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CONFERENCES

SEARCHING THE PRIMARY CAUSE OF DIABETES

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Clinical observations and biochemical investigations showed that diabetes is a complex disorder of the energy homeostasis of the human body, including alteration in all 3 branches of the fuels used in organism: carbohydrates, lipids and proteins. Beta cell dysfunction is an early indicator of diabetogenic process leading finally to the blood glucose regulation, manifested by postprandial and / or fasting blood glucose level. According to our data, published several times in the last years, one of the main indicators of £ cell dysfunction is an increased proinsulin level and an increase in proinsulin/insulin ratio, indicating that a part of proinsulin is not processed in its components: insulin and C peptide. The advantage of these parameters is that they can be obtained in fasting state, from only one blood sample in which glucose can also be determined.

As an increased proinsulin level characterized an immature secretory vesicle, we suppose that such vesicle cannot be promptly and efficiently exocyted, explaining some other early indicators of 3 cell defects: decrease or disappearance of physiological circadian and ultradian insulin oscillation, as well as a decrease or disappearance of early insulin response (to 3–5 min) after an iv secretogogue administration (glucose or arginine). Such defect cannot be detected only by fasting blood glucose, demonstrating that the 3 cell defect precedes and conditions the decompensation of blood glucose regulation. In our view, the main function of 3 cell is not that to produce insulin ("is not an insulin factory"), but can be view as a "*factory of secretory vesicle*" (SV). Because these secretory vesicles are nascent in endoplasmic reticulum (ER) and during the passage of them from c/s- to frans-Golgi apparatus (GA), the primary diabetogenic defect could be located in refined and multiple posttranslational changes of proinsulin and proamylin molecules in endoplasmic reticulum. In or view, steric repulsion and volume exclusion in ER increase the propensity of misfolding of proinsulin and/or proamylin, leading finally to the above mentioned secretory defects and also to the cell apoptosis, secondary to the formation of toxic amylin oligomers inside the SV. This explain the progressive lose of (3 cell mass which start months or years before the decompensation of blood glucose regulation, marking the onset of clinical stage of diabetes.

INDUCTION OF MESENCHYMAL STEM CELLS INTO INSULIN-SECRETING CELLS

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Introduction. Multipotent bone-marrow derived mesenchymal stem cells (MSCs) have shown promising immunomodulatory properties *in vivo* which include inhibition of proliferation and

function of auto-reactive T cells in type 1 diabetes. While the beneficial effect of MSC co-infusion on lowering autoimmune-mediated destruction of transplanted cells has been consistently documented, difficulties in differentiating MSCs to regulated insulin- secreting cells are still an issue.

Materials and methods. We report cloning the human proinsulin transgene into a lentiviral vector backbone and subsequent transduction of MSCs, while sequentially exposing them to HDAC inhibitor treatment. Rat and human mesenchymal stem cells were isolated from the bone-marrow of healthy donors, maintained in culture for 5-7 successive passages and analyzed by flow cytometry for specific markers and HLA-DR expression prior and following transduction and qPCR analysis of pancreatic specific gene expression.

Results. Insulin transcription in the genetically-modified MSCs coincided with the intracellular presence of the protein, as shown by the specific binding of anti-insulin antibodies, and with the detection of insulin in the cell culture supernatant. Preliminary data showed regulation of glycemia following the infusion of insulin-secreting MSCs into recipient diabetic rats. *Conclusion*: Although there are still several issues to be overcome before a successful clinical application is possible, genetically-modified MSCs, capable of long-term, controlled secretion of biologically-active mature insulin, represent a promising therapeutical solution for the treatment of diabetes mellitus.

GENETIC CHANGES DURING ADIPOCYTES ONTOGENESIS

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Introduction. Mammary epithelial cells are embedded in unique extracellular environment to which adipocytes and other stromal cells contribute, being critically dependent on this milieu for survival. Adipokines could play a role in ductal carcinoma tumorigenesis, when molecular promoters of their secretion are highly up-regulated. This study investigated the molecular mechanisms involved in tumorigenic potential of adipocytes, using in vitro mesenchymal stem cells (MSCs) and tumor-associated fibroblasts (TAFs) models of adipogenesis.

