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Abstracts

IMPACT OF GENOMIC PROFILING IN CANCER RESEARCH

Professor BIN TEAN THE

National Cancer Centre Singapore & Duke-NUS Graduate Medical School, Singapore

Cancer Genomics has been key to recent advances in cancer biology and therapeutics. Systematic genetic and molecular information captured from clinical samples has enabled the development of drugs designed to target specific molecular aberrations found in disease resulting in specific targeted therapies. Examples include the discovery of the BCR-ABL oncogene as a driver of Philadelphia-chromosome-positive chronic myeloid leukemia and development of a specific BCR-ABL tyrosine kinase inhibitor was another milestone. Using high-throughput Next-Generation Sequencing (NGS), we and others have identified frequent mutations in histone modifiers and chromatin remodellers in different cancer types. For example, in clear cell renal cell carcinoma, the most common type of kidney cancer, a gene called PBRM1, which encodes a member of the SWI-SNF complex, was found to be mutated in up to 40 percent of this cancer type. Preliminary cellular studies involving PBRM1 demonstrated its tumor suppressive role. Gene expression profiling points to involvement of known biological pathways but these require further investigations. Besides these two genes, frequent mutations of other chromatin modifiers have also been identified including UTX in multiple cancers, MLL family in pancreticobiliary cancer. The lecture will discuss the direction of future studies in this field and their clinical implications.

HCC AND ANGIOGENESIS: POSSIBLE TARGETS AND FUTURE DIRECTIONS

DAN DUDA

Radiation Oncology, Edwin L. Steele Laboratory for Tumor Biology, Department of Radiation Oncology,
Harvard Medical School, Massachusetts General Hospital

Hepatocellular carcinoma (HCC), the most common primary liver tumor, is notoriously resistant to systemic therapies, and often recurs even after aggressive local therapies. HCCs rely on the formation of new blood vessels for growth, and VEGF is critical in this process. A hallmark of new vessel formation in tumors is their structural and functional abnormality. This leads to an abnormal tumor microenvironment characterized by low oxygen tension. The liver is perfused by both arterial and venous blood and the resulting abnormal microenvironment selects for more-aggressive malignancies. Anti-VEGF therapy with sorafenib was the first systemic therapy to demonstrate improved survival in patients with advanced-stage HCC. This important development in the treatment of HCC raises hope as well as critical questions on the future development of targeted agents including other antiangiogenic agents, which hold promise to further increase survival in this aggressive disease.

PERSONALIZED TREATMENT OF MAJOR AFFECTIVE DISORDERS AND THE GENOMEWIDE STRATEGY OF THE INTERNATIONAL CONSORTIUM ON LITHIUM GENETICS

MARIA GRIGOROIU-SERBANESCU[1] and CARMEN C DIACONU[2]

[1] Biometric Psychiatric Genetics Research Unit, Alexandru Obregia Clinical Psychiatric Hospital, Bucharest,
[2] Stefan S. Nicolau Institute of Virology, Romanian Academy, Bucharest, Romania

Introduction. Lithium is a first-line mood stabilizer in the maintenance treatment of bipolar I disorder (BPI) (manic-depressive illness) and it is also used to augment antidepressant drug effect in the treatment of refractory unipolar depression. It is very efficient in many patients and it seems that the clinical response to lithium therapy is heritable (Grof *et al.*, 2002). This points to a genetic basis of lithium treatment response. Nevertheless, data from pharmacogenetic studies of lithium are sparse and these studies used small samples and varying definitions of the treatment response. Recently, an international consortium was built (ConLiGen) (Schulze *et al*., Neuropsychobiology, 2010), member of which is the Obregia Psychiatric Hospital.The objective of the ConLiGen is to establish a sample large enough to meet the power and genomewide significance requirements and to identify, in GWAS strategy, the genotypes/SNPs underlying the favorable response to lithium maintenance treatment in order to avoid the toxic effects of long-term lithium-treatment and to prevent relapse induced by inadequate treatment in non-responder patients.Method. Genotypes in candidate genes and copy-number-variants (CNV) are determined investigating 1.300.000 SNPs genomewide in lithium-responder and –non-responder patients. The genotyping is performed on the Illumina platforms at Bonn University (Germany) and NIMH, Bethesda. A sample of 158 Romanian BPI-patients treated with lithium was included in the international sample and genotyped. The response to lithium treatment was rated on the Alda scale. The DNA was extracted at the Institute of Virology, Bucharest.

