancreatic masses samples by qRT-PCR in endoscopic ultrasound guided fine needle aspiration (EUS-FNA) specimens.
Methodology: The prospective study included consecutive patients with pancreatic cancer and chronic pseudotumoral pancreatitis. EUS-FNA was performed in all the patients. Gene analysis was performed by extracting the mRNA and by determining the expression of DNA repair genes (MLH1, MLH3, MSH6) using a standard algorithm. Furthermore, we analyzed the expression profiles for VEGF-A, VEGF-A121, VEGF-A169 and VEGF-B genes
Results: Total RNA was successfully isolated form all the EUS-FNA pancreatic samples. We have analyzed the ROC curves in order to assess the significance of determining the expression of the MMR genes in the EUS-FNA samples, obtaining for cut-off values. In pancreatic cancer samples we detected a disregulation of expression for the angiogenesis genes compared to chronic pancreatitis specimens ($p<0.05$). The expression of VEGF isoforms was polymorphic in human pancreatic cancer and in chronic pancreatitis samples.
Conclusions: The quality and the amount of cellular sampling using pancreatic EUS-FNA allow the extraction of sufficient quantities of RNA to perform qRT-PCR analysis for the differentiation between pseudotumoral chronic pancreatitis and pancreatic.

CO-17

MIGRATIA POPULATIILOR ANTICE EXPLICA COMPOZITIA GENETICA A EUROPEI: COMPARATIE CU MIGRATILE ACTUALE STUDIATE DE PROGRAMUL MEDIGENE

Florin Grigorescu[1], Mihail Coculescu[2], Ramon Gomis[3]
[1]*Universitatea Montpellier, IURC Montpellier*
[2]*UMF Carol Davila Bucuresti, Endocrinologie*
[3]*Universitatea Barcelona, Endocrinologie*

Europa este rezultanta valurilor successive de colonizare cu populatii migratorii. Analiza predispozitiei genetice pentru bolile complexe depinde de capacitatea de a descompune componentele ancestrale si istorice a unei

populatii. In cadrul proiectului MEDIGENE (FP7¬279171) atentia noastra a fost concentrata asupra componentelor populatiilor latine ca Italienii, Spaniolii si Romanii. In acesta directie, studierea ethnogenezei Romaniilor antici si demografia Imperiul Roman ar fi esentiali in interpretarea caracteristiciilor metabolice ale populatiilor ancestrale succesive de vinatori-culegatori (paleoplitic), a fermierilor din Neolitic sau a populatiilor Indo-Europenene, toate cu o predispozitie diferita la carbohidrati (cereale), carne, toleranta la gluten sau lactose sau raspunsul metabolic la periode seculare de foamete. Pe linga genotipurile determinate de ADN-ul mitocondrial sau cromozomul Y, cercetatorii sunt azi capabili sa descompuna populatiile actuale prin utilizarea noilor marcari SNP (single nucleotide polymorphism), care ofera o informatie aditionala asupra amestecului de populatii. Asemenea studii, pot aduce o noua viziune asupra compozitiei Daciilor pe actualul teritoriu al Romaniei, de unde probe de mare calitate pot fi obtinute prin colaborarea intre antropologi, geneticieni si endocrinologi. Deoarece Spania a initiat studii interesante pe populatiile antice la IDIBAPS (Barcelona), studii detaliate de etnogeneza se impun pentru locuitorii Daciei Mediteraneene in comparatie cu regiunile din nordul Dunarii pe actualul teritoriu al Moldovei, Transivaniei sau Munteniei. Genotiparea densa a genomului cu metodele noi de izolare a marcarilor ancestrali (ancestral informative markers sau AIMs) sunt un pas esential in definirea factorilor care regleaza echilibrul endocrino-metabolic si speranta de viata a populatiilor native sau imigrante ale Europei.

MOVEMENTS OF ANTIQUE POPULATIONS EXPLAIN THE GENETIC MAKEUP OF EUROPE: COMPARISON WITH ACTUAL MIGRATIONS STUDIED BY THE MEDIGENE PROGRAM

Europe results from waves of colonization by human populations during millennia. Analysis the genetic susceptibility for complex diseases is pending on ability to decompose ancestral and historical components of the population. As part of MEDIGENE program (FP7-279171) we focused on components of Latin speaking populations such as Italians, Spanish and Romanians with the goal to define ethnic components. Along this line, understanding ethno-genesis of antique Romans and demography of the Roman Empire would be essential in interpretation of metabolic characteristics of successive ancestral hunters-gatherers (Paleolithic), Neolithic farmers or Indo-European people, all with different susceptibilities for carbohydrate (cereals) or meat, gluten or lactose tolerance and metabolic response to repetitive food shortage during centuries. Over mitochondrial (mt)DNA and chromosome Y lineages, researchers are able to decompose actual populations using new autosomal SNP markers (single nucleotide polymorphism), which offer additional information on admixture. Such studies may give a new insight in composition of Dacians on the actual territory of Romania, from which high quality samples may be obtained through collaboration between anthropologists, geneticists and endocrinologists. Since Spain initiated interesting studies in antique populations at IDIBAPS (Barcelona), detailed studies are imposed to unravel components of Dacia Mediterranea inhabitants compared to regions on the North of Danube on the actual territory of Moldova, Transylvania and Walachia. Genotyping at high density of the genome combined with new methods in describing ancestral informative markers (AIMs) are essential steps in defining factors regulating endocrino-metabolic equilibrium and life expectancy in native or immigrant European populations.

CO-18

PREDICTIVE POWER OF POLYGENIC RISK SCORE IN BIPOLAR DISORDER IN THE ROMANIAN POPULATION

Maria Grigoroiu-Serbanescu[1], Carmen C. Diaconu[2], Ana I. Neagu[2], David Curtis[3]
[1]*Alexandru Obregia Clinical Psychiatric Hospital, Bucharest*
[2]*Stefan S Nicolau Institut of Virology, Bucharest*
[3]*University College London, Dept. Psychiatry, London*

Introduction: Bipolar disorder (BP) and schizophrenia are severe, heritable psychiatric disorders. Genome-wide research suggests that the molecular basis of BP and schizophrenia overlaps (Cross Disorder PGC Group, Lancet,

2013). The objective of our work was to investigate whether polygenic scores based on schizophrenia-associated SNPs in the PGC sample (www.med.unc.edu/pgc/) might predict the age of onset (AO) in bipolar I disorder (BP-I), the most severe BP form. We hypothesized that schizophrenia-associated SNPs might predict the late AO of BP-I due to the common character of SCZ-associated variants.

Method: We selected 10,681 non-ambigous SNPs among the 102,637 SNPs present in the PGC SCZ-sample. Using these SNPs we derived polygenic scores in a Romanian sample of 389 BP-I patients with genome-wide data (604,064 SNPs) to predict the patient AO as dichotomous variable (early onset: AO≤24 years; late onset: AO>24 years). The genotyping of the Romanian patients was performed at the Institute of Human Genetics of Bonn. PLINK 1.07 (Purcell, 2009) was used for computing polygenic scores, means of which were compared by t-test between the early- and the late-AO patient groups.

Results: 2114 out of 10,681 schizophrenia-SNPs were informative in our sample contributing to polygenic scores in BP-I patients. There was no significant difference in mean polygenic scores between the early- and the late-onset group of BP-I patients (t=1.14, P=0.25).

Conclusion: The polygenic scores based on 2114 SCZ-associated common variants did not predict the onset group in our BP-I patients under the AO-cutoff 24 years. Other AO-cutoffs and phenotypic traits (e.g. incongruent psychosis) might be tested.

Acknowledgements: This study was supported by a grant from the National Authority for Scientific Research -PCCA PNII 89/2012.

CO-19

NOVEL IMAGING TECHNIQUES AND THEIR POTENTIAL FOR INDIVIDUALIZING CANCER TREATMENTS

Anca-Ligia Grosu

Department of Radiation Oncology, Medical Center - University of Freiburg, Germany

In radiation therapy (RT) staging, treatment planning, monitoring and evaluation of response are traditionally based on computed tomography (CT) and magnetic resonance imaging (MRI). These radiological investigations have the significant advantage to show the anatomy with a high resolution, being also called anatomical imaging. In recent years, so called biological imaging methods which visualize metabolic pathways have been developed. These methods offer complementary imaging of various aspects of tumour biology. To date, the most prominent biological imaging system in use is positron-emission tomography (PET), whose diagnostic properties have clinically been evaluated for years. The first rationale for using PET in target volume delineation for radiation treatment planning is the higher sensitivity and specificity of PET for tumour tissue, in comparison to CT and MRI, in some tumour entities. This has been demonstrated in many studies evaluating the results of PET with the results of radiological investigations and histology. The hypothesis tested in these studies was that using PET in addition to CT and/or MRI allows tumour tissue detection with a higher accuracy. The second rationale for integrating PET in the process of radiation treatment planning is the ability of PET to visualize biological pathways, which can be targeted by radiation therapy. The visualization of hypoxia, angiogenesis, proliferation, apoptosis, receptor expression, gene expression etc. leads to the identification of different characteristics of the tumours, of different sub areas of the gross tumour mass, which can be individually targeted. The aim of this review is to discuss the valences and implications of PET in RT. We will focus our evaluation on the following topics: the role of biological imaging for tumour tissue detection / delineation of the gross tumour volume (GTV) and for the visualization of heterogeneous tumour biology. We will discuss the role of FDG-PET and hypoxia-PET (FMISO-PET) in lung and head and neck cancer, the impact of amino-acids (AA)-PET in target volume delineation of brain tumours and the role of Choline-, Bombesine- and PSMA-PET in prostate cancer. Furthermore, we will discuss the impact of animal-PET in the visualisation of glioblastoma tumor stem cells. We conclude that, regarding treatment planning in radiotherapy, PET offers advantages in terms of tumor delineation and the description of biological processes.

CO-20

RS199508964 DELETION IN EXON 6 OF IRF5 GENE IS CORRELATED WITH IL28B GENE SNP RS12980275 AND PREDICTS SUSTAINED VIROLOGICAL RESPONSE IN PATIENTS WITH RECURRENT HEPATITIS C FOLLOWING LIVER TRANSPLANTATION

Speranta Iacob, Razvan Iacob, Codruta Vagu, Simona Ruta, Cristian Gheorghe, Liliana Paslaru, Irinel Popescu and Liana Gheorghe
Institutul Clinic Fundeni, Bucuresti, Romania

Background: In patients with recurrent HCV infection after liver transplantation (LT), analyses of single nucleotide polymorphisms of IL28B in recipient and donor tissues allows prediction of sustained virological response (SVR) to PEG-Interferon and ribavirin therapy. IRF-5, a member of Interferon Regulatory Factors, a transcription factor, functions as a key regulator in TLR4 cascade, and is capable of inducing inflammatory cytokines. IRF1 and IRF5 have antiviral roles that are IFN-independent and cell-type specific.
Aim: To investigate IL28B polymorphism and IRF5 mutations in Romanian LT recipients with recurrent hepatitis C in order to establish a possible functional explanation for the already proven association of IL28B gene polymorphism to SVR following double antiviral therapy in patients with recurrent hepatitis C following liver transplantation.
Methods: Fourty-five LT recipient DNA samples were screened for rs12980275 single nucleotide polymorphism near the IL28B gene and for rs199508964 deletion of 30 bases in IRF5- exon 6, using Sanger sequencing technique.
Results: There were analyzed 23 females and 22 males with a mean age of 52.5±6.9 years and a mean time since LT of 16.3±11.6 months. In our study group no other mutations than rs199508964 were identified in exon 6 of IRF5 gene. IRF genotypes were: wild type (WT) – 14%, heterozygous for the deletion – 44.2%, and homozygous for the deletion – 41.9%. Minor allele frequency (MAF) for rs199508964 in our study group was 64%, higher than - MAF according to Pubmed (48.4%). Distribution of IL28B genotypes were: C/C – 14%, C/T - 58.1%, T/T - 27.9%. There was an association between IRF5- non-WT and IL28B non-C/C genotypes (p=0.01). A significant association was found between SVR and WT genotype of IRF5 (p=0.01), however mutations in IRF5 gene were not associated to advanced fibrosis after LT.
Conclusions: There is a link between recipient IL28B and IRF5 genotypesthat could explain correlation to SVR following double antiviral therapy.

CO-21

LATE PRESENTATION IN HIV INFECTED INTRAVENOUS DRUG USERS

Irina Ianache[1], Emanoil Ceausu[1,2], Simona Ruta[2,3], Simona Erscoiu[1,2], Roxana Radoi[1], Gratiela Tardei[1], Cristiana Oprea[1,2]
[1] *"Victor Babes" Clinical Hospital for Infectious and Tropical Diseases, Bucharest, Romania*
[2] *"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[3] *"Stefan S. Nicolau" Institute of Virology, Bucharest, Romania*

Background: Untreated HIV infection and HIV-related immunosuppresion are usually associated with a high risk of acquiring opportunistic infections and significantly increase the probability of HIV transmission, the mortality rate and the healthcare costs.OBJECTIVE: The aim of our study was to evaluate the demographic and clinical characteristics and the prevalence of "late-presenters" (LP)among HIV infected injecting drug users (IDUs).
Methods: Prospective study on HIV infected IDUs, admitted at "Victor Babes" Clinical Hospital for Infectious and Tropical Diseases Bucharestbetween January 2009 - December 2014. IDUs with CD4 cell counts < 350/mm^3 were considered "late presenters" (LP) and those with CD4 <200/mm^3 as "advanced HIV disease" (AHD). Statistical analysis was performed using Graph pad Prism 4.01.

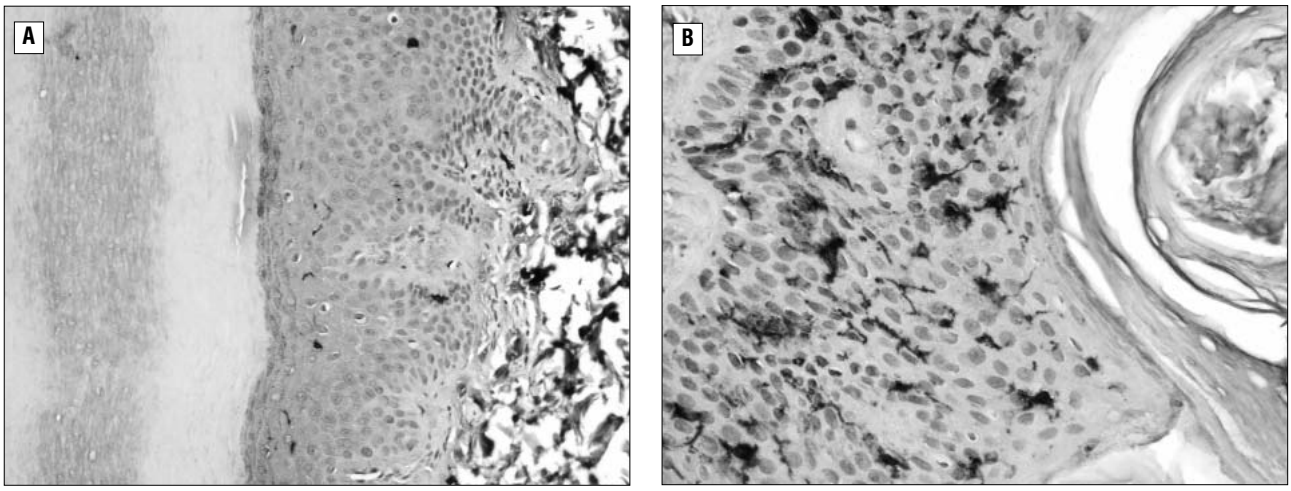
Results: Out of 563 IDUs diagnosed with HIV infection, 221 (39.2%) were LP and 156 (27.7%) had AHD. Among LP, the majority were males 188 (85%), the median age at HIV diagnosiswas 30 years (range 16-70). They were mostly from urban areas, 199 (90%) and 113 (51.1%) were unemployed. The median period of time for intravenous drug use was 10 years; more than half (56%), used both heroin and psychoactive injectable drugs. The median CD4 cell count at diagnosis was 105 cells/mm^3 (range 1-346) and the median viral load 5.41 log10 (range 2.6 – 7 log10). The median CD4 cell count decreased from 160.5 cells/mm^3 in 2009 to 55 cells/mm^3 in 2014, while the percentage of LP increased from 25% in 2009 to 50% in 2014. All but three (98.6%), were coinfected with HCV, while only 9.5% with HBV and 18% with other sexually transmitted diseases. More than half of IDUs, (57.4%), had AIDS defining diseases: (51.1%) tuberculosis, 3.1% cerebral toxoplasmosis and 1.8% AIDS-related malignancies. Severe bacterial infectionswere diagnosed in 141 (63.8%) IDUs: bacterial pneumonia in 81 (36.6%), sepsis 19 (8.5%), endocarditis 15 (6.7%). The overall mortality rate was 23.5% (52).
Conclusions: The number of LP among HIV infected IDUs rose over the last years, due to their high risk behavior and lack of addressability to the health care system. The management of HIV infection in IDUs is difficult due to their severe addiction and thelong term outcome is poor due to their lack of adherence to cART, drug-druginteractions and toxicities. Implementation of targeted prevention methods among IDUs and a pro-active policy of HIV testing among identified risk groups are absolutely necessary. Key words: injecting drug users, HIV, late-presenters.

CO-22

CELULELE LANGERHANS: 147 ANI DE LA DESCOPERIREA LOR THE LANGERHANS CELLS: 147 YEARS SINCE THEIR DISCOVERY

Constantin Ionescu-Tirgoviste[1,2], Sabina Zurac[1], Cristian Guja[1,2], Costin Căruntu, Paul Gagniuc[2], Mihaela Ghiță[1]
[1]*UMF "C. Davila", București, România*
[2]*Institutul Național de Diabet, Nutriție și Boli Metabolice N.C. Paulescu, București, România*

Introducere. In 1868, tânărul cerectător Paul Langerhans (1847-1888) a descris în piele o nouă și ciudată celulă, pe care a interpretat-o ca aparținând sistemului nervos. Folosind microscopia electronică, Michael Birbeck (1925-2005) a descoperit în interiorul insulelor câteva organele histologice numite ulterior "granulele" Birbeck, oarecum impropriu, intrucât structura lor seamănă cu bastonașe sau rachete de tenis. Aceste "microorganele" sunt formate din molecule de langerină, care sunt specifice acestor celule, care aparțin clasei mari de celule dendritice. Scopul acestui studiu este acela de a analiza statusul lor la pacienții diabetici, avaând în vedere rolul lor imunomodulator.
Material și metode. Am obținut (cu consimțământul informat) 16 fragmente de piele, din diferite zone (brațe, trunchi, scalp sau palme) de la 12 pacienți. Fragmentele de țesut au fost fixate în formaldehidă 10% și incluse, apoi, în blocuri de parafină. Secțiunile cu grosime de 3 micrometri au fost colorate cu Hematoxilină-eozină, PAS și Giemsa; a fost efectuată evaluarea imunohistochimică pentru proteinele S100, CD1a și Langerină.
Rezulate. În figurile A și B redăm 2 imagini, una cu un număr mic de celule Langerhans (CLs) și în alta (B) cu un număr mare de CLs. Semnificația distribuției variate a acestor celule în diferite regiuni ale pielii nu a fost încă bine explicată.
Abordări ulterioare Intenția noastră este de a realiza densitate celulelor Langerhans în diferite zone cutanate la pacienții diabetici. Urmează să evaluăm prezența lor (inclusiv densitate și distribuția lor) cu densitatea filetelor nervoase, precum și relația dintre ele. Intenționăm dezvoltarea în viitor a unui studiu prin care să urmărim la persoanele aflate la risc pentru diabet, relația dintre densitatea celulelor Langerhans în diferite regiuni cutanate.
Notă: "Acest studiu a fost efectuat în cadrul programului postdoctoral „CERO - Carees profile: Romanian Research", grant POSDRU /159/1,5/S/135760, cofinanțat de European Social Fund for Sectoral Operational Program Human Resources Development 2007-2013"



Figură: număr mic (A) și număr mare (B) de celule Langerhans în piele

THE LANGERHANS CELLS: 147 YEARS SINCE THEIR DISCOVERY

Introduction. In 1868, the young researcher Paul Langerhans (1847-1888) described in the skin a new strange cell which he interpreted as belonging to the nervous system. Using electron microscopy, Michael Birbeck (1925-2005) had discovered, inside these cells, several histologic organnelles named later, inappropriately, Birbeck "granules", as their structure is rod-like or shaped like a tennis racquet. This "microorganelles" are formed by the Langerin molecule, which is a specific for these cells belonging to the dendritic cells (DCs). The aim of our study was to analyze their presence in diabetic patients, having in view that LCs have a protective immune role.
Material and method. We harvested 16 fragments of skin from different areas (arms, trunk, scalp and palms) form12 patients. The tissue fragments were fixed in 10% buffered formalin and routinely processed into paraffin blocks. 3µ thick sections were stained with Hematoxilin-eosin, PAS and Giemsa; immunohistochemical tests for S100 protein, CD1a and Langerin were performed.Results: In the figure A and B, we give two images, one (A) with a small number of LCs, and the other (B) with a high number of LCs. The significance of the various distribution of these cells in different regions of the skin has not been yet explained.
Future developments: We intend to analyze the density of Langerhans cells in different cutaneous areas of diabetic patients. We will also correlate their presence (both as density and distribution) with the density of small nervous fibres. We intend also to develop a prospective study to analyze the possible correlation

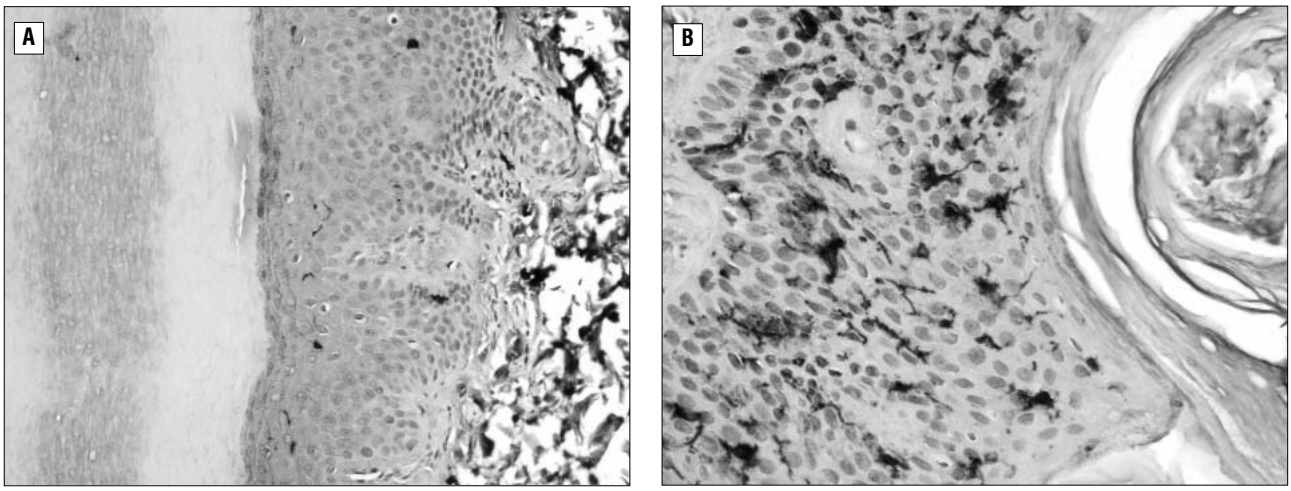


Figure: low number (A) and high number (B) of Langerhans cells in the skin

between density and/or distribution of Langerhans cells in different areas of skin and future development of a diabetes mellitus.
Acknoledgements: "This work was supported by the postdoctoral program "CERO - Career profile: Romanian Researcher", grant number POSDRU/159/1.5/S/135760, cofinanced by the European Social Fund for Sectoral Operational Programme Human Resources Development 2007-2013

CO-23

HYPOXIA-REGULATED NONCODING RNAS: FINE-TUNING THE MOLECULAR LANDSCAPE IN CANCER

Mircea Ivan
Department of Medicine Division of Hematology/Oncology, Department of Microbiology and Immunology, Indiana Univesity School of Medicine, USA

Our previous work extended the hypoxic response to noncoding RNAs, and some of these short transcripts are currently known to play important roles in cancer. miR-210, the prototypical hypoxia-miRNA, is a widely accepted HIF target, and has a significant impact on normal and cancer cell biology, including regulation of proliferation and energy metabolism. Clinically, miR-210 is upregulated in many solid cancers and generally correlated with adverse prognosis. More recently, we have identified long noncoding RNAs (lncRNAs) that respond to oxygen deprivation and potentially interfere with the activity of cancer-related pathways. The presentation focuses on how such noncoding transcripts reshape the molecular landscape in cancer and may influence responses to antineoplastic therapy.

CO-24

NEW ISONIAZID DERIVED HYDRAZONES WITH ANTI-TUBERCULAR ACTIVITY

Lilia Matei[1,2], Ioana M. Aldea[1], Coralia Bleotu[1], Irina Zarafu[2], Cristina Mambet[1], Ana I. Neagu[1], Ion Baciu[2], Anamaria Hanganu[2], Otilia Banu[3], Petre Ionita[2], Anca Paun[2], Arnaud Tatibouët[4], Carmen C. Diaconu[1]
[1]*Stefan S Nicolau Institute of Virology, Romanian Academy, Bucharest, Romania*
[2]*Faculty of Chemistry, University of Bucharest, Romania*
[3]*Institute of Cardiovascular Diseases Prof. Dr. C.C. Iliescu, Microbiology Department, Bucharest, Romania*
[4]*University of Orleans, ICOA-UMR7311, CNRS, France*

Introduction: Isoniazid is the most frequently prescribed antibiotic in the treatment of tuberculosis. Isoniazid-derived reactive species inhibit the synthesis of cell wall lipids and of nucleic acids and also interfere with bacterial respiratory metabolism. Compounds derived from isoniazid reported in literature have proved good anti-mycobacterial activity, some of them being more active than the prodrug they derived from. Thus, this class of substances might be useful in increasing the effectiveness of standard drug regiments in the therapy of M. tuberculosis infections and may serve as promising compounds for future anti-mycobacterial drug development.
Methods: A series of 7 new isoniazid derivatives, isonicotinic acid (2-hydroxy-8-substituted-tricyclo[7.3.1.02.7] tridec-13-ylidene)-hydrazides, were synthesized and their structures were confirmed by 1H- and 13C-NMR spectral analysis and by elemental analysis. Further, these derivatives, were analyzed for their antibacterial activity (against M. tuberculosis and other non-tuberculosis strains), cytotoxicity (apoptosis induction - Annexin V-FITC Apoptosis Detection Kit I and qRT-PCR) and cell cycle blocking (flowcytometry and qRT-PCR), and also for their activity on some metabolizing enzymes.
Results: The best anti-mycobacterial activity was observed in the case of compounds containing alkyl moieties in the 8 position of tricyclo[7.3.1.02.7]tridec-13-ylidene group. One compound (no. 6) has proved best activity on non-tuberculosis strains and was less potent against M. tuberculosis. This compound was the most toxic, inducing apoptosis and blocking the cell cycle in G0/G1 phase. The cell cycle was blocked in G0/G1 phase also by compound 3, but this compound did not show any toxic effect. All compounds induced the expression of

NAT1 and NAT2 genes in HT-29 cell line, and the expression of CYP1A1 in HT-29 and HCT-8 cell lines. Three compounds (1, 6 and 7) were able to increase the CYP3A4 expression level in HCT-8 cells. This effect indicate the activation of other metabolizing pathways, different from that of isoniazid, and the possibility to increase isoniazid acetylation ratio by co-administration with new compounds in slow acetylators.
Conclusions: Our results showed that the new isoniazid derivatives could represent potential candidates for the development of new anti-M. tuberculosis agents. However, more research is needed to improve their pharmacological properties, by increasing their antimicrobial activity and reducing the risk of side-effects.
Acknowledgements: Ioana M. Aldea was financed from the European Social Fund and the Romanian Government under the contract number POSDRU/159/1.5/S/137390 and Cristina Mambet received financial support through the project entitled "CERO – Career profile: Romanian Researcher", grant number POSDRU/159/1.5/S/135760, cofinanced by the European Social Fund for Sectorial Operational Programme Human Resources Development 2007-2013.

CO-25

ROLE OF INTERLEUKINE-17 (IL-17) IN RENALE AND CARDIOVASCULARE DAMAGE IN SYSTEMIC LUPUS ERYTHEMATOSUS

Adriana Metoni[1], Paul Balanescu[1,2], Carmen C Diaconu[3], Dumitru Matei[1,4], Cristian Baicus[1,2,5]
[1]*Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
[2]*Laboratory of Immunology, Colentina Research Center, Bucharest, Romania*
[3]*Stefan S. Nicolau Institute of Virology, Bucharest, Romania*
[4]*Institute of Mother and Child "Prof. Alfred Russescu", Bucharest, Romania*
[5]*Internal Medicine Clinic, Colentina Clinic Hospital, Bucharest, Romania*

Introduction: Atherosclerosis is a major problem in patients with lupus sistemic eritematos (SLE), atherosclerosis risk increases with duration and disease activity. Renal involvement in SLE remains a strong predictor of morbidity and mortality of patients.
Methods: We conducted a retrospective study on a group of 87 patients diagnosed with SLE, the purpose of this study consists in identifying the role of pro-inflammatory interleukin-17 (IL-17) in cardiovascular and renal impairment in patients with SLE. Serum levels of IL-17 were determined by ELISA.
Results: It was observed that 47 patients in the study group show positive values of IL-17 concentrations (from 1.12 pg/ml - 23.66pg/ml) and in 38 patients serum IL-17 expression was below the limit of detection of the assay. Our results showed a positive correlation of IL-17 with active disease lupus monitored by SLEDAI score. IL-17 was detected in SLE patients with renal, cardiovascular, joint, skin, blood impairments.
Conclusions: In our study, patients with positive IL-17 and receiving corticosteroids associated hypertension and dyslipidemia. Absence of IL-17 correlated with minimal or zero values of SLEDAI score.
Acknowledgement: This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390.

CO-26

CORRELATION BETWEEN TRYPTASE-POSITIVE MAST CELLS'S DENSITY AND H. PYLORI PRESENCE IN GASTRIC NEOPLASIA

Gianina Micu[1], Alexandra Bastian[1,2], Mirela Petre[1], Alexandra-Simona Iacob[1], Maria Bosa[1], Teodor Voiosu[2,3]
[1]*"Colentina" Clinical Hospital, Department of Pathology, Bucharest, Romania*
[2]*"Carol Davila" University of Medicine, "Colentina" Clinical Hospital, Department of Gastroenterology, Bucharest*

Background: Success in cancer prevention depends on our understanding of its origins. In the past several years,

we have gained insights into the pathogenesis of gastric cancer identifying Helicobacter pylori bacterium as a key factor in the etiology of gastric carcinoma and lymphoma. Only recently, the role of mast cells in the neoplastic process (direct or indirect) has been revealed both pro- and anti-tumor growth, and could provide ways for new therapeutic opportunities.Subjects and Methods:In the process of trying to find a link between gastric cancer, the density of mast cells (DMC-TP) and the presence of H.pylori infection, we studied samples from 30 pacients who underwent surgery for gastric cancer and compared them with samples from a control group (30 cases), age and sex-matched, with no history of malignancies. For each case, DMC-TP was performed, using 5 areas from the same gastrectomy specimen: intratumoral (IT), tumor invasion front in longitudinal axis (FTL), tumor invasion front in the in-depth of gastric wall (FTP), limit of gastric resection distal of the tumor (LRD), gastric wall away from the tumor (PGD). Results: The study shows a significantly lower value of DMC-TP in male patients rather than in female patients unconnected with the H.pylori presence or absence. In H.pylori infection associated with preneoplastic lesions and also in control cases from patients with other neoplasia than gastric cancer, the DMC-TP levels were above those cases who had only H.pylori inflammatory injury. Conclusion:Mast cells have a big role in pathology of tumor microenvironment, stimulating and/or inhibiting tumor growth, but H.pylori infection was not found to cause any significant changes like mobilizing mast cells in the gastric wall with advanced tumors and minimal stage III TNM.

Acknowledgement: This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390.

CO-27

POTENTIAL MOLECULAR TARGETS FOR GASTRIC CANCER THERAPY

Laura G. Necula[1], Mihaela Chivu Economescu[1], Coralia Bleotu[1], Denisa Dragu[1], Gabriel Constantinescu[2], Simona O. Dima[3], Irinel Popescu[3], Carmen C. Diaconu[1]

[1]*Stefan S Nicolau Institute of Virology and Nicolae Cajal Institute, Titu Maiorescu University Bucharest, Romania*
[2]*Floreasca Emergency Hospital, Bucharest, Romania*
[3]*Center of General Surgery and Liver Transplantation, Fundeni Clinical Institute, Bucharest, Romania*

Introduction: Gastric adenocarcinoma remains one of the most aggressive cancers, being the fourth most common type of cancer and the second leading cause of cancer-related death worldwide. Our study aims to analyze molecular pathogenesis of gastric adenocarcinoma by exploring aberrant signaling pathways in gastric tumor tissues and gastric cancer cell lines.

Methods: We collected tumor and adjacent normal tissue samples from 51 patients with gastric adenocarcinoma. cDNA and proteins have been used in exploring gene expression and signaling pathways by gene microarray and dot-blotting.

Results: Gene expression analysis has identified seven genes, significantly up-regulated, that seems to be associated with tumor progression: KRT17, COL10A2, KIAA1199, SPP1, IL11, S100A2, and MMP3. Results from proteomics highlighted STAT3 activation, simultaneously with JNK and p38 MAP kinase, Wnt/b-catenin, and Akt pathways in gastric tumor tissues. The RNA interference technology was used to inhibit S100A2, and KRT17 gene expression in two human gastric cancer cell lines in order to investigate the biologic significance of these two genes in gastric cancer pathogenesis; genes were selected based on their high expression level on gastric adenocarcinoma samples. The inhibition of specific mRNA expression was determined by quantitative PCR and the effect of gene down–regulation on signaling pathways was assessed by dot-blotting.

Conclusion: The results showed that siRNA knockdown of S100A2, and KRT17 genes decreased signaling on the pathways associated with proliferation, migration and angiogenesis, and these genes may be potential targets for developing new therapeutical strategies in gastric cancer.

Acknowledgements: Laura G. Necula was financed from the European Social Fund and the Romanian Government under the contract number POSDRU/159/1.5/S/137390. This study was supported by a grant from the National Authority for Scientific Research - PCCA PNII 90/2012.

CO-28

MOARTEA CARDIAC SUBITA CORDE INDEMNO: INTRE BLESTEMSI BINECUVANTARE

IN MEMORIAM STEWART G. WOLF, MD (1914 - 2005)

Radu Negoescu
Institutul National de Sanatate Publica, Bucuresti

Stresul psihosocial cronic (SPS) este suspectat a fi la originea deceselor subite cu miocard indemn ("autopsii albe") acoperind cel puțin 9 % din mortalitatea cardiovasculară a României - una dintre cele mai înalte din Europa. Punerea la punct a unor algoritmi eficienți de măsurare automată bătaie-cu-bătaie a intervalelor QT în facsimile digitale ECG de înaltă rezoluție (1-2 ms) a deschis o fereastră către controlul simpatic ventricular, a cărui exacerbare este pusă în legătură cu moartea cardiacă subită (SCD) la pacienți sau subiecți necardiaci sub SPS.
Metodă: Un mijloc direct de a stabili aptitudinea descriptorilor spectrali ai QT-gramelor de a caracteriza controlul simpatic idio-ventricular în situații stresante este comparația cu situații caracterizate de o comandă simpatică indubitabil crescută, precum efortul fizic. Într-un astfel de studiu, 15 subiecți cu miocard normal, cu vârsta medie 47,8 ani, au fost monitorizați în timpul unui interviu de stress (evocând evenimente stresante în viața lor recentă) şi în timpul unui test de efort sub-maximal pe bicicleta ergometrică (3-8 minute).
Rezultate: Stresul mental este statistic echivalent din perspectiva puterii spectrale de joasă frecvență (0,04 - 0,15 Hz) a variabilității intervalului QT pe epoci de 3 minute cu cardiotahograme (sau RR-grame) staționare, pe scurt a indicatorului QT-LF, cu un efort sub-maximal care nu poate fi menținut mai mult de câteva minute.
Discuție şi concluzii: Rezultatele sugerează că stresul psiho-social cronic la adulți maturi (evocabil prin interviu de stress) poate pune miocardul sub solicitare simpatică supranormală de durată (perceptibilă cu ajutorul metodelor neinvazive QT) - binecunoscută ca punând în pericol robustețea electrică a inimii. În funcție de o afecțiune cardiacă pre-existentă, de alți factori de risc prezenți în stilul de viață, precum şi de factori ereditari, stresul poate astfel declanşa incidente aritmice sau accidentele fatale – SCD, considerată a fi primul şi ultimul semn de boală cardiacă în aproximativ 25% din cazuri - care scapă mijloacelor de protecție oferite de cardiologia convențională. Astfel, variabilitatea QT bătaie-cu-bătaie este gata pentru utilizare în situații clinice si sub-clinice ca patognomonic pentru supraviețuire sau deces. Eu- sau dis- stresul mental pot juca un rol major în gestionarea riscului SCD atât la pacienți cardiaci cât şi la subiecți necardiaci cu simptomatologie aritmică vagă. Cu toate acestea, trebuie să distingem între aspectul catastrofal al SCD ce intervine la adulții relativ tineri aparent sănătoşi - fenomen ce trebuie studiat pentru a desprinde patognomonice utilizabile în prevenire - şi perspectiva mai nuanțată asupra SCD ca mijloc lesnicios si demn de a părăsi viața pentru pacienți incurabili în faze terminale sau pentru persoane de vârstă prea înaintată pentru a continua să viețuiască într-o manieră acceptabilă ("Iar zilele omului sunt 70 de ani şi pentru cei mai în putere 80, iar ce este peste 80 e durere si chin" – Psalmul 89). In aceste din urmă cazuri, trebuie să convenim, cu Robert Browning, asupra virtuților curative ale SCD care "…Răscumpără voios într-un minut/A vieții rămăşiță de chin, frig şi urât".