Previous results of the consortium members (Perlis *et al.*, 2009) indicated a region on chromosome 10 to be genomewide associated with positive response to lithium. Other regions provided suggestive results (chromosomes 6, 12, 21). Confirmation of previously reported genotypes as well as potentially new genotypes and CNV are expected to be associated with lithium response in the international sample. Biomarkers are also looked for.

CROSS TALK BETWEEN SMOOTH MUSCLE CELLS AND MONOCYTES AUGMENTS EXPRESSION OF PRO-ATHEROGENIC MOLECULES

ELENA DRAGOMIR BUTOI, ANA MARIA GAN, ILEANA MANDUTEANU, DANIELA STAN, MANUELA CALIN, MONICA PIRVULESCU, RORY RYAN KOENEN, CHRISTIAN WEBER and MAYA SIMIONESCU

Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania

Objective. In atherosclerotic lesions, fractalkine (CX3CL1) and its receptor (CX3CR1) expressed by smooth muscle cells (SMC) and monocytes/ macrophages, mediate the heterotypic anchorage and chemotaxis of these cells.We questioned whether, during the close interaction ofmonocytes

with SMC, the CX3CL1/CX3CR1 pair modulates the expression of pro-atherogenic molecules in these cells.

Methods and results. SMC were co-cultured with monocytes or LPS-activated monocytes (18 h) and then the cells were separated and individually investigated for the gene and protein expression of TNFα,IL-1β, IL-6, CX3CR1 and metalloproteinases (MMP-2, MMP-9). We found that SMC-monocyte interaction induced, in each cell type, an increasedmRNA and protein expression of TNFα, IL-1β, IL-6, CX3CR1, MMP-2 andMMP-9. Blocking the binding of fractalkine to CX3CR1 (by pre-incubation of monocytes with anti-CX3CR1 or by CX3CR1 siRNA transfection) before cell co-culture decreased the production of TNFα, CX3CR1 and MMP-9. Monocyte–SMC interaction induced the phosphorylation of p38MAPK and activation of AP-1 transcription factor. Silencing the p65 (NF-kB subunit) inhibited the IL-1β and IL-6 and silencing c-jun inhibited the TNFα, CX3CR1 andMMP-9 induced by SMC–monocyte interaction.

Conclusions. The cross-talk between SMC andmonocytes augments the inflammatory response in both cell types as revealed by the increased expression of TNFα, IL-1β, IL-6, CX3CR1 and MMPs. Up-regulation of TNFα, CX3CR1 and MMP-9 is further increased upon interaction of SMC with activated monocytes and is dependent on fractalkine/CXRCR1 pair. These data imply that the fractalkine/CX3RCR1 axis may represent a therapeutic target to impedethe inflammatory process associated with atherosclerosis.

DUAL ROLE OF CERULOPLASMIN IN OXIDATIVE PROCESSES

CAMELIA S. STANCU[1,2], ELENA CONSTANTINESCU[1] and ANCA V. SIMA[1]

[1] Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
[2] National Institute for Economic Research "C.C. Kiritescu", Bucharest, Romania

Introduction. Atherosclerosis is a complex disease, developing in the arterial wall in response to various stimuli, such as plasma hyperlipemia known to increased transcytosis and accumulation of atherogenic lipoproteins within the arterial wall. Oxidative modification of the low density lipoproteins (LDL), play an important role in human atheroma formation. Extensive LDL oxidation in the atherosclerotic lesions may require a source of iron or copper as catalysts. The main copper carrier in plasma is ceruloplasmin (CP), a protein whose pro- or anti-oxidant properties are unclear. Our aim was to study the interaction between LDL and CP in vitro, and in vivo in an animal model of diet-induced atherosclerosis.

Methods. The in vitro interaction between LDL and CP was evaluated by their incubation at neutral pH and 37^{o}C. The oxidative modifications of LDL were assessed by measuring the lipid peroxides level and the electrophoretic mobility. To visualize the pathway of LDL into the arterial wall, native fluorescent labeled LDL (LDL-DiI) was injected into male, Golden Syrian hyperlipemic hamsters. The presence of oxidized LDL and CP in the hamster arterial wall was detected immunohistochemically.