CO-29

IN VITRO ASSESMENT OF NOVEL TUMOR CELLS DEATH MECHANISM – ENUCLEATION

Virgil Paunescu, Florina Bojin, Alexandra Ivan, Mirabela Cristea, Alina Taculescu, Oana Gavriliuc, Gabriela Tanasie, Carmen Panaitescu, Calin Tatu
"Victor Babes" University of Medicine and Pharmacy Timisoara, Romania

Introduction. During tumorigenesis process, cancer cells proliferate without control, escaping the cellular death regulatory mechanisms. Several types of cell death have been described: apoptosis (type I), autophagy-associated cell death (type II), necrosis or oncosis (type III), mitotic catastrophe, anchorage-dependent mechanisms - anoikis, excitotoxicity, Wallerian degeneration, and cornification of the skin. This study aimed to investigate for the first time a possibly novel mechanism inducing tumor cell death under in vitro conditions - enucleation.
Materials and methods. We pursued the influence of colloidal suspensions of Fe3O4 nanoparticles on human

tumor cell lines (melanoma, breast, hepatic and pancreatic cancer) and non-tumor cells (adult mesenchymal stem cells, MSCs) grown according to standard cell culture protocols. Magnetite nanoparticles (MNPs) were prepared by combustion synthesis, double layer-coated with oleic acid and left to interact with tumor cells for various time intervals (2h, 6h, 12h, 24h, and 48h). The in vitro studies focused on morphological and ultrastructural changes, functional studies, immunophenotypical markers and gene expression of tumor and mesenchymal stem cells, employing appropriate methodologies.

Results: Scanning and transmission electron microscopy revealed that tumor cells developed a network of intracytoplasmic stress fibers, which induce extrusion of nuclei, and enucleated cells die. Normal adult mesenchymal stem cells, used as control, did not exhibit the same behavior. Intact nuclei were found in culture supernatant of tumor cells, and were visualized by immunofluorescence.

Conclusion: This study provides evidence that enucleation is a potential mechanism of tumor cell death, opening new horizons in cancer biology research and development of therapeutic agents capable of exploiting this behavior.

Key words: tumor cells, cancer cell death, cell enucleation, magnetite nanoparticles

---

CO-30

RAGE EXPRESSION, LOCALIZATION AND FUNCTION IN MELANOMA CELLS AT DIFFERENT STAGES OF MALIGNANCY

Ioana Popa, Elena Ganea, and Stefana Petrescu
Institute of Biochemistry, Bucharest, Romania

Introduction. The receptor for advanced glycation end products is a pattern recognition receptor which binds a wide range of ligands (AGEs, S100 proteins, HMGB1, amyloid fibrils). Its expression is regulated during development, but also under pathological conditions (inflammatory disorders, cancer, diabetes, Alzheimer's disease) when RAGE ligands accumulate and determine receptor up-regulation and a sustained activation. Over 20 splice variants of the receptor have been described in literature, however, they are less characterized at the protein level.In an attempt to better understand the regulatory mechanisms supporting RAGE involvement in tumorigenic processes we investigated RAGE expression in melanoma cells with distinct malignancy properties.

Methods. We used cultures of melanoma cell lines transfected or not with hRAGE to study RAGE overexpression and soluble RAGE (sRAGE) generation. RAGE was down-regulated by siRNA. A method based on SDS-PAGE under reducing and non-reducing conditions, followed by Western blotting with RAGE antibodies in the presence or absence of purified soluble RAGE was developed to study RAGE protein expression and oligomerization. Cells were further investigated by immunofluorescence microscopy, and cell migration was assayed by 'wound healing' and quantified.

Results. We showed an up-regulation of the full-length RAGE and the down-regulation of the soluble variants of RAGE, in a metastatic type of melanoma cell line (SK-Mel28) compared to a tumor cell line derived from a primary melanoma lesion (MelJuSo). These results suggest that the reduction in soluble RAGE and the increase in full-length receptor occur during the progression to a more aggressive tumor phenotype, feature which has not been previously uncovered. We have detected by SDS-PAGE under non-reducing conditions and Western blot that endogenous RAGE in melanoma cells forms oligomers of approx. 200 kDa through disulphide bridges. Our results of the competition Western blot with sRAGE suggest that constitutively, endogenous full-length RAGE is expressed most likely as a tetramer. In addition to the 200 kDa oligomer, higher (>260 kDa) SDS-resistant complexes are likely to form in primary melanoma MelJuSo cells, and that is a feature of this cell type unlike normal melanocytes and metastatic melanoma SK-Mel28 cells. RAGE had a distinct localization pattern in these cells, as assessed by immunofluorescence microscopy. Moreover, down-regulation of RAGE and soluble RAGE by siRNA inhibited cell migration in MelJuSo cells, and not in SK-Mel28, pointing to a different regulation of RAGE functions in the two melanoma types.

Conclusion. In the present study using cell culture models of melanoma we uncovered several differences in RAGE isoform expression, oligomerization, subcellular localization and generation of the soluble form, which

could potentially contribute to a better understanding of RAGE role through tumor progression.

CO-31

DINAMICA MITOCONDRIALĂȘI DIALOGUL MOLECULAR DINTRE ORGANITELE INTRACELULARE; SEMNIFICAȚII ÎN MIOCARDUL DIABETIC

Doina Popov
Institutul de Biologie și Patologie Celulară "N. Simionescu" al Academiei Române

Descoperirile recente asupra funcției de autoreglare a organitelor celulare și identificarea mecanismelor moleculare participante oferă o perspectivă nouă asupra implicării mitocondriilor în disfuncția miocardului ventricular asociată diabetului zaharat. Ne propunem examinarea următoarelor aspecte noi: (i) rolul mitocondriilor în "controlul calității", exemplificat prin modificările formeiacestor organite: procese de fuziune a mitocondriilor "disfuncționale" cu mitocondrii "sanatoase", conducând la constituirea unor mitocondrii alungite care conservă capacitatea bioenergetică și procese de fragmentare/fisiune care generează mitocondrii mici, urmare a scindării porțiunii "disfuncționale" de cea "sănătoasă". Moleculele implicate în aceste procese sunt proteinele de fuziune (Mitofusin 1 și 2, OPA-1) și cele de fisiune ("Dynamin-related protein1", Fis1); (ii) "turnover"-ul mitocondriilor, characterizat prin dezechilibrul dintre procesul de degradare a mitocondriilor prin autofagie ("mitofagie") și de refacere a organitelor "sănătoase", prin mitocondriogeneza; (iii) dialogul molecular dintre mitocondrii și nucleul celular (genomul nuclear codifică proteinele componente ale mitocondriilor), interacția mitocondrii- reticol endoplasmic/sarcoplasmic (ER/SR), asigurând transportul lipidelor între aceste organite celulare, interacția mitocondrii-picături lipidice (în miocardul steatotic), precum și interacția cu peroxizomii; (iv) activarea stressului ER/SR stress (corelată fibrozei) și inter-relațiile funcționale dintre fibroblaste și celulele inflamatorii din miocardul diabetic (cu implicarea citokinelor și a factorilor de creștere). Evaluarea strategiilor actuale de modulare a stresului la nivelul organitelor celulare permite identificarea unor strategii cu potențiale beneficii pentru aplicarea in clinică; ca exemple în acest sens menționăm înțelegerea mecanismelor de semnalizare de la mitocondrii către restul celulei ("semnalizarea retrogradă"), exploatarea dinamicii mitocondriale în vederea supraviețuirii cardiomiocitelor din miocardul diabetic, manipularea procesului de formare a picăturilor lipidice (corelată stresului ER/SR) folosind noi compuși cu efecte de redresare ale lezării miocardului în diabet.

MITOCHONDRIAL DYNAMICS AND ORGANELLES MOLECULAR CROSSTALK; PROMINENCE FOR DIABETIC MYOCARDIUM

The recent discoveries on organelles autoregulation and their molecular mechanisms motivate a novel perspective on mitochondria involvement in diabetic ventricular myocardium. We'll examine here the following issues: (i) the "quality controller" role of mitochondria, illustrated by the dynamic shape changes from fusion (resulting into elongated mitochondrial networks) to fragmentation/fission (generating smaller size individual organelles); the opposing effects of fusion proteins (Mitofusin 1 and 2, OPA-1) and fission proteins (Dynamin-related protein1, Fis1) will be highlighted; (ii) the turnover of mitochondria, characterized by compromissed autophagic degradation ("mitophagy"), and inadequate replenishment of a healthy pool of organelles by mitochondrogenesis; (iii) the molecular crosstalk between mitochondria and cell nucleus (as mitochondrial proteins are encoded by the nuclear genome), mitochondria¬endoplasmic/sarcoplasmic reticulum (ER/SR) dialogue, ensuing lipid transport at contact points between the two organelles, lipid droplets (of steatotic myocardium), and peroxysomes; (iv) the activation of ER/SR stress (related to fibrosis) and the functional relationship between fibroblasts and inflammatory cells within diabetic myocardium (by mechanisms involving released cytokines and growth factors). A critical evaluation of strategies aiming modulation of organelles stress, allows identification of several conducts with potential clinical benefits; among these we quote understanding mitochondria signalling to the rest of the cell ("retrograde signaling"), targeting mitochondrial dynamics in diabetic heart (as a key therapeutic target towards cardio-

myocytes survival), and manipulation of ER/SR stress-associated lipid droplets formation by new drugs aimed to reverse diabetes-induced cardiac damage.

CO-32

INTERFERENTE MOLECULARE IN DETERMINAREA TIROGLOBULINEI, UN RISC PENTRU GHIDURILE DE CONSENS IN ENDOCRINOLOGIE

Mariana Purice[1], Mihail Coculescu[1,2]
[1]*Institutul National de Endocrinologie " C.I.Parhon", Bucuresti, Romania*
[2]*Academia Romana*

Tiroglobulina (Tg) este o glicoproteina sintetizata exclusiv in celulele foliculare tiroidiene normale sau canceroase. Determinarea Tg serice aduce informatii pretioase recurenta tumorala sau a metastazelor tiroidiene, fiind un marker sensibil in monitorizarea pacientilor cu neoplasm tiroidian diferentiat (DTC) in timpul tratamentului cu radioiod (RAIT). In anii '90 metodele imunometrice (IMA) de determinarea tiroglobulinei aveau o sesibiltate analítica (Sa) scazuta (1-2 ng/ml), erau imprecise iar interferenta moleculara cu anticorpii endogeni anti Tg (TgAb), anticorpi heterofilici, cu fragmente sau isoforme de Tg, au un impact major negativ asupra interpretarii rezultatelor. Metoda alternativa, este spectrometria de masa care inca nu este standardizata si are o sensibilitate inadecvata. Din experienta noastra, la pacientii cu DTC in timpul RAIT si stimularea secretie de TSH, valorile Tg se coreleaza cu uptake ul 131I, existind un procent considerabil de interferente moleculare (12 %) care face dificila interpretarea clinica a valorilor chiar si in cazul metodei folosite, cu Sa = 0.5 ng/ml. Nu există un consens privind nivelul minim de anticorpi endogeni de la care s-ar exclude interferentele moleculare. Generatia noua IMA, are o Sa< 0.1 ng/ml, performanta, sugerind clinicienilor ca pentru evaluarea corecta a Tg, nu mai este necesara stimularea TSH lui la pacienti cu risc scazut. Acest lucru are implicatii considerabile asupra practicii medicale dar un este inclus in ghidurile clinice curente. Atit European Consensus cit si American Thyroid Society, fac recomandari pe baza studiilor cu metode neperformante, deci se impune revizuirea performantelor clinice si modificarea algoritmului de management al pacientilor cu DTC.

MOLECULAR INTERFERENCES IN THYROGLOBULINE MEASURMENT, A RISC FOR CLINICAL GUIDELINES IN ENDOCRINOLOGY

Thyroglobulin (Tg), is a glycoprotein synthesized in normal or malignant thyroid follicular cells. Measurements of serum Tg provide important information about the presence or absence of residual, recurrent, or metastatic disease, making of it a sensitive marker for post surgical follow up of differentiated thyroid cancer (DTC) patients. For decades, Tg measurements have relied on immunoassay methods that has poor analytical sensitivity Sa (1–2 ng/ml) and poor precision or are subject to molecular interference with Tg autoantibodies (TgAb), heterophilic antibodies, Tg isoforms, with impact on result. Alternative method is mass spectrometry, still not standardized and with similar Sa. From our experience, serum Tg levels are usually well correlated with the results of 131I uptake, but changes in immunoassay method can disrupt the serial monitoring of DTC patients. Molecular interferences of TgAb on Tg interpretation were significant (12 %). The new generation for Tg assay has been developed following further technologies and initial reports suggest that their sensitivity < 0.1 ng/ml is sufficient to obviate the need for TSH stimulation in patients with DTC and undetectable Tg during L-T4 replacement therapy. This has considerable implications for clinical practice, but is not yet included in clinical guidelines. Most current clinical guidelines (European Consensus and American Thyroid Society) base their recommendations on studies performed using methods with a poor functional sensitivity. The improvements of it is important for clinicians to review the clinical performance of the assays for their population and to formulate a new DTC management algorithm.

CO-33

MIR-29A INFLUENCES HIV-1 INFECTION PROGRESSION THROUGH MULTIPLE MECHANISMS

Adelina Rosca[1], Luminita Ene[2], Gabriela Anton[3], Iulia Iancu[3], Claudia Dita[3], Carmen Diaconu[3], Aura Temereanca[1,3], Cristian Achim[4], Simona Ruta[1,3]
[1]*Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
[2]*Dr Victor Babes Hospital for Infectious and Tropical Diseases, Bucharest, Romania*
[3]*Stefan S. Nicolau Institute of Virology, Bucharest, Romania*
[4]*University of California San Diego, La Jolla, USA.*

Introduction: Chronic HIV infection is characterized by an early onset and increased frequency of comorbidities, largely related to chronic inflammation and immune activation, despite successful antiretroviral treatment. There is increasing evidence that microRNAs (miRNAs) play important roles in inflammation, apoptosis and cell differentiation processes. We aimed to explore the relationship between cellular miRNA expression and markers of inflammation in a group of antiretroviral experienced patients with chronic HIV-1 infection. We focused on mir-29a, a cellular miRNA that can target the nef viral protein.
Methods: Plasma levels of inflammation biomarkers (IFNγ, IL-1β, IL-6, IL-8, IP-10, monocyte chemoattractant protein-1 (MCP-1), fraktaline and TNFα) were measured using multiplex assays on the MesoScale Discovery (Meso Scale Diagnostics, LLC. Gaithersburg, MD) platform. Expression levels of miR-29a were measured by quantitative real-time PCR (Life Technologies - TaqMan® MicroRNA Assays) and levels found in HIV patients were normalized against those in an age- matched healthy subjects.
Results: A total of 90 patients (median age 24 years, 48.9% males) were included in the study. The median time from HIV diagnosis was 14.6 years and the median time on combination antiretroviral therapy 11.2 years. The median CD4 value was 464 cells/mmc, 20% of the patients had severe immunosuppression. The median viral load was 2.78 log10 copies/mL, only 33.3% had undetectable HIV RNA in plasma. Significant diferences in IL-8 (p=0.005), IP10 (p=0.002) and MCP1 (p=0.001) plasma levels were found in immunocompromised versus immunocompetent patients. Patients with treatment failure (HIV viral load >50 copies/ml) had significant higher IP10 (p=0.005) and MCP1 (p=0.01) plasma levels. MiR-29a expression correlated both with CD4 level (rho=0.23, p=0.02) and with HIV viral load (rho= -0.41, p=0.001). Significant lower miR-29a expression was present in patients with virologic failure (HIV VL>50 copies/mL) compared with those with viral suppression (undetectable HIV VL) - 0.62 fold change vs 1.39 fold change, p=0.02. In the subgroup of patients with lower levels of miR-29a, we found a significant inverse correlations between miR-29a expression and plasma levels of IFNγ (rho= -0.54, p=0.009), IL-1β (rho= -0.51, p=0.01), IL=6 (rho= -0.46, p=0.02), IL-8 (rho= -0.46, p=0.03) and TNF-alpha (rho= -0.49, p=0.02).
Conclusion: MiRNA expression is a potential marker for the progression of HIV infection, influencing both HIV-1 replication and chronic immune activation through inflammation mediators.

CO-34

APLICAȚII ALE NANOMATERIALELOR ÎN DETECȚIA ELECTROCHIMICĂ PE PROBE BIOMEDICALE ȘI DE MEDIU

Robert Săndulescu, Cecilia Cristea, Mihaela Tertiş, Andreea Cernat, Adela Maghear, Bogdan Feier, Anca Florea, Luminița Fritea
Chimie analitică, Facultatea de Farmacie, Universitatea de Medicină și Farmacie Iuliu Hațieganu, Cluj-Napoca

Nanomaterialele pe bază de carbon, cel mai frecvent utilizate, pentru elaborarea biosenzorilor sunt nanotuburile de carbon, nanofibrele de carbon și grafenele. Conductibilitatea electrică și flexibilitatea nanotuburilor de carbon le recomandă pentru dezvoltarea biosenzorilor enzimatici, deoarece facilitează reacțiile de transfer de electroni. Având proprietăți electrochimice similare, nanofibrele de carbon și grafenele pot fi de asemenea folosite la fabricarea biosenzorilor enzimatici. Diverse nanoparticule metalice, cum ar fi Au, Ag, Pd și Pt au fost folosite pentru a imobiliza biomolecule pentru construcția de biosenzori amperometrici pe bază de enzime redox

(elemente sensibile) și nanomatrici conductive (pentru imobilizarea enzimelor). Prin modificarea electrozilor cu argile, a fost realizată adsorbția proteinelor pe suprafața minerală și facilitat procesul de transfer de electroni între proteine și suprafața electrodului. Mai multe tipuri de polimeri conductori (polipirrol, polianilină) sau neconductori au fost utilizați pentru a obține suprafețe nanostructurate cu aplicații în domeniul biosenzorilor. Datorită avantajelor lor, particulele magnetice prezintă un interes crescut, ca suport în biodetecție. Biomolecule diverse ca, enzime, anticorpi sau oligonucleotide, imobilizate pe particule magnetice pot fi reținute în apropierea, sau chiar pe suprafața unui electrod cu ajutorul unui câmp magnetic. O celulă electrochimică în flux, folosind electrozi poroși de grafit, ca atare sau modificați a permis detecția Cu (II) și Zn (II), din suplimentele alimentare și apă de robinet. Vor fi prezentate tendințele viitoare în proiectarea și fabricarea senzorilor electrochimici și cercetările care vor avea un impact deosebit în acest domeniu, cum ar fi tehnicile de imobilizare, nanotehnologia, miniaturizarea și analizele multiple în matrici complexe.

APPLICATIONS OF NANOMATERIALS IN BIOMEDICAL AND ENVIRONMENTAL ELECTROCHEMICAL SENSING

The most frequently used carbon based nanosized materials for biosensor construction are carbon nanotubes, carbon nanofibers and graphenes. The electrical conductivity and flexibility of the carbon nanotubes recommend them for the development of enzyme biosensors, because it facilitates electron transfer reactions. Having similar electrochemical properties, carbon nanofibers and graphenes can be also used in the fabrication of enzyme biosensors. Various metal nanoparticles such as Au, Ag, Pd and Pt have been employed to immobilize biomolecules for the construction of amperometric biosensors based on redox enzymes (as sensing elements) and nanoparticle arrays (as conductive matrices where enzyme molecules are implanted). By modifying the electrode surface with clays, adsorption of proteins on clay mineral surfaces and the heterogeneous electron transfer process between the protein and the electrode surface was facilitated. Several types of conductive (polypyrrrole, polyaniline) or insulating polymers were used to obtain a nano/micropatterned surface with applications in the biosensors field. Because of their advantages, magnetic particles have been increasingly used as biomaterial support in biosensing, enzymes, antibodies or oligonucleotides immobilized onto magnetic particles can be advantageously trapped by magnets and retained close to, or onto an electrode surface. A special flow electrochemical cell, using the unmodified or modified graphite felt as working electrode was designed, Cu(II) and Zn(II), being detected in food supplements and tap water. The future trends in sensor research activities and areas of development that are expected to have an impact in biosensor performances, like immobilization techniques, nanotechnology, miniaturization, and multisensor array determinations will be presented.

CO-35

CANCERUL TIROIDIAN DIFERENTIAT: INTRE GENETICA SI EPIGENETICA

Sorina Schipor[1], Suzana Vladoiu[1], Andra Caragheorgheopol[1], Dana Manda[1], Sabina Oros[1,2], Andrei Goldstein[1], Dumitru Ioachim[1], Cosmin Giulea[2], Corin Badiu[1,2]
1 National Institute of Endocrinology and 2."C. Davila" Unversity of Medicine and Pharmacy

Thyroid carcinoma is the most common endocrine malignancy and represents ~1% of all types of human cancer. **Objective:** As the molecular pathogenesis of thyroid cancer still remains to be clarified, the goal of our study was to find new molecular markers that could improve the diagnostics, follow-up protocols, treatment outcome, prognosis and the quality of life of differentiated thyroid cancer patients. BRAF V600E mutation occurs in 28 to 83% of papillary thyroid cancer, being associated with increased tumor aggressivity. RET gene rearrangements are often involved in PTC occurrence. RNA microarray was used for comparative analysis of normal and tumor tissue from each case.
Subjects and methods: Matched tumor and normal thyroid tissue samples were obtained from patients (n=125)

who were enrolled for surgery after they gave their informed consent: 57 patients with papillary thyroid carcinoma (PTC), 41 patients with follicular adenoma, 21 patients with hyperplastic thyroid nodule and 6 patients with autoimmune thyroiditis. DNA was isolated from thyroid tissue using High Pure DNA Template (Roche). BRAF V600E mutation was determined by PCR-RFLP using TspRI as restriction enzyme and confirmed by sequencing on Beckman Coulter CEQ8000 genetic analyser. RNA was extracted using the RNeasy Mini Kit from Qiagen and the quality was checked with the Infinite® 200 NanoQuant (Tecan) and with the 2100 Bioanalyzer (Agilent). 24 samples with RIN>7 were chosen for microarray gene expression analysis (6 with classical papillary thyroid carcinoma and 6 with papillary thyroid carcinoma folicullar variant). Microarray analysis was performed following Agilent One-Color Microarray-Based Gene Expression protocol, ver 6.6, using SurePrint G3 Human Gene Expression arrays 8x60K v2.

Results: Patients with PTC were divided into following histological subtypes: Classical PTC – 21 patients, PTC „follicular variant" – 29 patients, aggressive types – 7 patients. BRAF V600E analysis was done in all enrolled patients. We didn't find this mutation in patients with follicular adenoma, hyperplastic thyroid nodule or thyroiditis. In PTC group we found 8/57 mutations (14.04%): 6/21 (28.57%) in classical PTC and 2/7 (28.57%) in the histological aggressive forms. There were no mutations in PTC follicular variant. 6 patients carrying BRAF V 600E mutation were T3 (75%) and 2 were T4 (25%). In one case we identified RET/PTC rearrangements in 92% of the analyzed nuclei. Using GeneSpring ver 12, we identified 25 genes and 2 lincRNA (long intergenic non-protein coding RNA 1140 and BROAD Institute lincRNA (XLOC_005062)/lincRNA [TCONS_00010536]) down regulated in the tumour tissue compared with the normal one, p value<0.05 and fold change ≥ 2. When accounting for the two thyroid cancer types studied, we identified 3 genes up-regulated (COL13A1, EDA2R, KLHDC8A) and 8 down regulated (SLITRK5, CCL21, TFPI, TBX1, LOC389033, ADH1C, MMRN1, F10) in both subtypes. The level of gene expression dis-regulation is much higher in the case of classical papillary thyroid carcinoma.

Conclusions: Gene expression is altered in papillary thyroid carcinoma. Beside BRAF status analysis, RET/PTC rearrangements identification is a complementary method aiming to individualize the therapy in aggressive forms of PTC. Our study identified by RNA microarray, 3 hyper-expressed genes and 8 genes with low expression in tumour tissue compared to normal one. Further studies are undergoing for gene expression data validation by qPCR. This study was funded by UEFISCDI grant PN-II- PT-PCCA-2011-3.2 no.135/2012.

CO-36

RISCUL REACTIVARII VIRUSULUI HEPATITEI B SI C IN ERA TERAPIILOR BIOLOGICE

Coman Tanasescu
UMF Carol Davila Clinica Medicala Colentina

Medicatia biologica reprezinta o adevarata revolutie in domeniul tratamentelor imunomodulatorii. Ea foloseste anticorpi monoclonali indreptati impotriva unor molecule implicate in raspunsul inflamator cronic si in procesele autoimune. Riscul major al acestei terapii il reprezinta reactivarea unor infectii. Printre acestea se afla infectia cronica cu virusul hepatitic B sau C. Deoarece mecanismele patogenice ale bolii hepatice produse de cele doua virusuri sunt diferite, riscul de reactivare al acestora nu este identic. In timp ce riscul reactivarii virusului B este mai crescut, riscul reactivarii virusului C este determinat mai ales de pleiotropismul citokinic si este influientat de asa-numitul „paradox al TNF-alfa". Riscul reactivarii virusului C este mai redus decat al reactivarii virusului B, iar terapia anti-TNFalfa ar putea uneori sa aiba chiar un rol benefic asupra afectarii hepatice concomitente. Este prezentata experienta personala referitoare la acest risc precum si opiniile din literatura.

RISK OF HEPATITIS B AND C VIRUS REACTIVATION IN THE AGE OF BIOLOGIC THERAPY

Biologicals revolutionized the immunomodulatory treatment of chronic viral hepatitis. Monoclonal antibodies are directed towards molecules involved in chronic inflammation and autoimmune loops. The major risk related to biological treatments is reactivationof infection. B and C hepatitis viruses are amongst the viruses that can reactivate during those treatments. Disease mechanisms and reactivation risk differ between B (HBV) and C viral

hepatitis (HCV). Whilst risk of reactivation is higher for HBV, viral reactivation in HCV is correlated with pleiotropic cytokine effects and depending on the so-called 'TNF-alpha paradox'. Risk of HCV reactivation is smaller than of HBV. Anti-TNF therapy may have a beneficial effect in case of concomitant HCV infection. Personal experience and current state of knowledge on reactivation of HBV and HCV infections related to biologic therapy will be presented.

CO-37

PERSONALIZED/PRECISION MEDICINE – TRANSDISCIPLINARY AND TRANSLATIONAL APPROACH; OMICS TECHNOLOGY

Cristiana Tanase[1,2], Cristian Stan[1], Radu Albulescu[1,2,3]
[1]*"Titu Maiorescu" University, Faculty of Medicine, Bucharest, Romania*
[2]*"Victor Babes" National Institute of Pathology,Proteomics, Bucharest, Romania*
[3]*National Institute for Chemical Pharmaceutical Research and Development, Bucharest, Romania*

Personalized/precision medicine will focus on an individual approach and its nature will be proactive. Personalized medicine promotes the concept of the right therapy for the right group of patients, at the right moment, with the right cost. Systems biology and systems medicine has led to a "P4 medicine" that is predictive, preventive, personalized and participatory. The future of medicine relies on such personalization. Personalized medicine is designed medicine based on "Omics" contribution. The need to apply molecular screening in order to improve the diagnosis is crucial in most of the pathologies. The incorporation of proteomics in the further development of the personalized medicine concept is a more recent phenomenon and it has given rise to a complete image of the health/disease status of an individual, especially at functional level. In order to identify new circulating biomarkers, high throughput proteomic technologies, such as mass spectrometry (SELDI-ToF, MALDI-ToF), 2D-DIGE, multiplexed and protein microarray are being used. Proteomics can generate new and useful information by identifying and establishing protein-protein interactions at intra- and intercellular level. The most recent tendency in personalized/precision medicine approach relies on the "P4/P5 medicine", which constitutes a health concept focused on each individual patient.
Acknowledgment: Partially supported by project PN 09.33-03.10 and 4SEE/2014.

CO-38

REBALANCINGCYTOKINE LEVELS WITH THE USE OF HAEMOADSORPTION DEVICE IN SEVERE ACUTE RESPIRATORY DISTRESS SYNDROME AFTER LIVER TRANSPLATATION. A CASE REPORT

Dana Tomescu[1,2], Mihai Popescu[2], Simona Dima[3], Irinel Popescu[2,3]
[1]*Clinica ATI 3 Institutul Clinic Fundeni, Bucuresti, Romania*
[2]*UMF 'Carol Davila' Bucuresti, Romania*
[3]*Centrul de Chirurgie Digestiva si Transplant Hepatic "Dan Setlacec", Bucuresti, Romania*

Introduction: Severe acute respiratory distress syndrome (ARDS) represents a life-threatening condition especially in the immuno-compromised patients such as liver transplant (LT) recipients.
Methods: We report the case of a 53 years old LT recipient with early severe postoperative ARDS (postoperative day 3) due to infectious pneumonia. A haemoadsorption device (CytoSorb) was applied in combination with standard continuous veno-venous haemofiltration in three consecutive sessions. Cytokine levels, C reactive protein (CRP) and procalcitonine (PCT) levels were measured before and after each session. Clinical response, haemodynamic parameters and radiological findings were also noted.
Results: The use of CytoSorb was associated with a decrease in inflammatory cytokines, especially interleukine (IL) 6 from 664.4 to 123.2 pg/mL and tumor necrosis factor (TNF)-alpha from 87.0 to 20.4 pg/ml, while anti-inflammatory cytokines IL-4 and IL-13 remained constant: 13.2 pg/mL and 18.4 pg/mL respectively. A sub-

sequent decrease in inflammatory markers was also noted: CRP from 190 to 101 mg/L and PCT from 11.1 to 5.27 ng/mL. These findings correlated with regression of bilateral pulmonary infiltrates, a rise in arterial oxygen pressure and improvement of haemodynamics (a decrease in vasopressor support from a maximum of 4.2mg/h to discontinuation after the third CytoSorb session).
Conclusion: The use of CytoSorb shifted the inflammatory response from a severe pro-inflammatory response to a compensated anti-inflammatory response that translated into remission of severe ARDS and a stable haemodynamic profile.

CO-39

RARE CASES OF BIRTH DEFECTS IN HIV VERTICALLY EXPOSEDCHILDREN

Ana Maria Tudor[1,2], Mariana Mărdărescu[2], Ioana Alina Anca[1,3], Cristina Petre[2], Cosmina Cristea[1], Ruxandra Neagu Drăchicenoiu[2], Rodica Ungurianu[2]
[1]*„Carol Davila" University for Medicine and Pharmacy, Bucharest*
[2]*„Matei Bals" National Institute for Infectious Diseases, Bucharest*
[3]*„ Alfred Rusescu" Institutefor Mother and Child Protection, Bucharest*

Objective: to present rare types ofbirth defects in HIV vertically exposed children followed up inPediatric Department of Matei Bals" National Institute for Infectious Diseases, Bucharest.
Methods: we analyzed the data recorded for HIV perinatal exposed children followed up in our hospital from January 1st 2006 to December 31st 2012.and compile the information regarding birth defects.
Results: From 262 children followed up in studied period, 44 cases were diagnosed with vertical HIV infection, 25 children did not complete the 18 month virologic assessment to sustain the non HIV infected status. More than 37% of studied had at least one congenital condition. We have noticed that 63%of birth defects involved heart, 23% involvedmusculoskeletalsystem, the kidney was involved in 10% cases, neurologic defects were present in 10% children, the digestive tract was involved in 5% cases. We identifymetabolic and storage disordersand genetic disorders in 2% cases. 9% from studied children with birth defects had more than one organ involvement. Among studied patients we identify four cases of very rare congenital diseases: one case of chromosomal hermaphroditism in the offspring of parents treated for more than 15 years with antiretrovirals since childhood (parents are part of Romanian Pediatric Cohort), one case of gangliosidosisin a girl born by untreated iv drug user women, one case of Niemann Pick syndrome (less than 1/100000live births) in a girl born by a treatedwomen and one case of Dandy Walker syndrome (cerebellar hypoplasia) in a boy born from an former pediatric patient. This type of rare birth defects are estimated to be present in 1/30 000 - 1/1 million persons in Europe.
Conclusion: the rate of malformations is relative high among HIV exposed children followed up in our hospitaland some of the cases are severe or very rare. This aspect raises the problem of special care needsto be offered to HIV pregnant women and their offsprings.

CO-40

"IMPORTANȚA BIOMARKERILOR ÎN DIAGNOSTICUL BOLII ALZHEIMER"

Catalina Tudose[1], Cristina Popescu[2], Magdalena Budisteanu[3], Ioan Codorean[4], Roxana Dinu[5], Ana Giurgiuca[1]
[1]*Disciplina Psihiatrie, UMF "Carol Davila", București, Romania*
[2]*Spitalul Clinic de Psihiatrie "Prof. Dr. Alex. Obregia" , București, Romania*
[3]*Neurologie Pediatrica, Spitalul Clinic de Psihiatrie "Prof. Dr. Alex. Obregia", București, Romania*
[4]*Disciplina Imagistica și Medicina Nucleara, București, Romania*
[5]*Disciplina Psihologie, Univ. "Spiru Haret", București, Romania*

Boala Alzheimer este cea mai frecventă tulburare neurodegenerativă, afectând îndeosebi populația peste 65 de

ani și conducând la un declin progresiv cognitiv și funcțional. Depistarea bolii Alzheimer, în cele mai precoce stadii posibile, a devenit principaluldeziderat pentru cercetători și clinicieni. Astfel ar fi posibilă intervenția asupra procesului etiopatogenic, demonstrându-se deja faptul că modificările neuropatologice se instalează cu multe decade înaintea apariției simptomatologiei clinice. S-au făcut eforturi considerabile pentru identificarea de biomarkeri care să susțină diagnosticul, să permită evaluarea severității bolii și să aibă valoare prognostică, prezicând conversia de la tulburări dismnezice discrete spre demență. Noile ghiduri de diagnostic (2011) elaborate de Institutul Național de Studiu al Îmbătrânirii și Asociația Alzheimer au integrat biomarkerii în algoritmul diagnostic de cercetare, ceea ce a reprezentat un important pas în stabilirea diagnosticuluiprecoce și al unor posibile strategii privind prevenția bolii Alzheimer. Biomarkerii actualmente recunoscuți includ în mod obișnuit măsurarea proteinei Tau și amiloidului beta în LCR, evidențierea atrofiei cerebrale folosind tehnici neuroimagistice (MRI și PET) și a tulburărilor memoriei episodice (examinare neuropsihologică). Perspectiva neuropatologilor a modificat însă intr-o maniera importantăconceptia despre boala Alzheimer, posibilitățile diagnostice și semnificația biomarkerilor. Numeroase studii de autopsie au demonstrat că mai putin de jumătate din cazurile diagnosticate cu boala Alzheimer reprezinta forma pura de boala, majoritate prezentând leziuni cerebrale mixte, plăci de amiloid, degenerescență neuro-fibrilară asociate cu numeroase alte tipuri de leziuni (vasculare, depozite de proteine toxice cum ar fi alfa–synucleina si TDP-43 etc). Reuniunea AARR (Alzheimers Associations Research Roundtable) (oct 2014) a reconsiderat calitatea corelatiilor dintre leziunile neuropatologice si aspectele clinice dovedind faptul ca biomarkerii demonstreaza prezenta leziunilor cerebrale dar nu sunt patognomonice pentru boala Alzheimer. Corespondenta dintre prezența, amploarea, tipul leziunilor cerebrale cu tabloul clinic rămâne destul de slaba. Prezentarea analizează rolul al biomarkerilor recunoscuți pentru diagnosticul, evaluarea progresiei si severității bolii Alzheimer, luând în discuție dezbaterile actuale privitoarea la limtele corelațiilor dintre aspectele clinice si modificările valorilor acestora. Sunt de asemnena prezentate rezultatele activității de aproximativ 15 ani a Centrului Memoriei din București, servici specializat în diagnosticarea precoce a tulburărilor cognitive, care a reușit realizarea unei baze de date care cuprinde peste 7000 de cazuri de tulburări neurocognitive. Adresabilitatea la Centrul Memorie este extrem de ridicată, iar structura populației care solicită consultul demonstrează o pondere importantă a cazurilor de tulburări neurocognitive minore (MCI si SCC). Metodele de diagnostic și investigație s-au limitat la aspectele clinice psihiatrice, neurologice și neuropsihologice coroborate cu datele explorărilor structurale neuroimagistice. Sperăm ca realizarea proiectului in curs de desfășurare „Centru de cercetare translațională în psihiatrie și neuroștiințe” să ofere cercetătorilor români, prin laboratoarele care se vor crea, posibilitatea abordării integrate și multidisciplinare a patologiei neurodegenerative, promovând includerea României în comunitatea științifică internațională din domeniu.

CO-41

SPECTRULFUNCȚIONAL CEREBRAL AL OXITOCINEI.

Leon Zăgrean, Ana-Maria Zăgrean, Anca Panaitescu, Ioana F. Grigoraș, Gheorghe Peltecu

Oxitocina, unnonapetid ce a precedat apariția filogenetica asistemului nervos, are o paletă complexa de acțiuni legate de controlul mecanismelor fundamentale de menținere a vieții începând cu organismele apărute cu peste 700 milioane de ani în urma. Dacă studiile clasicede fiziologie au identificat acțiunile oxitocinei în susținerea funcției de reproducere (naștere, alăptare), studiile din ultimele două decenii au extins implicațiile oxitocinei la mecanismele complexe ale comportamen-tului uman în condiții fiziologice si patologice. Studiul prezintă integareare zultatelor personale în literatura stiințifică actuala.