Results. In vitro experiments demonstrated that slightly degraded CP has an oxidative potential against LDL at neutral pH. In vivo, LDL-DiI was taken up by the enlarged intima and fatty streaks of the arterial wall. The oxidized LDL and CP were colocalized in the same segments of the arteries that take up LDL-DiI. These data demonstrate that CP has an oxidative potential against LDL at neutral pH and that enhanced permeability of the endothelium induced by hyperlipemia leads to the accumulation of both LDL and CP in the arterial intima, thus enabling the subendothelial oxidation of LDL.

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IN SEARCH OF A GASTRIC CANCER MOLECULAR SIGNATURE

MIHAELA CHIVU-ECONOMESCU[1], LAURA G. NECULA[1], CORALIA BLEOTU[1], DENISA DRAGU[1], SIMONA O. DIMA[2], ADRIANA DUMITRU[3], GABRIEL CONSTANTINESCU[3], IRINEL POPESCU[2] and CARMEN C. DIACONU[1]

[1] Stefan S. Nicolau Institute of Virology, Bucharest, Romania
[2] Center of Digestive Disease and Liver Transplantation, Fundeni Clinical Institute, Bucharest, Romania
[3] Floreasca Emergency Hospital, Bucharest, Romania

Introduction. While extensive research focus on biological mechanisms details of cancer cells motility and invasiveness, these processes remains largely unknown. This study aims to detect an invasion related gene signature and pathway, bringing light on these mechanisms.

Methods. We conducted genomic and proteomic studies analyzing differential expressed genes relating with TNM staging. Several aspects were considered: 1) the possible role of key point mutations in Tyr from IL6ST exon 17 that encode for the catalytic domain of gp130, and of its respective activators (IL-6 family member cytokines) in human gastric cancer initiation and development; 2) gene expression profiling, investigated though cDNA microarray and relative quantification by RT-PCR; 3) signal transduction activated pathways using proteome profiler array kit - human phospho-kinase.

Results. Sequencing analysis does not identified mutations in gp130 key positions (Y759, Y767, Y814, Y905 and Y915). An increased IL-6 and IL-11 level in gastric mucosa was observed, correlated with staging, indicating this cytokines as gp130 activators in tumor epithelial cell. Those variations were consistent with increased IL-6 level in plasma, significant correlated with patient's survival time.

Gene expression profiling revealed a complex remodeling of normal gastric epithelium morphology and function associated with the tumorigenesis and metastasis. A large number of proteases are being over-expressed, together with keratins, genes associated with morphogenesis and anti-apoptosis. We report also, the identification of seven genes, significant up-regulated, that seems to be associated with tumor progression: KRT17, COL10A1, KIAA1199, SPP1, IL11, S100A2, and MMP3.The analysis of signal transduction pathways revealed a difference between primary and advanced adenocarcinoma. In primary adenocarcinoma, PI3K/AKT/mTOR and Ras/p38/JNK/MAPK were activated, mainly supporting cell division and survival. Additionally, advanced adenocarcinoma distinguished by main activation of Wnt/b-catenin pathway, which initiates transcription of c-myc, cyclin D1, MMPs and CD44 genes, with an important role in proliferation, migration and adhesion. In contrast, gastric ulcer was characterized by Ras/Raf/MEK/ p38/MSK pathway activation.

Conclusion. These results are preliminary steps in developing highly specific biomarkers for gastric adenocarcinoma invasion, predicting response to therapy, as well as developing new targeted therapeutic strategies addressed to biological mechanism.

MOLECULAR MARKERS IN SPORADIC COLON CANCER

L. PASLARU[1,2], A. NASTASE[2], V. HERLEA[3], M. IONESCU[2], C. CALIN[1], C. GHEORGHE[2], A. MACOVEI[4], S. DIMA[2], T. DUMITRASCU[2] and I. POPESCU[2]

[1] University of Medicine and Pharmacy "Carol Davila"
[2] Center of Digestive Diseases and Liver Transplantation, Fundeni Clinical Institute
[3] Pathology Department, Fundeni Clinical Institute
[4] Biochemistry Institute, Romanian Academy

Introduction. The aim of our study was to assess as possible biomarkers a panel of candidate genes (INHBA, CLDN1, TCN1, LCN2, KLK10, GREM2, GDF15, ASCL2, CXCL2, CXCL3,

RNF43, DEFA5, DEFA6, INHBA, IL8, MMP1, MMP3, MMP7, SPP1, CLDN1, NNMT) in sporadic colon cancer.