P-01

CLINICOPATHOLOGICALSTUDY OF KRAS-MUTATED ANDWILD-TYPE COLORECTAL CANCERS

Mariana Aşchie[1,2], Georgeta Cozaru[2], Anca Mitroi[2], Gabriela Bălțătescu[2], Ionuț Poinăreanu[1,2]
[1]*Faculty of Medicine, „Ovidius" University, Constanta, Romania*
[2]*„Sf. Apostol Andrei" County Emergency Clinical Hospital, Constanta, Romania*

KRAS mutation are frequently found in colorectal cancer(CRCs) (between 30% to 50%), although the precise molecular and cellular mechanisms that constitute the oncogenic effects of activating KRAS mutations remain incompletely understood. Until now, there are still important gaps in our understanding of CRC carcinogenesis. So, we have proposed to compared cases of CRCs with mutated KRAS and wild-type KRAS and to identify any possible clinico-histologic features associated with mutated KRAS in CRCs. In this study, weanalyzed the profile of KRAS mutations on 17 CRCs using formalin-fixed, paraffin-embedded tissue by polymerase chain reaction (PCR) and cycle sequencing of the amplified PCR products. We performed a detailed analysis of the frequency of each mutation within codons 12 and 13. Clinicopathologic parameters were obtained, and a detailed histomorphologic analysis was performed. About one third of patients with colorectal cancer had KRAS gene mutation. Our study showed that the KRAS gene mutation is not associated with age, sex, tumor location, depth of tumor invasion, lymph node metastasis, distant metastasis, tumor grade, tumor size, or angiolymphatic invasion.
Key-words: KRAS gene, colorectal cancer, paraffin-embedded tissue

---

P-02

MOLECULAR CHARACTERIZATION OF SCCMEC AMONG METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS CLINICAL STRAINS ISOLATED FROM DIFERENT SPECIMENS AND INFECTIONS OCCURRED IN PATIENTS WITH CARDIOVASCULAR SURGERY

Otilia Banu[1], Bogdan Ionescu[2], Diana Ionescu[2], Irina Gheorghe[2], Ani Ioana Cotar[3], Grigore Mihaescu[2], Mariana Carmen Chifiriuc[2], Coralia Bleotu[4]
[1]*Emergency Institute of Cardiovascular Diseases "Prof. Dr. C.C. Iliescu", Bucharest, Romania*
[2]*Faculty of Biology, University of Bucharest, Research Institute of the University of Bucharest, Romania*
[3]*INCDMI Cantacuzino, Bucharest, Romania*
[4]*Institute of Virology Stefan S. Nicolau, Bucharest, Romania*

Methicillin-resistant Staphylococcus aureus (MRSA) is well established as a major pathogen associated with nosocomial infections. Some of these strains present the mecAgene, located in a staphylococcal chromosome cassette (SCCmec). The most frequent SCCmec types are I, II and III, which are hospital-acquired, and IV, which is community-acquired. Their remarkable ability to acquire antimicrobial resistance limits therapeutic options and may contribute to patient morbidity and mortality. The aims of this study were to evaluate the antibiotic susceptibility and to characterize at molecular level the methicillin resistant Staphylococcus aureus (MRSA) clinical isolates.
Materials and methods: The study was performed on a group of 144 S. aureus clinical isolates collected from various sources: venous blood, peritoneal fluid, ocular secretion, wound, nasal secretion in hospitalized patients from Emergency Institute of Cardiovascular Diseases "Prof. Dr. C.C. Iliescu, between 2011 and 2013. The identification of Staphylococcus strains was done with conventional tests, API STAPH tests and Vitek 2 Compact Sistem. Minimal inhibitory concentration (MIC) of oxacillin, penicillin, vancomycin, teicoplanin, gentamicin, rifampicin, trimethoprim-sulfametoxazole, tetracycline, and ciprofloxacin was established by using Vitec2 Compact Sistem. Susceptibility to erythromycin and clindamycin and evaluation of MLSB resistance mechanism

was done by disc diffusion method and Vitek2 Compact Sistem. Methicillin resistance was detected by cefoxitin disc diffusion test, Vitek 2 Compact Sistem and the presence of mecA gene was confirmed by PCR. All MRSA strains were characterized by SCCmec typing using PCR based methods.

Results: Among all examined Staphylococcussp. isolates, 34 strains (23,6%) were MRSA and 110 (76,4%) exhibited macrolide-lincosamide-streptogramin B (MLS(B)) phenotypes (MRSA/MLSB). All examined strains were susceptible to vancomycin, teicoplanin and trimethoprim-sulfamethoxazole. There was no significant difference of antibiotic MIC values observed between MRSA and MRSA/MLSB strains. Approximately 61.8% of MRSA were susceptible to most of the examined antibiotics. However, 35.3% of MRSA were resistant to gentamicin, 38.2% to tetracycline, and 23.5% to rifampicin. The resistance profile of MRSA/MLSB was extended to almost all antibiotic classes, excepting glicopeptides (vancomycin and teicoplanin) and trimethoprim-sulfametoxazole: rifampin 8.9%, ciprofloxacin 23.4%, mupirocin 24.3, gentamicin 68.5% and tetracycline 97.3%. The ccrB2 genetic element was associated with mec A and Type IVa. SCCmecVJ1 was never associated with SCCmecIIIJ1 in both MRSA and MRSA/MLSB strains. Also ccrC was observed in gentamicin resistant strains. However, a significant number of the MRSA strains from the both groups were nontypeable.

Conclusion: The study enabled to evaluate the baseline susceptibility to antibiotics of S. aureus MRSA and MRSA /MLSB strains isolated from various sources in 2011 - 2013. A high prevalence of strains with SCCmec type IV and V was observed in the analysed clinical isolates.

Acknowledgments: Dr. Otilia Banu was supported through the project entitled "CERO - Career profile: Romanian Researcher", grant number POSDRU/159/1.5/S/135760, cofinanced by the European Social Fund for Sectoral Operational Programme Human Resources Development 2007–2013.

P-03

PROTEOMIC PROFILES OF THE INTRACYSTIC PAPILLARY CARCINOMA OF THE BREAST

Gabriela Izabela Bălțătescu[1], Mariana Aşchie[1,2], Georgiana Cozaru[1], Manuela Enciu[1,2], Ionut Poinăreanu[1,2], Oana Cojocaru[1], Anca Chisoi[1], Sînziana Ghițoi[1]

[1]*Clinical Service of Pathology, Emergency County Hospital, Constanta, Romania*

[2]*Department of Pathology, Faculty of Medicine, "Ovidius" University, Constanta, Romania*

Introduction: Intracystic papillary carcinoma (IPC) is a rare subtype of breast carcinoma. It is considered by some authors as an intermediate stage between intraductal breast carcinoma and invasive breast carcinoma. Other authors consider this entity as a low-grade invasive breast carcinoma with a compressive pattern of invasion and with potential metastasis to the regional lymph nodes.

Material and Method: We reported two cases of IPC and representative samples were submitted for histo-pathological, immunohistochemical and chromogenic in situ hybridisation examination (CISH). A pannel of eight biomarkers (ER, PR, HER2neu, Ki67, p53, CD10, SMA, colagen IV) was used to evaluate the imunohistochemical profile and in order to detect human HER2 gene by CISH was used ZytoDot SPEC HER2 Probe Kit from Zytovision.

Results: Immunohistochemical examination revealed a high expression of hormonal receptors associated with overexpression of HER2neu oncoprotein. Amplification of HER2/neu gene was proved by chromogenic in situ hybridisation for the case with an equivocal immunohistochemical expression. The evaluation of nuclear proliferation using Ki-67 index showed a high expression and p53 oncoprotein assessment proved to be positive. The presence of myoepithelial cells or the basement membrane have not been highlighted by specific biomarkers (SMA, CD10, colagen IV) at the periphery of the lesions.

Conlusions: Histopathological, immunohistochemical and molecular profile of intracystic papillary carcinoma was consistent with luminal B-HER2pozitive molecular subtype. In its pure form these lesions are an indolent biological behavior and should not be treated with aggressive methods.

P-04

EXPRESIA PROTEINELOR S100 IN CANCERUL PANCREATIC EVIDENTIATA PRIN NANO-LC-MS/MS

Raluca M Boteanu[1], Luminita Ivan[1], Elena Uyy[1], Viorel I Suica[1], Florentina Safciuc[1], Nicolae Bacalbasa[2], Valeria Tica[3], Simona Dima[3], Irinel Popescu[3], Felicia Antohe[1]
[1]*Institutul de biologie si patologie celulara "N. Simionescu"Academia Româna, Bucuresti, România*
[2]*UMF Carol Davila, Bucuresti, România*
[3]*Centrul de Chirurgie Generala si Transplant Hepatic "Dan Setlacec" Institutul Clinic Fundeni, Bucuresti, România*

Introducere: Proteinele S100 fac parte din familia alarminelor endogene, care semnalizeaza prezenta unui stress tisular, inclusiv in agresiunea asociata cancerului. Scopul acestui studiu a fost de a identifica un potential grup de biomarkeri apartinand familiei de proteine S100, exprimate in cancerul pancreatic, prin analiza de spectrometrie de masa.
Material si Metoda: Probele de tesut uman recoltatein mediu steril in timpul operatiei de la pacienti (n=16) cu adenocarcinom pancreatic ductal au provenit atat din zona tumorala (T)cat si din zona adiacenta tumorii (NT), folosite drept control.Probele au fost procesate corespunzator si cantitati echivalente de proteina provenite din omogenatele totale tisulare au fost separate si analizate prin cromatografie in faza lichida cuplata cu spectrometria de masa (LC-MS/MS), in vedereaidentificarii si cuantificarii proteinelor de interes.
Rezultate: Utilizarea programului informatic Proteome Discoverer 1.4 a condus la identificarea a treisprezece membri ai familiei S100 (A2, A4, A6, A8, A9, A10, A11, A12, A13, 14, A16, P si B) in probele investigate. Analiza bioinformatica efectuata cu programul SIEVE 2.1,care permite cuantificarea relativa a acestor proteine, a aratat ca proteinele S100-P si A11 isi modifica expresia proteicain toate probele. Astfel, in 87,5 % din cazuri S100-P creste in medie de 3,5 ori in tesutul tumoral, iar in 75%din cazuri expresia proteinei A11 creste in medie de 4 oriin T vs NT. Tot de 4 ori au crescut si proteinele S100A6 si A9 in 62,5% din cazurile T comparativ cu cele NT. De asemenea s-a observat ca proteina A10 a crescut de 2,2 ori in 68,75% din probele tumorale, iar nivelul proteinei A8 a fost de 3,5 ori mai mare in tesutul tumoral comparativ cu cel adiacent tumorii in 75% din cazuri.
Concluzii: Acest studiu reprezinta primul screening al proteinelor S100 realizat la nivelul tesutului pancreatic al pacientilor cu adenocarcinomductal. Rezultatele obtinute indica proteinele S100-P, A4, A6, A8, A9, A10 si A11 ca potentilai biomarkeri in diagnosticarea cancerului pancreatic.
Studiul a fost finantat de Academia Romana si Ministerul Educatie si Cercetarii proiect UEFISCDIPN II PCCA 2011-3, Nr. 90/2012.

P-05

ANTIGENGLYCOSYLATION AND CYTOTOXIC T CELLS ACTIVATION: CASE STUDY OF TYROSINASE MUTANTS EXPRESSING HLA-A2 RESTRICTED YMD EPITOPE

Gabriela N. Chiritoiu[1], Cristian V.A. Munteanu[1], Camilla Jandus[2], Simona Ghenea[1], Pedro Romero[2], Stefana M. Petrescu[1]
[1]*Institute of Biochemistry, Romanian Academy*
[2]*Ludwig Cancer Research Center, Faculty of Biology and Medicine, University of Lausanne*

Malignant melanoma is one of the most aggressive skin cancers. [1] Immunotherapy has emerged as a promising method in melanoma treatment, mainly by the rejection of the cancer cells by cytotoxic T lymphocytes (CTLs), that specific recognizecell surface antigenic peptides associated with the major histocompatibility complex (MHCI). An important melanoma antigen is tyrosinase, a key-enzime in melanogenesis, normally found in melanocytes and up regulated in melanomas. Tyrosinase is a type I transmembrane protein, with 7 potential glycosylation sites that are critical in the proper folding of the protein. [2] The MHCI restrictedantigenic peptides,are generated throughproteasomal degradationof tyrosinasein endoplasmic reticulum associated degradation (ERAD). Using CT Lactivation assays and mass spectrometry we have investigated the role of

glycosylation and protein folding in the generation of a HLA-A02 antigenic peptide derived from tyrosinase (Tyr369-377): YMDGTMSQV (YMD). A 375 amelanoticmelanoma cells stable transfected with WT tyrosinase or glycosylation mutants were analyzed in terms of peptide generation efficiency, T cell recognition and degradation status. Degradation efficiency of these mutants was assessed using proteasome inhibitors and the immune response was estimated using CT Lactivation assays as specificlysis and interferon secretion. Quantification of the YMD peptide was performed using high-resolution mass spectrometry of cell surface acid-elution of the stable transfected melanoma cells. Our results reveal that triple glycosylation mutants can generate an increased amount of the Tyr369-377 epitope through an efficient ubiquitin-proteasome degradation of the polypeptide chain. This is confirmed by the IFN secretion assay that shows specificrecognition andcell lysis oftyrosinase glycosylation mutants by multiple CTL clones. High-resolution mass spectrometry analysis has revealed that the YMD antigenic peptide is subject to methionine oxidation. Even more, there is an increased amount of the peptide at the cell surface of melanoma cells stable transfected with glycosylation mutants compared with the WT protein. Our data suggest that protein glycosylation and folding is linked with epitope generation, presentation and T cell recognition efficiency in melanoma. Moreover, C-terminus glycosylation mutants of tyrosinase, generate an increased amount of the peptide at the cell surface, concomitant to it's folding state.[2] Here, we also show that the Tyr369-377 epitope is oxidized and this could possible impact the immunologic response.

Acknowledgements: This work received financial support through the projects entitled "CERO – Career profile: Romanian Researcher", grant number POSDRU/159/1.5/S/135760, cofinanced by the European Social Fund for Sectoral Operational Programme Human Resources Development 2007-2013 andSwiss Enlargement Contribution in the framework of the Romanian-Swiss Research Programme, grant no. IZERZO_142216/1.

References: 1. Gray-Schopfer V, Wellbrock C, Marais R (2007) Melanoma biology and new targeted therapy. Nature 445: 851-857. 2. Cioaca D, Ghenea S, Spiridon LN, Marin M, Petrescu AJ, et al. (2011) C-terminus glycans with critical functional role in the maturation of secretory glycoproteins. PLoS One 6: e19979.

P-06

MAJOR ROLE OF K-RAS GENE MOLECULAR ANALISYS IN COLORECTAL CARCINOMA ANTI-EGFR THERAPY

F. Cionca[1], G. Cardos[2]
[1]*The National Institute for Research and Development in the Field of Pathology and Biomedical Sciences "Victor Babeș", Bucharest, Romania*
[2]*Personal Genetics - Medical Genetic Centre, Bucharest, Romania*

Background: Gain-of-function K-ras point mutations, present in 30–50% of the metastatic colorectal carcinomas (CRC), maintain the active form of the ras p21 protein and lead to epidermal growth factor receptor (EGFR) independent activation of intracellular signaling pathways, making the anti-EGFR tumor therapy ineffective.
The aim of our study is to identify possible correlations between the mutational status of the K-ras gene and the histopathological findings in patients with CRC.
Methods: We studied 45 patients with CRC, 39 primary tumors and 6 metastases (2 liver metastases, 2 pulmonary, 1 ovarian and 1 cutaneous). The formalin-fixed paraffin-embedded tissue samples were analyzed using an indirect bistadial immunohistochemical (IHC) technique, performed with a Dako EnVision+ Dual Link System-HRP, with antibodies for the EGFR and the ras protein. Mutations in exon 2, codons 12 and 13 of the K-ras gene were detected by Polymerase Chain Reaction -Restriction Fragment Length Polymorphism (PCR-RFLP) analysis.
Results: K-ras mutations were present in 46, 66 % of the cases (21 cases), 19 primary tumors and 2 metastases (a liver and a pulmonary one). EGFR was positive in 10 cases in tumor cells and in 11 cases in the vessels, with 3 cases of double positivity. Immunohistochemical overexpression of ras protein was detected in 14 samples. The relationship between the positivity of the K-ras mutation and the positive immunohistochemical reaction for EGFR and the ras protein did not reach statistical significance.
Conclusions: There were no significant correlations between the mutational status of the K-ras gene and the IHC

reaction for EGFR and ras p21 protein, proving once more the major role of molecular analyses in colorectal carcinoma anti-EGFR therapy.

P-07

PROTEOMIC ANALYSIS OF LARYNGEAL CARCINOMAS

Oana Cojocaru[1], Mariana Aşchie[1,2], Ionut Poinăreanu[1,2], Anca Mitroi[1], Anca Chisoi[1], Alexandra Maiorean[1]
[1]*Clinical Service of Pathology, Emergency County Hospital, Constanta, Romania*
[2]*Department of Pathology, Faculty of Medicine, "Ovidius" University, Constanta, Romania*

Introduction: In Europe, laryngeal cancer ranks 20th in frequency of malignant tumors, with approximately 39,000 new cases diagnosed in 2012. The most common type of carcinoma of the upper airway in adults is laryngeal squamous cell carcinoma, which represents approximately 1.5% of all malignancies. P 53 tumor suppressor gene expression has been correlated with reduced survival period and the progression from dysplastic lesion to invasive carcinoma. The expression of the Ki 67 protein in the tumoral cells is considered a prognostic factor, including laryngeal malignant neoplasms.
Material and Method: Our study is a histopathological and immunohistochemical analysis (Ki 67 and p53) of the 55 cases of squamous cell carcinoma (44 men and 1 woman) diagnosed during 01.01.2011-31.12.2011 in the Clinical Service of Pathology, Emergency County Hospital "Sf. Apostol Andrei" Constanta, in order to determine the relationship between the expression of Ki 67 and p 53 and clinicopathological aspects. There were analyzed issues such as sex, age, location, tumor size, degree of differentiation, mitosis, tumor stage. Data were processed using χ2 test and statistically analyzed using SPSS software (Statistical Package for the Social Sciences). Data were considered statistically significant at p value <0, 05.
Results: The location of the tumor at the glottis in 49 cases (90%), supraglottic in 2 cases (4%) and the epiglottis in 3 (6%). In terms of the degree of differentiation, 27 carcinomas were well differentiated, 19 moderately differentiated and 8 poorly differentiated (without keratinization). There was a statistically significant correlation between the degree of differentiation and the number of mitosis (p <0.01). P53 expression was present in 35 cases (64.81%). Statistically significant correlation was observed between p53 expression and tumor location (p = 0.04) and tumor stage (p = 0.02). Ki67 proliferative index was <50% in 42 cases (78%) and >50% in 12 cases (22%). Statistically significant correlation was observed between tumoral Ki67 expression (p = 0.017) and the degree of differentiation and the rate of mitosis (p = 0.008).
Conclusions: The imunohistochemical expression of p53 and Ki 67 represent an important prognostic factors for patients with laryngeal squamous cell carcinoma. Even though it is considered a disease with poor prognosis, identification of prognostic and predictive factors can improve quality of life and may help „tailor" new therapies for each patient.

P-08

HER2/NEUAMPLIFICATION IN GASTRIC ADENOCARCINOMA

Georgeta Camelia Cozaru[1], Mariana Aschie[1,2], Anca Mitroi[1], Gabriela Bălțătescu[2], Ionuț Poinăreanu[1,2], Costel Brînzan[1], Sînziana Ghițoi[1]
[1]*„Sf. Apostol Andrei" County Emergency Clinical Hospital, Constanta, Romania*
[2]*Faculty of Medicine, „Ovidius" University Constanta, Romania*

Recent studies suggest that the HER2 oncogene was reported to be amplified and over expressed in gastric adenocarcinoma and can be used as a marker to identify aggressive disease. Using the standard slide-based techniques of immunohistochemistry (IHC) and Chromogenic in situ hybridization (CISH), the HER2 protein is over-expressed or the HER2 gene is amplified in approximately 7–34% of primary tumours. The current study included 28 gastric adenocarcinoma who underwent diagnostic tissue biopsy or surgical resection. The

HER2 status was tested by IHC in histological samples. The HER2 IHC results were confirmed by CISH on available histological sections. We noted also the clinicopathological parameters and a detailed histo-morphologic analysis was performed. The HER2 amplification was observed in 7 of the 28 (25%) gastric tumours. HER2 testing in gastric carcinoma is a new field, opening several opportunities: for patients with gastric cancer, this is a new promising therapeutic option; for pathologists, strengthening our role in therapy selection and emphasizing our duty of providing accurate and reproducible HER2 testing results

Key-words: HER2 gene, gastric cancer, overexpressed

P-09

INFLUENCE OF PROBIOTICS UPON THE EXPRESION OF VIRULENCE AND ANTIBIORESISTANCE FEATURES IN STRAINS ISOLATED FROM GASTROENTEROLOGICAL INFECTIONS

Cristina Delcaru[1], Paulina Podgoreanu[1], Ionela Alexandru[1], Violeta Corina Cristea[2], Raluca-Madalina Staiculescu[2], Mariana Carmen Chifiriuc[3], Veronica Lazar[3], Coralia Bleotu[4], Otilia Banu[5]

[1]*Private Iancului Laboratory, Bucharest, Romania*
[2]*Department of Microbiology Reference Laboratory Central Synevo, Bucharest, Romania*
[3]*Faculty of Biology, University of Bucharest, Romania*
[4]*Institute of Virusology Stefan S. Nicolau, Bucharest, Romania*
[5]*Emergency Institute of Cardiovascular Diseases "Pro. Dr. C.C. Iliescu, Bucharest, Romania*

Introduction: In the digestive apparatus, each cavity presents its own microbiota, which reaches its target niches during the perinatal period, and comprises complex and stable communities (homeostasis). Digestive resident microbiota is normally present only at the extremities of the digestive tract, especially in the terminal area, is very complex and includes aerobic and anaerobic bacteria, fungi, protozoa and viruses. The generally symbiotic relationships can sometimes turn from mutualistic to parasitic, causing opportunistic endogenous infections and being responsible of the generation of carcinogenic compounds. Currently, antibiotic treatment of gastrointestinal infections is designed to cover a broad spectrum and often polymicrobial etiology (ex. penems, third generation cephalosporins, being associated with disbiosis, which could be prevented or diminished by using probiotics.

Purpose: In vitro investigation of the probiotic potential of Bifidobacterium sp. recently isolated strains.

Materials and Methods: In this study there were used 17 strains of Bifidobacterium sp. and 27 enteropathogenic strains (EPEC, EIEC, Klebsiella sp., Salmonella sp., Yersinia enterocolitica, P. aeruginosa). Most of Bifidobacterium sp. strains were isolated from healthy patients stool specimens. Evaluation of the probiotic potential was performed by studying the influence of whole liquid cultures/ sterile supernatants on the expression of virulence factors and antibioresistance features of enteropathogenic strains after cultivation for 24 h. The evaluated parameters were: adherence to the cellular substrate using Cravioto modified method, the expression of soluble virulence factors using special media with specific incorporated substrate and antibioresistance profiles (CLSI, 2015).

Results: In terms of antibiotic resistance, one could observe a slight increase in the diameters of growth inhibition zones in the presence of various fractions of Bifidobacterium sp. cultures. Adhesion of pathogenic strains was significantly reduced in the presence of whole cultures and sterile supernatants of Bifidobacterium sp., demonstrating their ability to compete with pathogenic strains colonizing the intestinal epithelium.

Conclusion: These results demonstrate that the recently isolated Bifidobacterium sp. exhibit a probiotic potential, lowering the pathogenic features of enteropathogenic bacteria.

Acknowledgements: This work was supported by the strategic grant POSDRU/159/1.5/S/133391, Project "Doctoral and Post-doctoral programs of excellence for highly qualified human resources training for research in the field of Life Sciences", Environment and Earth Science cofinanced by the European Social Fund within the Sectorial Operational Program Human Resources Development 2007-2013.

P-10

PERSISTANCE OF SUBCLINICAL CEREBRAL INFLAMMATION IN HIV-1 INFECTED PATIENTS WITH LOWCEREBROSPINAL FLUID (CSF) HIV-1 VIRAL LOAD

I.A. Diaconu[1], L.M. Stratan[2], V. Arama[1,2], L. Nechita[3], S. Zurac[2,3], Anca Negru[1], Alina Lobodan[2], C. Popescu[1,2], R. Moroti[1,2], S. Ruta[2,4], D.A. Ion[2]

[1]*Institutul National de Boli Infectioase "Prof. Matei Bals", Bucuresti*
[2]*Universitatea de Medicina si Farmacie "Carol Davila" Bucuresti*
[3]*Spitalul Clinic Colentina, Bucuresti*
[4]*Institutul de Virusologie "Stefan S. Nicolau", Bucuresti*

In a study that we have previously conducted, post-mortem histopathological examination of the brain tissue of 33 HIV-1 infected subjects revealed morphological proofs of HIV-induced brain damage, opportunistic infections or secondary neoplasms in all cases, regardless of whether the patients received antiretroviral therapy or not. A plethora of diagnoses were established, ranging from the apparently benign hyperaemia associated with edema to more serious infections such as progressive multifocal leukoencephalopathy or cryptococcal meningo-encephalitis. HIV encephalopathy was also a cardinal finding, linked consistently with brain tissue samples prelevated from patients that were either untreated or underwent antiviral therapy for less than 5 months. Before the advent of combination antiviral therapy (cART), severe and disabling dementia (HIV-associated dementia, HAD) affected ~20% of patients with advanced HIV disease. Following cART, in opposition to the decline of the incidence of HIV-associated neurocognitive disorders, prevalence has remained stable, due either to longer survival rates of individuals with milder disorders, subpar efficacy of cART in the brain, or the combination of both. HIV encephalopathy is characterized by gliosis, microglial nodules, perivascular macrophage accumulation, and the presence of multinucleated giant cells. Despite plasma level suppression, in HIV-treated patients, the virus can continue to replicate in brain tissues, an important virus sanctuary, with a rate of 3-10%. This replication (as low as 2 copies/ml in CSF) is capable to maintain a persistent immune activation, often out of proportion to the amount of HIV virus present in the brain. Consequently, for the better understanding of the underlying HIV-induced brain damage, it is of utmost importance to study the intricate mechanisms of HIV replication in the central nervous system (CNS) and the biomolecular interactions with the cells within. The neuronal injury can result from a direct mechanism by interaction with viral proteins, such as gp120 (neurodegeneration induced directly via interaction with N-Methyl-D-Aspartate receptor), Tat (transcriptional transactivator, which contributes to the disruption of the blood-brain barrier by affecting endothelial permeability) and Vpr (viral protein R, which can induce apoptosis of human neuronal precursor cells and mature, differentiated neurons by increasing the activation of caspase-8) produced by infected cells, or by an indirect effect resulting from the inflammatory process involving activated monocytes, macrophages and astrocytes (Tat promotes TNF-α and interleukin IL-1 production by monocytes and macrophages, stimulates the production of several cytokines and chemokines, including IL-8, RANTES, MCP-1 and TNF-α in astrocytes and gp120 interferes with the receptors, both phenomena leading to neurotoxicity). In addition, in order to improve the outcome of HIV infected patients and the efficiency of the antiretroviral therapy, further studies must focus on the details of viral persistance in the sanctuary of the central nervous system.

P-11

URETRITE SEXUAL INDUSE: PANEL ETIOLOGIC

A. V. Dragodan[1], F. Isopescu[2], C. Giurcăneanu[3]
[1]*International Medical Center, București,*
[2]*Universitatea de Medicină și Farmacie"Carol Davila", Catedra de Obstetrică - Ginecologie, Spitalul Clinic "Nicolae Malaxa", București,*
[3]*Universitatea de Medicină și Farmacie"Carol Davila"- Catedra de Dermatologie Oncologică și Alergologie, Spitalul Universitar de Urgență "Elias", București*

Uretritele, procese inflamatorii ale uretrei, sunt frecvent întâlnite în practica medicală, majoritar la bărbați. Având în vedere frecvența mare acestor afecțiuni in arealul bolilor transmisibile sexual (BTS), de cele mai multe ori delimitarea originii veneriene sau urologice a acestor afecțiuni este dificilăşi implică colaborarea mai multor specialități medicale. Stabilirea etiologiei, amplificată, în continuă diversificare, potențată de o multitudine de factori de risc, implică din ce în ce mai multe dificultăți în validarea precoce şi țintită a unui diagnostic etiologic prezumtiv, ca accesul dificil şi, uneori, tardiv, la investigații complexe şi extrem de costisitoare chiar şi pentru un pacient asigurat. Actualizarea etiologiei uretritelor alături de accesul facil la investigații paraclinice complexe, va potența adecvarea terapiei, consilierea şi profilaxia eventualelor recidive cu diminuarea complicațiilor şi, mai ales, a extinderii BTS.

P-12

FETAL HYDROTHORAX: CASE REPORT - POSDRU/159/1.5/S/137390

Mihai Dumitrescu, Dinu Florin Albu, Crenguta Albu

Introduction: To determine whether or not a fetus with hydrothorax has Down syndrome or CMV infection.
Materials and methods: Fetal hydrothorax refers to a collection of fluid within the fetal thoracic cavity as a result of leakage or generalized fluid retention from a variety of causes.
Case report: A 27-year-old Caucasian female, pregnant for the first time, was referred at 27 weeks' gestation for a routine prenatal ultrasound. Routine ultrasonography, triple test (AFP,uE3, hCG), selective ultrasonography for detection of fetal abnormalities, thoracocentesis and amniocentesis were performed.
Results: Ultrasound examination at 27 weeks of gestation revealed a single fetus with a large hydrothorax (~200ccm) compressing the lung and pushing down the diaphragm. A sample of 70ccm pleural fluid was obtained at 28 weeks of gestation through fetal thoracocentesis by ultrasound guided puncture. Infection with CMV was excluded after testing, while karyotype and QF-PCR from both amniotic fluid and thoracic fluid indicated the presence of Trisomy 21.
Conclusion: Prenatal diagnosis was useful in management, prognosis and detection of Down Syndrome through analysis of thoracic and amniotic fluid, in a fetus with hydrothorax. POSDRU/159/1.5/S/137390

P-13

IMMUNOHISTOCHEMICAL ASSESSMENT OF BASAL CELLS IN SMALL GLANDS PSEUDONEOPLASTIC LESIONS OF THE PROSTATE

Manuela Enciu[1,2], Mariana Aschie[1,2], Anca Chisoi[2], Gabriela Baltatescu[2], Sinziana-Andra Ghitoi[2]
[1]*Department of Pathology, Faculty of Medicine, ''Ovidius'' University, Constanta*
[2]*Clinical Service of Pathology, Saint Apostle Andrew Emergency County Hospital, Constanta*

Introduction: In histopathological practice, are described a series of small glands pseudoneoplastic benign lesions, which are difficult to differentiate, by common techniques, from prostatic adenocarcinoma and requires special techniques for the diagnosis of certainty. These are represented by proliferative lesions such as atypical adenomatous hyperplasia, sclerosing adenosis, basal cell hyperplasia and atrophy of the prostate. 34βE12 or CK903 is a high molecular weight cytokeratin relatively specific for prostate basal cells - present in basal cells of normal prostate glands and absent in adenocarcinomas, which reacts with CK1, CK5, CK10 and CK14.
Material and methods: Have been studied prostatic tissue excised by transurethral resection of the prostate. Histopathology exam and nuclear measurements were performed in the Clinical Service of Pathology, Saint Apostle Andrew Emergency County Hospital of Constanta. Tissue fragments were fixed in 10% formalin, included in paraffin, sectioned and stained with hematoxylin-eosin. The study group was represented by 60 atypical adenomatous hyperplasia cases, 33 cases of sclerosing adenosis, 19 cases of atrophy and 21 cases of basal cell hyperplasia. The control group was composed of 69 prostatic adenocarcinomas with small glands, 1 and 2 Gleason grade. Monoclonal mouse anti-Human High Molecular Weight Cytokeratin (HMWCK), Clone 34βE12, Isotype IgG1, Kappa (DAKO) were applied.
Results: Immunohistochemistry highlighted the presence of continuous or discontinuous basal cell in mimic adenocarcinoma lesions. The cases of adenocarcinoma were negative for 34βE12. The results were statistically significant ($p < 0.05$).
Conclusions: The immunohistochemichal techniques has been extremely useful in the diagnosis of certainty and exclusion of adenocarcinoma, with important therapeutic implications, especially as benign lesions do not require treatment, only follow-up.
Acknowledgements: This work benefited from financial support through the project,, CERO-CAREER PROFILE: Romanian researchers, contract no. POSDRU/159/1.5/S/135760, a project co-financed by European Social Fund through the Sectoral Operational Programme Human Resources Development 2007-2013.

P-14

TRANZIȚIA EMT DEPENDENTĂ DE DOZA DE CITOSTATIC CA REZULTAT AL SUPRAEXPRESIEI FOXC1 ÎN LINIILE CELULARE UMANE DE HEPATOCARCINOM

Raluca Florea[1], Madalina Veronica Ilie[1], Andrei Sorop[1], Valeria Tica[1], Liliana Livia Paslaru[1,2], Nicolae Bacalbasa[2], Razvan Iacob[1], Simona Dima[1], Irinel Popescu[1]
[1]*Fundeni Clinical Institute, București, Romania*
[2]*University of Farmacy and Medicine "Carol Davila", București, Romania*

Introducere: Hepatocarcinomul celular este una dintre cele mai comune boli hepatice din întreaga lume şi reprezintă a treia cauză a mortalității, fiind responsabil de 13% dintre cazurile de deces. Rata crescută a mortalității este relaționată în principal cu rata recurențelor după intervențiile terapeutice. Mecanismele celulare ale tumorii conduc la inducerea unui fenotip de tranziție specific tipului oncogenic, din epitelial în mezenchimal, reprezentând dobândirea unor caracteristici ale fenotipului mezenchimal invaziv care sunt responsabile de metastazare. Tranziția epitelial-mezenchimală este un proces morfogenetic în mai mulți paşi în timpul căruia celulele epiteliale îsi subexprimă proprietățile lor epiteliale şi supraexpimă caracteristici mezenchimale. Acest mecanism poate fi indus in vitro cu ajutorul doxorubicinei, unde se observă o abilitate crescută de migrare a celulelor can-

ceroase, care îşi schimbă fenotipul morfologic în cultură mimând un aspect de tip fibroblastic.
Scop: În acest studiu am realizat o analizăin vitro a efectelor diferitelor doze de doxorubicină asupra liniilor celulare de hepatocarcinom celular uman bine diferențiat în ceea ce priveşte promovarea tranziției epitelial-mezenchimale.
Materiale şi metode: Pentru analiza tranziției epitelial-mezenchimale, s-au analizat expresia genica a factorilor transcripționali Snail, Slug, FOXC1 si a proteinelor Vimentină, E-caderină in urma tratamentului liniilor celulare Huh7 si HepG2 cu diferite doze de doxorubicina la 24 si la 48 de ore.
Rezultate si discutii: În liniile celulare de hepatocarcinom uman bine diferențiat, doxorubicina la doze de peste 0.0025 µg/µl, induce apoptoza şi necroza celulară. Rezultatele au arătat că doze scăzute de doxorubicină (între 0.0003125µg/µl şi 0.0025µg/µl) promovează tranziția epitelial¬mezenchimală prin supraexpresia marcantă a factorilor transcripționali FOXC1, Slug, Snail, promovarea expresiei de Vimentinăşi scăderea expresiei de E-caderină.
Concluzii: La diferite momente de timp, acțiunea citostaticului la doze mici induce modificări morfologice şi implicit modificări la nivelul mecanismelor celulare. Aceste modificări morfologice conduc la procesul de tranziție epitelial-mezenchimală activată prin efectul doxorubicinei, ca rezultat al supraexpresiei factorilor transcripționali Snail, Slug şi FOXC1, a Vimentinei şi prin subexpresia E-caderinei. Astfel, se poate recomanda adaptarea concentrației de doxorubicină utilizată în procedurile TACE în funcție de volumul tumoral.
Acest studiu ştiințific a fost finanțat din granturile de cercetare UEFISCDI: 125/2011 (PN-II-ID¬PCE- 2011-3-0605), PD 23/ 2011 (PN-II-RU-PD-2011-3-0137), EEA-JRP-Romania-Norvegia nr.4SEE/30.06.2014.

CITOSTATIC DOSE DEPENDENT EMT TRANSITION AS A RESULT OF FOXC1 OVEREXPRESSION IN HUMAN HEPATOCELLULAR CARCINOMA CELL LINES

Introduction: Human hepatocellular carcinoma is one of the most common liver diseases worldwide and is the third cause of death, responsible for 13% of deaths. High mortality rate is related primarily with the recurrence rate after therapeutic interventions. Epithelial-mesenchymal transition is a morphogenetic process in several steps during which epithelial cells downregulate their epithelial proprieties and overexpress their mesenchymal features. This mechanism can be induced in vitro by doxorubicin, where is observed an increased ability of cancer cells migration, changing the culture morphological phenotype mimicking a fibroblast-like aspect.
Aim: In this study we performed in vitro analysis of the effects of different doses of doxorubicin on human hepatocellular carcinoma cell lines in terms of EMT promotion.
Methods and materials: For the analysis of EMT we analyzed by Real Time PCR the gene expression of the transcriptional factors Snail, Slug, FOXC1 and proteins Vimentin, E-cadherin following treatment of Huh7 and HepG2 cell lines with different doses of doxorubicin at 24 and 48 hours.
Results and discussion: In HCC cell lines, doxorubicin at doses greater than 0.0025 µg/µl, induces apoptosis and cell necrosis.The results showed that low doses of doxorubicin (between 0.0003125µg/µl and 0.0025 µg/µl) promote EMT marked by overexpression of transcription factors FOXC1, Slug, Snail, promotion of vimentin expression and decreasing the expression of E-cadherin.
Conclusions: At various points in time cytostatic action at low doses induce morphological changes and also changes in the cellular mechanisms. These morphological changes lead to EMT process activated by the effect of doxorubicin as a result of overexpression of transcription factors Snail, Slug and FOXC1, of Vimentin and E-cadherin downregulation. Thus, we can recommend the adjustment of doxorubicin concentration used in TACE procedures based on tumor volume.
This study was financially supported by 125/2011 (PN-II-ID-PCE- 2011-3-0605), PD 23/ 2011 (PN-II-RU-PD-2011-3-0137), EEA-JRP-Romania-Norvegia nr.4SEE/30.06.2014.