Methods. Normal, adenoma and tumor colon samples from 40 patients with colon cancer were analyzed for gene expression profiling using qPCR and for protein expression by immunohistochemistry and by ELISA which was done on patients` sera.

Results. qPCR results showed that DEFA5, DEFA6, TCN1 and LCN2 are expressed in adenoma at a higher level that in tumoral tissue and can be assessed as markers of the onset of the disease. The other studied genes are higher expressed in tumoral tissue compared with adenoma and can be assessed as markers of disease progression.

Immunohistochemistry showed MMP7 is expressed both in adenoma and in tumoral tissue, while DEFA6 is expressed more in adenoma than in tumor.

ELISA test pointed out that IL8, INHBA, DEFA6 have higher concentrations in sera of the patients with colon cancer compared with control subjects reviling a good sensibility and specificity of the test. DEFA6 and INHBA sera concentrations are correlated with the adenoma diameter, while IL8 is correlated with tumor diameter.

Conclusions. Our results show that serum levels of IL8, DEFA6, SPP1 and INHBA represents a valuable panel of biomarkers and can be used in laboratory for early diagnostic of sporadic colon cancer.

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INTROSPECTION IN MOLECULAR EXPRESSION PATTERN OF HUMAN MESENCHYMAL STEM CELLS-DIFFERENTIATED ADIPOCYTES

FLORINA BOJIN, VALENTIN ORDODI, OANA GAVRILIUC, MIRABELA CRISTEA, SIMONA ANGHEL, ALEXANDRA GRUIA, GABRIELA TANASIE, CARMEN TATU, CARMEN PANAITESCU and VIRGIL PAUNESCU

Department of Functional Sciences, "Victor Babes" University of Medicine and Pharmacy, Timisoara

Introduction. Human mesenchymal stem cells (MSCs) obtained from adult bone marrow are characterized by *in vitro* ability to differentiate towards at least three cellular lineages: adipocytes, osteoblasts, and chondrocytes. When considering the adipogenic potential of MSCs, 21 days after induction, these cells are capable of accumulating cytoplasmic lipid vacuoles, similar to native adipose tissue-isolated adipocytes. There are few data regarding the composition of fatty acids within the *in vitro*-differentiated adipocytes and the regulation of the metabolic functions.

Material and method. We used gas chromatography/mass spectrometry to identify fatty acids (FA) species in MSC-derived preadipocytes/ adipocytes and compared them to FA profile found in adipocytes isolated from adult adipose tissue. For identification of molecular expression we used microarray technique, and one-color data obtained from our microarray experiment were extracted using Agilent Feature Extraction Software and normalized to the 75th percentile signal value. Further downstream analysis was performed with the Genespring GX 11.0 program.

Results. We found that differentiation media did not contain essential fatty acids, and MSCs differentiated preadipocytes and mature adipocytes contain significant smaller amounts of arachidonic acid (C20:4) compared to native adipose tissue. MSCs, adipocytic precursors and mature adipocytes lacked linolenic acid (C18:3) and contained almost insignificant amounts of linoleic acid (C18:2) compared to native adipose tissue-derived adipocytes. Microarray analysis showed that there are different expression profiles in genes along the adipocytic differentiation pathway, level of expression being not similar to the adipose tissue, mainly in genes involved in lipids metabolism, and poly unsaturated fatty acids synthesis from essential fatty acids.

Composition of fatty acids in adipocytic differentiation media and MSCs inability to functionally convert these acids into appropriate lipidic end-products required for normal functioning of mature adipocytes makes the MSCs poor candidates of *in vitro* adipogenesis process.

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AN INTRINSICALLY DISORDERED DOMAIN OF EDEM1 CONTROLS ERAD IN MAMMALIAN CELLS

MARI B. MARIN[1], SIMONA GHENEA[1], LAURENTIU N. SPIRIDON[2], GABRIELA N. CHIRITOIU[1], ANDREI JOSE PETRESCU[2] and STEFANA-MARIA PETRESCU[1]

[1] Institute of Biochemistry, Molecular Cell Biology Department,
[2] Bioinformatics and Structural Biochemistry Department,