P-15

ENHANCED GREEN FLUORESCENT PROTEIN (EGFP) AND COUMARIN LABELING OF HEPATITIS B VIRUS

Paula E. Forian[1], Ramona N. Galantonu[2], Catalin Lazar[2], Simona Ruta[3], Anca Roseanu[1], Norica Nichita[2]
[1]*Institute of Biochemistry of the Romanian Academy, Ligand-Receptor Interactions Dept., Bucharest, Romania*
[2]*Institute of Biochemistry of the Romanian Academy, Viral Glycoproteins Dept., Bucharest, Romania*
[3]*Stefan S. Nicolau Institute of Virology, Emergent Diseases Department, Bucharest, Romania*

Hepatitis B virus (HBV) which infects human hepatocytes is a small enveloped double-stranded DNA virus. The envelope of HBV contains three viral proteins: large (L), middle (M) and small (S) and is important for assembly, secretion and infectivity of the virions. The aim of this work is to label M envelope protein to visualize the HBV traffic inside infected hepatocytes. Firstly we created a recombinant M protein containing enhanced green fluorescent protein (EGFP) tag in N-terminal region. Our results demonstrated that M (EGFP.M) was incorporated into virion envelope and secreted as shown by spectrofluorimetry experiments. Microscopy analyses revealed a cellular distribution of recombinant EGFP. M characteristic to wild-type envelope viral proteins. However the proper secretion of HBV virions was achieved only in the presence of S protein. Therefore we constructed a mutant M protein (MN3) containing a 13 aminoacids specific sequence for PRobe Incorporation Mediated by Enzyme (PRIME) labeling with blue fluorophore coumarin. The recombinant MN3 protein is efficiently expressed and secreted in hepatoma cells and does not affect neither HBV replication nor HBsAg secretion as revealed by qPCR and ELISA experiments. Further transcomplementation experiments will be performed in order to show the incorporation and secretion of recombinant MN3_HBV coumarin labeled virus;
Acknowledgements: This work was supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number OSDRU/159/1.5/S/137390 and by Romanian Academy Project 3 of the Institute of Biochemistry.

P-16

PREVALENȚA LEZIUNILOR ATEROSCLEROTICE ASOCIATE LA PACIENȚII CU STENOZĂ DE ARTERĂ RENALĂ UNI- VERSUS BILATERALĂ

Dana-Oliviana Geavlete[1], Carmen Beladan[1,2], Andreea Călin[1], Marian Croitoru[1], Ruxandra Jurcuț[1,2], Bogdan A. Popescu[1,2], Carmen Ginghină[1,2]
[1]*Institutul de Urgență pentru Boli Cardiovasculare "Prof. Dr. C.C Iliescu", București*
[2]*Universitatea de Medicină și Farmacie "Carol Davila", București*

Introducere: Co-existența leziunilor aterosclerotice în teritorii vasculare adiacente a fost demonstrată la pacienții cu stenoză de arteră renală (SAR). Studiul actual își propune să compare extensia afectării aterosclerotice la pacienții cu SAR unilaterală versus bilaterală.
Material și metodă: Am fost înrolați prospectiv 58 de pacienți diagnosticați cu SAR unilaterală (n=31) și bilaterală (n=27). S-au evaluat: istoricul bolii cardiace, factorii de risc cardiovascular, semnele și simptomele afectării vasculare și comorbidități asociate. Toți pacienții au fost evaluați ecocardiografic și electrocardiografic. Boala coronariană a fost confirmată prin coronarografie. S-a efectuat ecografie Doppler vasculară pentru boala carotidiană și arterială periferică, leziunile semnificative fiind confirmate ulterior prin arteriografie.
Rezultate: Prezența factorilor de risc tradiționali, a afectării coronariene și a arteriopatiei periferice a fost similară în ambele loturi. Prevalența stenozei carotidiene semnificative în ambele loturi a fost de 62,06 %, semnificativ mai mare în grupul SAR bilaterală (63.88 % vs. 36.11%, $p<0.001$). În plus, afectarea bilaterală carotidiană a fost semnificativ crescută în grupul SAR bilaterală (66.7 % vs. 33.3%, $p<0.001$).
Concluzii: Pacienții cu SAR bilaterală comparativ cu SAR unilaterală au demonstrat o prevalență crescută atât a afectării aterosclerotice extinse (mai mult de 2 teritorii vasculare) cât și a stenozei carotidiane semnificative atât unilaterală, cât și bilaterală.

Mențiune: Această lucrare este efectuată în cadrul Programului Operațional Sectorial pentru Dezvoltarea Resurselor Umane (POSDRU), finanțat din Fondul Social European şi Guvernul României prin contractul nr. POSDRU/159/1.5/S/137390.

P-17

IN VITRO BIOLOGICAL SCREENING OF POTENTIAL INSULINMIMETIC METAL-FLAVONOID COMPLEXES

Ariana Hudita[1], Bianca Galateanu[1], Alexandra Cristina Munteanu[2], Valentina Uivarosi[2], Marieta Costache[1]
[1]*Department of Biochemistry and Molecular Biology, University of Bucharest, Romania*
[2]*Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davila" of Bucharest, Romania*

Intoduction: The scientific community has focused on developing novel therapeutic approaches for the efficient management of metabolic disorders, as obesity has become amajor globalconcern. Recent studies revealed the quercetin'sability to ameliorate themetabolic syndrome in vivo. Additionlly, Zn, Cr and V ions might contribute to the improvement of diabetes due to theirantioxidant properties andability to activate the insulin receptor.
The aim of our study was to determine the cytotoxicity ofsomeoriginal metal-quercetin complexes upon adipose-derived stem cells(hASCs) in order to identifythe least toxic candidate for further studies.
Materials and methods: The median lethal dose (LD50) of Zn/Cr/V complexes with querceitin was assessed in terms of cell viability. In this view, hASCswere incubated for 24 h with various concentrations of the complexes and then subjected to the MTT spectrophotometric assay and the Live/Dead fluorescence assay.
Results: After the statistical analysis of the spectrophotometric data, the LD50 for all the complexes was determined.
Conclusions: Since the LD50 value for the Zn and V complexes with quercetin was relatively low, we concluded that they exert atoxic effect on hASCsand that only the Cr-quercetin complex could be employed in further studies.

P-18

IDENTITATEA DUPA MASTECTOMIE

Iulia Mihaela Iacob[1], Carmen Giuglea[1], Crenguta Coman[2], S. Dumitrache[2], I. P. Florescu[3]
[1]*Spitalul Clinic de Urgenta "Sf. Ioan", UMF Carol Davila, Bucuresti, Romania*
[2]*Spitalul Clinic de Urgenta "Sf. Ioan", Bucuresti 3)Spitalul Clinic de Urgenta "Bagdasar Arseni", Bucuresti, Romania*

Introducere: Mastectomia determina modificarea imaginii corporale, astfel incat o femeia care a trecut printr-o astfel de operatie – de necesitate – sufera ulterior si modificari ale structurii psihologice. Perturbarea imaginii corporale vulnerabilizeaza pacienta, determinand dificultati in reintegrarea sociala, profesionala si familiala. Echilibrarea imaginii corporale - prin metode de reconstructie mamara – aduce o stabilitate psihica si fizica necesara pacientei.
Material si metoda: Prezint cazul unei paciente in varsta de 53 ani, cu mastectomie de tip Halsted la nivelul sanului drept in antecedente, efectuata pentru un carcinom invaziv ductal, asociat cu carcinom intraductal, chimio si radiotratata. In acest caz au fost necesare mai multe interventii chirurgicale pentru a restabili forma si conturul sanului, precum si simetrizarea cu sanul contralateral.
Rezultate: Rezultatul a fost foarte bun, acest lucru fiind demonstrat atat prin imbunatatirea aspectului fizic, cat si prin ameliorarea statusului psihologic.
Concluzii: Reconstructia de san ar trebui privita si la noi in tara ca o etapa in tratamentul cancerului de san, fiind necesara pentru restabilirea integritatii corporale si mentinerea unui echilibru psihic.
Proiect cofinanțat din Fondul Social European prin POSDRU/159/1.5/S/137390, Cercetarea doctorală şi post-doctorală prioritate a învățământului superior românesc (Doc-Postdoc)

P-19

PREVALENTA INFECTIILOR DIGESTIVE VIRALE LA COPII IN SEZONUL CALD

S. Lazar[1], M. Nica[1], Nicoleta Tudor[2], Dana Obretin[2], Carmen Marcu[2], Rodica Rusu[2], Em. Ceausu[1]
[1]*UMF "Carol Davila" Bucuresti*
[2]*Spitalul de Boli Infectioase si Tropicale " Dr. V. Babes" Bucuresti*

Introducere: Bolile diareice acute de natura infectioasa reprezinta o cauza majora de imbolnavire la copii, multe dintre ele necesitand spitalizare, datorita in primul rand deshidratarii acute care apar frecvent la aceasta categorie de varsta. Obiective: In studiul de fata am incercat sa stabilim prevalenta bolilor diareice acute de etiologie virala in contextul infectiilor digestive ingrijite in clinica noastra in sezonul cald.
Materiale si metode: Am efectuat un studiu retrospectiv al cazurilor de enterocolita acuta diagnosticate in perioada 1 mai 2014- 30 septembrie 2014.Cazurile au fost investigate pentru douaetiologii : Rotavirus si Adenovirus.
Rezultate: In perioada mai-septembrie 2014 am inregistrat un numar de 1849 episoade de imbolnavire, din care, pe baza definitiilor de caz am stabilit 1385 au fost de cauza virala si 464 de cauza bacteriana. Dintre entero-colitele considerate ca virale, etiologia certa a fost stabilita in 73 de cazuri (57 produse de Rotavirus si 16 de Adenovirus). Majoritatea cazurilor a fost la grupa de varsta 1-3 ani (685 cazuri), cu o corelatie buna intre sexe (pearson=0.99) si cu o usoara predominenta la sexul masculin. Cele mai multe din episoadele de boala au fost de intensitate moderata, cu o durata medie de spitalizare de 3,4 zile.
Discutii si concluzii: Cele mai multe episoade de boala diareica acuta in perioada mai-septembrie 2014 au prezentat etiologie virala, au avut intensitate medie si perioade relativ scurte de spitalizare. Datorita sezonalitatii si manifestarilor clinice sugestive, asocierea diareei cu fenomene respiratorii si eruptii cutanate, in majoritatea cazurilor am suspicionat etiologia enterovirala.
Cuvinte cheie: sezon cald, boala diareica acuta, etiologie, Rotavirus, Adenovirus, Enterovirus

---

THE PREVALENCE OF THE VIRAL DIGESTIVE INFECTIONS IN CHILDREN DURING THESUMMER TIME

Introduction: Acute diarrheal infectious diseases are a major cause of ilness in children, many of them requiring hospitalization due primarily to the acute dehydration that occurs frequently in this age.
Objectives: In the present study we tried to determine the prevalence of acute diarrheal diseases with viral etiology in the context of the digestive infections treated in our clinic during the summertime.
Methodes: We performed a retrospective study of the cases of acute enterocolitis diagnosed between May 2014 to September 2014. The cases were investigated for two etiologies : Rotavirus and Adenovirus.
Results: In the period May-September 2014 we recorded a total of 1849 episodes of illness, which, based on the case definitions established in 1385 cases the viral etiology and in the rest of the cases (464) the bacterial etiology. Ofenterocolitis considered viral, etiology was established in 73 definite cases (57cases products by Rotavirus and 16 cases by Adenovirus). Most cases were in the age group 1-3 years (685 cases) , with a good correlation between the sexes (Pearson = 0.99) and with a slight predominance in males . Most of the episodes had moderate intensity, with an average duration of hospitalization of 3 to 4 days.
Discussion and conclusions: Most episodes of acute diarrheal diseasesduring May-September 2014 had viral etiology, were mild and needed relatively short periods of hospitalization. Because of seasonality and suggestive clinical manifestations(association with rash and respiratory phenomena), in most of the cases we suspected the enteroviraletiology.
Key words: summer time, acute diarrheal disease , etiology, Rotavirus, Adenovirus, Enterovirus

P-20

PERSISTENT EFFECT OF AUTOLOGOUS BONE MARROW STEM CELL THERAPY ON LEFT VENTRICULAR GLOBAL LONGITUDINAL STRAIN IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND IMPAIRED LEFT VENTRICULAR FUNCTION – A PILOT STUDY

Miruna Mihaela Micheu, Nicoleta Oprescu, Lucian Calmac, Daniela Pitic, Maria Dorobantu
Department of Cardiology, Clinical Emergency Hospital of Bucharest

Introduction. Aim: left ventricular global longitudinal strain (GS) assessment 6 months after autologous bone marrow stem cell (ABMSC) therapy in ST-segment elevation myocardial infarction (STEMI) patients with low left ventricular ejection fraction (LVEF).
Methods: Twelve patients with a first STEMI and LVEF < 40% were treated by successful primary percutaneous coronary intervention (PCI). In the ABMSC group, collection of 50 ml of bone marrow aspirate was performed 7 to 13 days after PCI, the bone marrow cell suspension being delivered via intracoronary route during the same day.
Results: There were no adverse effects during follow-up period. At 1 month, the GS absolute improvement was significantly greater in the ABMSC group than in the placebo group (-2.72 ± 2.6 vs. 0.89 ± 3.1, $P < 0.05$). This improvement was also maintained at 6 months follow-up (-2.78 ± 2.16 vs. 0.46 ± 1.32, $P < 0.05$).
Conclusions: We observed an early improvement of left ventricle global longitudinal strain starting with the first month after stem cell therapy, possibly due to paracrine action. This early beneficial effect was maintained 6 months after autologous bone marrow stem cell transplant.
Acknowledgement: This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390.

P-21

HER 2 STATUS IN BREAST CARCINOMAS DETERMINED BY CISH AND IHC: COMPARATION OF THE RESULTS

Anca Mitroi[1], Mariana Aşchie[1,2], Georgeta Cozaru[1], Gabriela Bălţătescu[1], Ionuţ Poinăreanu[1,2], Anca Chisoi[1], Manuela Enciu[1,2], Anca Nicolau[1], Oana Cojocaru1, Costel Brînzan[1]
[1]*Pathology Department, Emergency Clinical County Hospital of Constanta, Romania*
[2]*Faculty of Medicine ,"Ovidius" University of Constanta, Romania*

Identification of HER2 status is important for determining the prognosis of patients who have invasive breast carcinoma, as well as for selecting a subgroup of patients for personalized therapy. 81 cases diagnosed with breast carcinoma were selected from the Pathology Department of Emergency Clinical County Hospital of Constanta. These cases were pathological and immunohistochemical re-evaluated. HER2 status was determined of formalin fixed paraffin embedded sections by immunohistochemistry with CERB2 antibody followed by identification of gene status using HER2 gene probe by chromogenic in situ hybridization (CISH). Staining was scored according to manufacturer's criteria. The mean age of patients diagnosed with breast carcinomas was 58.69±10.21. All cases selected presented moderate membrane expression of CERB2 glycoprotein by immunohistochemistry. HER2 gene status determined by CISH revelled no gene amplification in 57% cases, low level amplification in 25% cases and high level amplification in 14% cases. Tumour heterogeneity was observed only in 4% of cases. Our study revealed a strong positive correlation between HER2 protein expression determined by immunohistochemistry and HER2 gene status determined by CISH.

P-22

ASPECTE IMUNOHISTOCHIMICE IN NEOPLASMELE COLORECTALE- REZULTATE PRELIMINARE ALE UNUI STUDIU PROSPECTIV

Stelian Stefanita Mogoanta[1], Olivia Garofita Mateescu[2], Antoanela Crafciuc[1], Liliana Streba[2], Laurentiu Mogoanta[2]
[1]Departamentul Chirurgie, UMF Craiova, Romania
[2]Departamentul Histologie, UMF Craiova, Romania

Tehnicile imunohistochimice reprezinta la momentul actual o metoda aflata in plina expansiune în descrierea caracteristicilor si comportamentului tumoral, devenite uneori obligatorii şi determinante în vederea aplicării unui management terapeutic adecvat. Tendinta convergenta catre personalizarea terapiilor chirurgicale și oncologice implică o cat mai buna caracterizare morfo- și fizio-patologică a tumorilor,corelată cu elemente clinice dinamice conjucția acestor aspecte având impact semnificativ asupra indicilor prognostici și de rezultat terapeutic. Studiul nostru s-a desfăşurat pe piesele de rezectie de la 57 de pacienți operați pentru neoplasme colorectale în cursul anului 2015 în Clinica II Chirurgie Generală a Spitalului Clinic Județean de Urgență Craiova. Piesele recoltate au fost supuse analizei macroscopice cu identificarea ex-vivo a ganglionului sentinelă, disecției ganglionare extensive, studiului anatomopatologic convențional și prin tehnici imunohistochimice. S-a urmărit corelarea unor markeri imunohistochimici cu gradul de diferențiere tumoralăşi statusul limfoganglionilor. Pentru studiul imunohistochimic am utilizat ca markeri VEGF-c, caspaza-3 şi E-caderina. Pentru VEGF-c am remarcat o corelație cu gradul de diferențiere în sensul unei expresii intense în adenocarcinoamele colorectale bine diferențiate ce descreşte progresiv pentru a deveni redusă în adenocarcinoamele slab diferențiate. De asemenea E-caderina a generat reacție puternică în neoplasmele bine diferențiate în timp ce caspaza 3 a generat reacție redusă indiferent de gradul de diferențiere tumorală. La examinarea ganglionară am constatat existența unei relații inverse între numărul de ganglioni ce adapostesc metastaze și expresia E-caderinei în tumora primară. Paternul imunohistochimic al metastazelor ganglionare a urmat în general acelaşi profil cu tumora primară. Putem concluziona ca in majoritatea adenocarcinoamelor colorectale apoptoza este semnificativ diminuată, aproape absenta. In neoplasmele bine diferențiate expresia VEGF-c a fost puternică, contrastând cu adenocarcinoamele moderat sau slab diferențiate ce au prezentat o reacție redusă sau absentă la VEGF-c. Acest aspect sugerează fie un metabolism tumoral preferențial hipoxic în tumorile slab diferențiate, fie existența unor căi alternative de stimulare a angiogenezei. E-caderina ar putea fi un marker util în evaluarea capacității de metastazare precoce, în special în tumorile puțin avansate local, în perspectiva selecției pacienților pentru tratament minim invaziv.