EDEM1 is a mannosidase-like protein that recruits misfolded glycoproteins from the calnexin/calreticulin folding cycle to downstream endoplasmic reticulum associated degradation (ERAD) pathway. Here, we investigate the role of a EDEM1 in the processing of tyrosinase, a tumour antigen overexpressed in melanoma cells. First, we analyzed and modeled EDEM1 major domains. The homology model raised on the crystal structures of human and *S.cerevisiae* ER class I α1, 2-mannosidases reveals that the major mannosidase domain located between aminoacids 121-652 fits with high accuracy. We have further identified an N-terminal region located between aminoacids 40-119, predicted to be intrinsically disordered (ID) and susceptible to adopt multiple conformations, hence facilitating protein-protein interactions. To investigate these two domains we have constructed an EDEM1 deletion mutant lacking the ID region and a triple mutant disrupting the glycan-binding domain and analyzed their association with tyrosinase. Tyrosinase is a glycoprotein partly degraded endogenously by ERAD and the ubiquitin proteasomal system. We found that the degradation of wild type and misfolded tyrosinase was enhanced when EDEM1 was overexpressed. Glycosylated and non-glycosylated mutants co-immunoprecipitated with EDEM1 even in the absence of its intact mannosidase-like domain, but not when the ID region was deleted. In contrast, calnexin/ calreticulin and SEL 1L associated with the deletion mutant. Our data suggest that the ID region identified in the N-terminal end of EDEM1 is involved in the binding of glycosylated and non-glycosylated misfolded proteins. Accelerating tyrosinase degradation by EDEM1 overexpression may lead to an efficient antigen presentation and enhanced elimination of melanoma cells.

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MOLECULAR MECHANISM OF HDL SECRETION FROM LIPID-LOADED MACROPHAGES

LOREDAN S. NICULESCU[1,2], GABRIELA M. SANDA[1] and ANCA V. SIMA[1]

[1] Institute of Cellular Biology and Pathology "N. Simionescu", Bucharest, Romania
[2] National Institute for Economic Research "C.C. Kiritescu", Bucharest, Romania

Introduction. Cholesteryl ester transfer protein (CETP) and apolipoprotein E (apoE) are secreted by macrophages. Apolipoprotein A-I (apoA-I) is a potent inducer of apoE secretion from lipid-loaded macrophages, but the effect on CETP is not known. We aimed to identify the signaling pathways involved in apoA-I and HDL-mediated regulation of CETP and apoE secretion from lipid-loaded macrophages.

Material and methods. THP-1 macrophages were loaded with lipids by incubation with human copper-oxidized LDL, then exposed to human purified apoA-I or HDL_3 with/without inhibitors for NF-κB (TPCK) or PKA (H89). CETP and apoE

in the cultured cells and media were quantified by real-time PCR and Western blot.

Results showed that in lipid-loaded macrophages: (i) CETP and apoE gene expression and secretion were increased in the presence of apoA-I, and furthermore by inhibition of NF-κB with TPCK; (ii) CETP and apoE gene expression and secretion were reduced by the inhibition of PKA with H89; (iii) PKA-gamma subunit was activated by oxidized LDL and moreover by apoA-I. We also showed that: (i) siRNA-mediated CETP gene silencing diminished apoE secretion from both non-loaded and lipid-loaded macrophages; (ii) addition of apoA-I partially recovered apoE secretion from lipid-loaded macrophages with the silenced CETP gene.

In **conclusion**, our data suggest a new mechanism by which apoA-I stimulate CETP secretion, in addition to apoE, from lipid loaded macrophages, a process involving NF-κB inhibition and/or PKA pathway activation.

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A NUCLEOTIDE GENOMIC SIGNAL APPROACH TO MAMMAL MTDNA COMPARATIVE ANALYSIS

PAUL DAN CRISTEA and RODICA TUDUCE

University "Politehnica" of Bucharest, Spl. Independentei 313, 060042, Romania

Among other attractive features, nucleotide genomic signal (*NuGS*) methodology allows a molecular level approach to determine distances between homologous genes or between conserved equivalent non-coding genome regions in various species, or in individuals of the same species. Based on this result, molecular level distances between species or between individuals can be computed. The paper illustrates the use of the nucleotide imbalance (*N*) and nucleotide pair imbalance (*P*) signals to determine the distances between mitochondrial DNA (mtDNA) genes of several mammals, including humans. The results are in accordance with those of other genetic or phylogenetic approaches to establish distances between genes, individuals and species.

Index Terms – Nucleotide Genomic Signals (NuGS), Nucleotide sequence representation, Mitochondrial DNA, Distances between homologous genes.