Recunoastere: Această lucrare si activitatea de cercetare ce sta la baza elaborarii sale sunt susținute prin proiectul POSDRU/159/1.5/S/137390 cu titlul ”Cercetarea doctorală şi postdoctorală prioritate a învățământului superior românesc (Doc-Postdoc)”, proiect cofinanțat din Fondul Social European prin Programul operațional sectorial “Dezvoltarea resurselor umane 2007-2013”.

P-23

INTERLEUKINA 22 IN BOLILE INFLAMATORII INTESTINALE

Alexandru Constantin Moldoveanu[1], Mircea Diculescu[2]
[1]Clinica Gastreoenterologie si Medicina Interna II – SUUB, Universitatea de Medicina si Farmacie „Carol Davila” Bucuresti
[2]Catedra de Gastroenterologie si Hepatologie Fundeni, Universitatea de Medicina si Farmacie „Carol Davila” Bucuresti

Introducere: Bolile inflamatorie intestinale pot cauza modificari semnificativela nivelul mucoasei colonice. Recent s-a descoperit rolul patogenetic al Interleukinei 22 (IL-22) in aceste afectiuni.
Material si metoda: Au fost evaluate61 de articole din literatura internationala, indexate in baze de date electronice (PubMed), publicate pana in martie 2015.Din acestea 52au fost publicate in ultimii 5 ani.

Rezultate: Interleukina 22 actioneaza prin cresterea productiei de citokine pro-inflamatorii si are nivele serice crescute atat in boala Crohn cat si in colita ulcerativa. Cu toate acestea, IL-22are un rol protector in bolile inflamatorii intestinale, prin inductia sintezei de factori anti-apoptotici, de proteine implicate in proliferarea celulara si a unei membrane mucoase protective. Experiente efectuate pe soareci knockout pentru IL-22sau prin administrarea de anticorpi anti IL-22 la animale de experienta, au aratat agravarea semnificativa a simptomatologiei, in timp ce inducerea sintezei de IL-22 prin transfer de ADN in celulele din mucoasa colonica duce la imbunatatirea simptomatologiei. Mutatiile la nivelul genelor care codifica IL-22 si a receptorului acestuia se asociaza cu aparitia bolilor inflamatorii intestinale.
Concluzii: Efectul benefic al Interleukinei 22 a fost demonstrat in mai multe articole realizate de cercetatori independenti. Terapia bazata pe IL-22 ar putea reprezenta o tinta terapeutica pentru viitor in aceste afectiuni.
Multumiri: Studiu finantat prin proiectul POSDRU/159/1.5/S/137390.

P-24

COMPLICATIONS OF SYNDACTYLY RELEASE BY A MODIFIED FLATT METHOD

Doina-Iulia Nacea[1,2], D.M Enescu[1,2], Simona Stoicescu[1,2]
[1]*Plastic Surgery and Reconstructive Microsurgery Clinic , Clinical Emergency Hospital „Grigore Alexandrecu", Bucharest, Romania*
[2]*"Carol Davila " University of Medicine and Pharmacy, Bucharest, Romania*

Introduction: Syndactyly is one of the two most common congenital hand abnormalities, the other being polydactyly. It occurs in about 1: 2500 live birts, more commonly in males and is most often seen in the third weeb space.
Materials and methods: We reviewed 75 webs in 47 patients, operated for primary and secondary syndactyly during the period of 2002-2015 in the Plastic Surgery and Burns Department "Grigore Alexandrescu'' Clinical Emergency Hospital for Children. All the webs have been corrected by the same surgeon, using the same indication and timing. Developed of local infection, loos of skin graft, flap necroses, development of web creep, flection contracture, finger deviation, scars, sensation and function were assessed clinically and documented.
Results: Of the 24 operated patients, there were no cases of loos of skin graft or flap necroses, and none of the patients developed local infection due the surgery. Web creep was seen in 12 of the 41 webs operated before 1 year of age, while from the 34 webs operated later, only 7 developed weeb creep. The most of the patients operated in the first year of life had complex or complicated syndactlyly, which means that the difference in the incidence of late complications correlate not only with the timing of surgery but also with the complexity of this congenital anomaly. The scar quality evaluation reaveald a height below 2 mm in 72 of the 76 spaces , normal or supple pliabiliti in 68 of the 75 webs. There was no evidence of flection contracture. All patients had good finger-tip sensation.
Conclusions: The incidence of complications is low. To minimize the rate of complications, this type of surgery should be performed in a plastic surgery department of a hospital for children, by a surgical team experienced in treating the child syndactyly, using appropriate surgical instruments and loupe magnification. Revisional surgery for weeb creep can be done using the same technique, without the use of skin grafts.
Acknowledgement: This paper is supported by Sectoral Operational Programme Human Resources Development (SOPHRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390

P-25

EFFECT OF B-CYCLODEXTRINSBASED NANOSPONGES ON THE SOLUBILITY OF PHARMACOLOGICAL ACTIVE SUBSTANCES

Andreea Alexandra Olteanu, Corina-Cristina Arama, Crina Maria Monciu
University of Medicine and Pharmacy "Carol Davila" Bucharest, Department of Analytical Chemistry, Romania

Cyclodextrin (CD) based nanosponges are nanostructured cross-linked polymers, usually obtained by reacting cyclodextrin with a cross-linker such as carbonyldiimidazole, organic carbonates or (±) epichlorohydrin, (1), (2)They can overcome problems such as insolubility, permeability, sensitivity, and facilitate safe and efficient delivery of drugs. (3) Several β-CD, sulfobutylether-β-CD and hydroxypropyl-β-CD nanosponges were prepared using epichlorohydrin as cross-linker. After liophylization, the polymers were purified and characterized by FT-IR and 1H NMR, respectively. The complexing properties of the CD polymers were investigated against repaglinide (a hypoglycemic agent, practically insoluble in water) in order to improve the drug solubility, and also enalapril, captopril and cilazapril (effective angiotensin converting enzyme inhibitors used to control blood pressure and to prevent congestive heart failure and stroke), in order to improve their stability and bioavailability and to overcome the possible drug-excipient interaction. Solubility studies were performed according to the method reported by Higuchi and Connors and the the phase solubility diagrams were plotted. The phase solubility diagrams are AL-type (with good correlations coefficients, higher for the CD-NS), indicating the formation of soluble complexes. The solubility of repaglinide increases linearly with the amount of nanosponge in the solution, from 1.8 to7.2 mg/100 mL for 20 to 100 mg added βCD-NS, and from 3.0 to 10 mg/100 mL for 20 to 100 mg added SBEβCD-NS. The solubility is easily improved in case of captopril, cilazapril and enalapril.

Acknowledgments: This paper was supported by the Sectorial Operational Program Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390.

References: 1. Amber Vyas, Shailendra Saraf, Swarnlata Saraf. Cyclodextrin based novel drug delivery systems. J Incl Phenom Macrocycl Chem. 2008; 62: p. 23-42. 2. Samia Daoud-Mahammed, Patrick Couvreur, Kawthar Bouchemal, Monique Cheron, Genevieve Lebas, Catherine Amiel, Ruxandra Gref. Cyclodextrin and Polysaccharide-Based Nanogels: Entrapment of Two Hydrophobic Molecules, Benzophenone and Tamoxifen. Biomacromolecules. 2009; 10: p. 547-554. 3. R. Cavalli, F. Trotta and W. Tumiatti. Cyclodextrin-based nanosponges for drug delivery. J. Incl.Phenom. Macrocycl. Chem. 2006; 56: p. 209-213.

P-26

CERCETAREA TRANSLAȚÍONALĂ: O NOUĂ FRONTIERĂ PENTRU LABORATOARELE CLINICE

Alexandra Dana Maria Panait, Manole Cojocaru
"Titu Maiorescu" University, Faculty of Medicine, Bucharest, Romania

Au crescut exponențial studiile care utilizează termenul de cercetare translațională, reflectând creşterea interesului în domeniu. Au apărut noi concepte cum ar fi cunoaştere translațională, medicină translațională. Medicina translațională reprezintă cel mai mare potențial de dezvoltare şi cuprinde informații noi referitoare la prevenția, diagnosticul şi tratamentul bolilor. Scopul cercetării translaționale este de a accelera transferul noilor descoperiri ştiințifice în practica clinică. Există două căi translaționale în direcția îmbunătățirii sănătății publice, unul este transferul tradițional cercetărilor legate de investigațiile biomedicale, altul este transferul ultimelor noutăți între diagnostic şi terapie legat direct de trialurile clinice. Din punct de vedere al laboratorului clinic, ultimele căi de cercetare sunt relevante datorită unui număr crescut de tehnici noi şi de studii aprofundate propuse a fi translaționate în practica clinică. Cercetarea translațională reprezintă dezideratul unor noi idei generate de investigațiile biologice, răspunzând nevoii de a identifica ipoteze ştiințifice de ultimă oră relevante pentru patologie. Pentru specialiştii din laboratorul clinic, cercetarea translațională răspunde nevoii de a obține date noi din cercetarea cu aplicare în practica medicală. Cercetarea translațională reprezintă efortul creşterii

eficienței în care utilizarea noilor descoperiri în ştiințele medicale pot fi testate în clinică. În concluzie, testele de laborator necesită construcția unui sistem nou şi complex de cercetare translațională fiind motorul acestor idei aplicate din domeniul cercetării fundamentale de laborator în domeniul clinic.

P-27

MORPHOMETRY AND IMMUNOHISTOCHEMISTRY IN TESTICULAR TUMORS DIAGNOSIS

Ionut Poinareanu[1,2], Mariana Aschie[1,2], Georgeta Camelia Cozaru[2], Anca Florentina Mitroi[2], Antonela Anca Nicolau[2], Oana Cojocaru[2]
[1]*Pathology Department, Faculty of Medicine, "Ovidius" University Constanta, Romania*
[2]*Pathology Reserch Team, Emmergency Clinical County Hospital Constanta, Romania*

Introduction: Testicular tumors is a subject of particular interest in clinical problems gives and their development to younger age, those beeing malignant in over 90% of cases. They are often diagnosed late although they are located in an accessible tight of body and may allow direct examination of lesion biopsies extemporaneous early explorers. Their evolution may be complicated leading to infertility or death, following the occurrence of chemotherapy complications.
Means and methods: Early diagnosis of cancer is the ultimate desire of any physician, regardless of specialty. Knowledge about cancer today reached a high level, any discovery or deepening of what was researched was relevant. Morphology, immunohistochemistry and morphometry are methods used in research necessary for early diagnosis in testicular tumors. In this paper are presented 101 germinative testicular tumors operated and diagnosed in Clinical County Hospital Constanta.
Results: Using a panel of mono and polyclonal antibodies represented by alphafetoprotein (AFP), human chorionic gonadotropin (beta-HCG), placental alkaline phosphatase (PLAP), CAM5.2, OCT ¾, CD 30, CD 117, p53, EMA, by which it was possible to establish immunohistochemical profiles of tumors studied. Morphometric examination results in the following three distinct sections of nuclear and cellular changes in intraepithelial neoplasia, embryonic carcinoma and classic seminoma. It was examined morphometric a batch of 15 cases selected of total 101 on the basis of clear and well defined.
Conclusions: These findings demonstrate the usefulness of research proposed by achieving the main goal: to draw up a protocol for complex histopathological diagnosis of testicular germ tumors, morphological, immunohistochemical and morphometric. Acknowledgements – This work benefited from financial support through the project "CERO-CAREER PROFILE: Romanian researchers, contract no. POSDRU/159/1.5./S/135760, a project co-financed by European Social Fund through the Sectorial Operational Programme Human Resources Development 2007-2013.

P-28

DIFFICULTIES IN THE ETIOLOGICAL DIAGNOSIS OF VIRAL MENINGOENCEPHALITIS

C.P. Popescu, S. Ruta, S. Lazar, R. Rusu, M. Homos, V. Simion, M. Nica, G. Tardei, S. Purice, A. Gutan, S. Florescu, E. Ceausu

Background and aims: Viral CNS infections may be suspected on the basis of epidemiologic clinical features, and initial cerebrospinal fluid (CSF) studies, but the diagnosis requires positive identification of a viral pathogen from CSF or other patient samples. The aim of our study was to evaluate the challenges for the etiological diagnosis and treatment of acute viral central nervous system (CNS) infections.
Methods: We conducted a retrospective analysis of patients hospitalized in "Dr Victor Babes" Clinical Hospital of Infectious and Tropical Diseases, Bucharest with acute viral CNS infections between January 2012 and December 2013.
Results: 208 patients (56.7% males, median age 16 years.) were admitted. Most cases (69,2% ,144/208) were from

urban areas, out of which 69.4% (100/144) came from the capital city, Bucharest. The clinical features were suggestive for aseptic meningitis (55.2%- 115 cases), encephalitis (25%- 52 cases), meningo-encephalitis (17.3%- 36 cases). The etiology was confirmed in only 28 (13,5%) cases. The most frequently identified etiological agents were: Herpes simplex virus – 10 cases, Varicella-zoster virus - 8, rubella – 5, Epstein-Barr virus – 2, West-Nile virus, Influenza B virus and Enterovirus group B in one case). Most of the etiological diagnosis were made through serological tests (for HSV, rubella, EBV and WNV), nucleic acid testing was used only in 2 cases, and confirmed Influenza B and enteroviral infection. Etiological diagnosis was not available in 86,5% of the cases. As a consequence, empirical treatment with antibiotics was instituted in 56.2% of these cases due to potential overlapping with the initial stage of bacterial meningitis and with Acyclovir in 60 cases. The outcome was favourable in all cases.
Conclusions: Poor viral identification from cerebrospinal fluid highlights the utility of molecular techniques in the diagnosis of acute CNS infections. A good etiological diagnosis may reduce antibiotic use, hospitalization lengths and associated costs.

P-29

IN VITRO BIOLOGICAL SCREENING OF POTENTIAL ANALGESIC AND LOCAL ANAESTETIC BENZANILIDES

Mirela Serban[1], Bianca Galateanu[1,2], Diana Nuta[3], Ariana Hudita[1], Cornel Chirita[3], Anca Hermenean[2], Aurel Ardelean[2], Marieta Costache[1]
[1]Department of Biochemistry and Molecular Biology, University of Bucharest, Romania
[2]Institute of Life Sciences, VasileGoldis Western University of Arad, Romania
[3]Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davila" of Bucharest, Romania

Intoduction: Hydrogels are widely used as modern wound dressings, as they possess some notable advantages: they are flexible, durable, antigenic, and water vapor permeable, provide good coverage of the wounds, preventing bacterial infections. Such (bio)materials that combine a natural macromolecular component with a synthetic one offer interesting perspective for chronic wound healing applications. Drugs are easily incorporated in these hydrogels, which become also reservoirs of active principles released during wound treatment. The aim of our study was to determine the cytotoxicity of some original benzanilides upon adipose-derived stem cells (hASCs) in order to identify the least toxic candidate for further studies.
Materials and methods: The median lethal dose (LD50) of the original benzanilides was assessed in terms of cell viability. In this view, hASCswere incubated for 24 h with various concentrations of the compounds and then subjected to the MTT spectrophotometric assay and the Live/Dead fluorescence assay.
Results: After the statistical analysis of the spectrophotometric data, the LD50 for all the substances was determined.
Conclusions: Two original benzanilides were selected for further studies based on their high value of DL50 on hASCs.
Acknowledgements: This work was supported by the strategic Grant POSDRU/159/1.5/S/133391 and by the Romania-Moldova bilateral project 13/23.12.2013.

P-30

THE PATHWAY OF DEGRADATION OF HEPATITIS B VIRUS (HBV) ENVELOPE PROTEINS

Mihaela Uta, Catalin Lazar, Stefana Petrescu, Norica Nichita
Institute of Biochemistry of the Romanian Academy

Current antiviral therapies have no direct effect on the covalently closed circular DNA (cccDNA) minichromosome, the nuclear form of hepatitis B virus (HBV). The accumulation of cccDNA in the host cell nucleus is associated with development of hepatocellular carcinoma (HCC). It has been shownthat the envelope proteins

of HBV may regulate synthesis of cccDNA. We hypothesized that significant degradation of envelope proteins of HBV during the Unfolded Protein Response (UPR), triggered by HBV infection, may be a key component of this regulatory mechanism. We investigated the interactions between components of the endoplasmic reticulum associated degradation (ERAD) and the HBV envelope proteins. Expression of the ERAD regulators, EDEM1-3 was modulated in Huh7cells, a human hepatoma cell line which supports virus replication after transfection with HBV DNA. A Huh7 constitutively expressing tetracycline repressor (TetR) was first obtained and characterized. Lentiviral constructs expressing shRNAs targeting EDEM1-3, as well as a scrambled sequence, were cloned and further used to transduce the Huh7 TetR cell line. The newly established cell lines were induced to express the corresponding shRNA and the EDEM1-3 down-regulation was evaluated by Western Blot and Immunofluorescence assays with specific antibodies. The results have shown that EDEM1, EDEM2 and EDEM3 protein level decreased by 90% in the cell lineexpressing the correspondingshRNA. EDEM1-3 was also modulated by overexpression. Further investigation of the relationship between cccDNA amplification and the envelope proteins levels is in progress.

P-31

INTERACTOMICS AND LIVE IMAGING AS TOOLS FOR IDENTIFICATION AND CHARACTERIZATION OF ENDOGENOUS FACTORS INVOLVED IN HEPATITIS C VIRUS LIFE CYCLE

Ovidiu Vlaicu[1], Florin Pastrama[1], Cristian Munteanu[1], Georgiana Toma[1], Simona Paraschiv[2], Dan Otelea[2], Costin-Ioan Popescu[1]

[1]*Institute of Biochemistry of the Romanian Academy, Bucharest, Romania*
[2]*National Institute of Infectious Disease „Matei Bals", Bucharest, Romania*

Hepatitis C Virus (HCV) represents a global health problem with 170 million people infected worldwide. Although HCV therapy has improved tremendously in the recent years, side effects, resistance and cost are still issues to be solved by new antiviral therapies based on potential new endogenous targets. To identify new targets/biomarkers involved in HCV life cycle we are using affinity purification (AP) of viral proteins interactomes followed by protein identification by mass spectrometry (MS). NS2 and NS5A viral proteins were tagged with foreign epitopes suitable for AP or TAP (tandem affinity purification) in the context of infectious virions. In order to couple proteomics with a cell biology approach, we have constructed a recombinant virus with a tetracystein tag (TC) in the HCV core protein and EGFP in NS5A protein. The recombinant viruses presented similar levels of infectivity to the wild type virus. Further we have used the recombinant viruses in AP-MS experiments in the context of infectious virus particles producing cells showing their value in identification new endogenous factors potentially involved in HCV life cycle. In conclusion, we constructed fully functional tagged HCV genomes and optimized AP-MS protocols for viral proteins interactome identification. The functional genome tagged with TC and EGFP will be a valuable tool in live imaging the dynamics of viral proteins and endogenous factors in the context of infectious virions producing cells.