IMPLANT PARTICLES WITH THERAPEUTIC POTENTIAL AGAINST MALIGNANT TUMORS

SURDU-BOB C.C., C. COMAN, A. CALUGARU, L. CREMER, M. BADULESCU and E. VLASE

"Carol Davila" Universitary Emergency Central Military Hospital, Bucharest

Introduction. In the context of a modest success on cancer therapy at an international level, better materials and approaches are continuously under investigation. An important research effort has been made in recent years to study the potential of *nanomaterials* in treating cancer. The limited success registered with nanomaterials is due to poor selective toxicity, poor penetration control within the tumor and also poor localization

control within the body,leading to accumulation andtoxicity to vital organs.

A great number of chemical compounds and complexes were found to posess anticancer activity, but due to thenon-selective toxicity, they cannot be used in patients. We have found a material that has an important degradation effect on B16 tumoral cellsand has no effect on normal cells for 72 hours.

Materials. In search for better performance and control of our anticancer material in vivo, we designed a particle which, implantedat the tumor site, is capable of releasingtiny amounts ofthe active material in a controlled manner.The size of the designed particle (about half a millimeter) was chosen so that free circulation within the body is not possible,in contrast with nanomaterials.

Method and Results. The particles were injected in tumor-bearing C57 Black mice using an in vivo experimental model.We found that,while 86% of the mice presented visible tumors eleven days after inoculation of tumoral cells (TC), only 22% of the tumor-induced mice treated with our particleshad a visibile tumor. The treatment was given just after TC inoculation. Moreover, on the 23rd day after TC inoculation, all mice in the positive control group died whereas in the treated group only 44.3% of them died. Peri-tumoral and intra-tumoral inoculation of five particles per mouse resulted also in an increase of survival rate of 32% and 28%, respectively.

It is also important to mention that no macroscopic modifications of any organ or tissue were observed as a result of the presence of our particles in the body. Inoculation of particles in healthy mice resulted in the growth of a granuloma containing fatty tissue around the particles. This suggests that the immune system is able to protect the healthy tissue from this chemical. This result, together with the in-vitro findings on the interaction of our particles with normal cells, shows that the proposed treatment is both effective and non-invasive.The designed particles represent a therapeutic potential against cancer.

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SNARE PROTEINS - NOVEL REGULATORS OF MELANOSOME BIOGENESIS AND TRANSPORT

VIORICA IVAN and STEFANA M. PETRESCU

Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

Melanosomes are specialized organelles of pigment cells where melanins are synthesized and deposited. Defects in melanosome biogenesis and/or transport in melanocytes are the cause of certain human genetic diseases that affect pigmentation of the skin, hair and eyes. Little is known about proteins involved in membrane fusion events leading to the formation of melanosomes. In the present study we performed a targeted RNAi approach on several t-SNARE proteins. We identified syntaxin 13 as being involved in cargo transport to melanosomes and syntaxin 8 that is required for melanosome dispersion towards the periphery of the cells, for subsequent transfer to keratinocytes. Knock-down of syntaxin 13 induced a redistribution of the key melanogenic enzyme tyrosinase from throughout the cell in enlarged vesicles that tend to accumulate underneath the plasma membrane. Depletion of syntaxin 8 caused a selective perinuclear aggregation of melanosomes, accompanied by a change in cellular morphology. Our work has identified novel proteins involved in melanosome trafficking, with potential impact on skin pigmentation.

REDUCING PATERNALISM IN GENETIC COUNSELING SERVICE OF REPUBLIC OF MOLDOVA

MARIANA SPRINCEAN[1,2]

[1] National Center of Reproductive Health and Medical Genetics, Chisinau, Republic of Moldova,
[2] State University of Medicine and Pharmacy "N. Testemitanu", Chisinau, Republic of Moldova

In medical-genetic practice in Republic of Moldova, patients are often subjects of abuses and benefits for physicians because of their low medical and legal culture, including awareness on patients rights and liberties, phenomenon amplified by socially persisting poverty and informational isolation of people from group of genetic risk.

Efficiency in medical-genetic counseling might be evaluated according to a complex set of applied procedures as standard used methods and techniques, rated individual results and high level of communitarian prophylaxis of genetic pathologies. Paternalistic medical relationships are decreasing efficiency of national service of medical-genetic assistance by admitting indirect overriding of autonomous decision of the patient in order to benefit of him through directing the patient toward unnecessary for him genetic procedures, tests and analyses, fulfilling institutional research and treatment plans. Decreasing of ampleness of paternalistic elements in medical-genetic counseling diminishes the level of social genetic risk of next generations as well as assigns a new liberty of the individual patient to healthy offspring.

Reducing paternalization of patient-physician relationships in medical-genetic counseling could be achieved by: 1) survey of bioethical committees on fairness, moral equity and professionalism of medical decisions and involving patients in medical decision-making process, reflected in diagnostics protocols, in bio-bank databases, registers of patients with genetic diseases and direct observations of patient as well of physician behaviors; 2) periodical training by experts of bioethical committees of physicians involved in medical-genetic counseling regarding to bioethical and legal provisions prohibiting medical paternalism; 3) precise defining of all possible forms and ways of paternalization in medical genetic counseling and elaborating strategies of reducing of paternalism in medicine; 4)infixing in legal hard national as well in soft international regulation of provisions and enforcement against infringements of patient interests, autonomy and dignity by medical paternalistic attitude.

ANTI-CCP3 IGG ANTIBODIES: BIOMARKER FOR EARLY RHEUMATOID ARTHRITIS

MANOLE COJOCARU

Bucharest

It is important for disease management to diagnose and treat people with rheumatoid arthritis (RA) as early as possible. Diagnostic markers of RA ideally fulfil three requirements: good sensitivity, good specificity and early presence. Antibodies from some RA patients who are negative for cyclic citrullinated peptide 2 (anti-CCP2 IgG) are reactive with other citrullinated proteins suggesting that there are additional epitopes that are not present in the second generation CCP antigen sequence. Recently, the third generation test (called anti-CCP3 IgG) has been established to be the marker of choise for diagnosing early RA. This presentation will focus mainly on anti-CCP3 IgG test. The CCP3 IgG ELISA is an assay for the detection of anti-CCP3 IgG antibodies in patient sera or citrated or EDTA plasma. The literature scan indicated that, 77% of patients with RA are positive for anti-CCP3 IgG, while an average of 3% of non-RA subjects are

positive. This test has a 5% increase in sensitivity compared to the previous second generation test. We found the clinical sensitivity of anti-CCP3 IgG antibodies in early RA patients group of 75% with specificity of 97% (95% confidence interval). We classified the samples as negative (<20 U), weak positive, moderate positive or strong positive (≥60 U). In conclusion, anti-CCP3 IgG test shows superior performance compared to anti-CCP2 IgG, hold promise for earlier and more accurate diagnosis of disease. Anti-CCP3 IgG is currently the most wide used anti-citrullinated peptide assay. The presence of anti-CCP3 IgG antibodies early in disease development opens a window of opportunity for early treatment of RA.

THIAMINE HYDROCHLORIDE PREVENTS DIABETES COMPLICATION AT STREPTOZOCIN DIABETIC RATS

C. CONSTANTIN, A. TUBAC, G. CONSTANTIN and A.E. RANETTI

"Carol Davila" Universitary Emergency Central Military Hospital, Bucharest

Introduction. Murine models of diabetes are a good tool for reproduction of diabetes complications and a solution to test various treatments in this direction.

Material. 60 Wistar rats were injected with streptozotocin. The dose was 1mg/kgc. Initially we used insulin to reduce streptozotocin effects, then for adequate metabolic control. After 10 days of injection of streptozotocin a model of diabetes was obtained and were made three groups of rats as follows: a good glycemic control 130–150 mg/dl (20 rats – 2 groups A, A'), medium glycemic control 150–170 mg/dl (10 rats – group B), poor glycemic control 170–190 mg/dl (20 rats – 2 lots C, C').

Method. These lots receive thiamine hydrochloride in drinking water in quantities of 2mg/L (lots A, B, C) or 4 mg /L (lots A ', C') and were monitored: clinical aspects, blood sugar, weight, amount of drinking water and microalbuminuria. Tail tissue was taken at the beginning of the experiment. After six months of the experiment the lots were sacrificed and some parts from the liver, kidney and heart were analyzed.Results: Thiamine hydrochloride was no toxic in given doses (2 mg/L or 4 mg/L) and no toxicity effects were observed (clinical, kidney, liver or heart). For rats with type 2 diabetes with poor control receiving thiamine in large dose (C') the rate of renal impairment was lower compared with those who received a smaller dose of thiamine hydrochloride (group C *vs*. group C', $p < 0.05$).