Materials and Methods. Human TAFs were isolated from 10 breast cancer surgical pieces, and adipocytes differentiation media was used for induction of TAFs at passage 2. In days 3, 5, 7 and 21 of adipogenesis, total RNA extraction and qRT-PCR quantified gene expression for PPARy, C/EBPa, LPL, Leptin, Leptin receptor, OB-R, huB219.1, huB219.2, huB219.3 and FABP4. Histochemical (Oil Red O) and immunocytochemical (FABP4) staining procedures revealed differentiation rate. Culture supernatants were analyzed for presence of VEGF (leptin-induced) and TNF-a using ELISA method. MSCs – derived adipocytes were subjected to similar procedures, while total RNA of normal and peri-tumoral adipose tissue was used for qRT-PCR gene expression control.

Results. Various proportions of MSCs and TAFs differentiated towards adipocytic lineage (30-50%). Molecular markers are present even in early passages of both MSCs and TAFs, being downregulated with passage number and upregulated in mature adipocytes. C/EBPa and LPL are not present in undifferentiated cells. Leptin is upregulated in TAFs along adipogenic induction, being highly expressed in peri-tumoral adipose tissue. FABP4 and OB-R genes are upregulated in peri-tumoral adipose tissue. VEGF and TNF-a secretion increased in mature adipocytes, normal and peri-tumoral adipose tissue.

Conclusion. Since these cells seem to play important roles in cell proliferation and angiogenesis, it is critical to understand how they may be involved in promoting/inhibiting tumor growth and how they interact with TILs and other immune cells recruited in tumor.

INDUCTION OF DIFFERENTIATION CHANGE THE PHENOTYPE AND INVASION'S POTENTIAL OF GLIOBLASTOMA CANCER STEM CELLS

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Aims. Isolation and characterization of cancer stem cells (CSCs) from human glioblastoma opened new perspectives in primary brain tumors research and offered an alternative therapeutically approach for this severe disease. The purpose of this study was to evaluate the genes expression and the tumorigenic potential of CSCs before and after exposure of these cultures to differentiation induction factors.

Material and Methods. Tumors from patients with confirmed glioblastoma multiforme (GBM) were mechanically and enzymatically dissociated and grown in neural stem cell expansion medium to generate neurospheres (DMEM supplemented with 10 ng/ml EGF; l×B27; l×N2). In order to induct the differentiation of CSCs, fetal serum and all trans-retinoic acid have been added in CSCs cultures. The changes in stem cell markers, matrixmetalloproteases, cadherins and Notch pathway expression have been assessed. The *in vivo* tumorigenic potential of glioblastoma cell cultures (neurospheres) and CSCs exposed to differentiation medium was assessed by intracranial injection of glioblastoma-derived CSC into the right striatum of CD1 nude mice.

Results. Some of the initiated cultures formed the free-floating structures generated by these cells in vitro, the "neurospheres", considered to be a characteristic feature of tumor neural stem cells, which were morphologically and functionally heterogeneous. We successfully developed tumor xenografts in nude mice using these primary cultures. The using of serum-free culture for selection (neurosphere assay) allowed the selection of CSCs containing subpopulation that were able to reproduce original tumour aspect in orthotropic xenografts. The expression of genes regulating neurogenesis on Notch pathway (DLL1, DTX1, HEYL, JAG1, NEURL, NOTCH2, PAX5) were increased from 1.5 to 6 times. The sternness biological feature was correlated with increased of metalloproteases, cadherins and catenin expression and with tumour contra-lateral invasion. The expression of stem cells markers, metalloproteases and cadherins decreased after exposure of the CSCs cultures to fetal serum and trans-retinoic acid. *In vivo* experiments demonstrated also the inhibition of tumorigenic potential of differentiated CSCs cultures.

Conclusions. Serum-free culture allowed the selection of a subpopulation containing CSCs with increased tumorigenic potential. When exposed to differentiation induction factors, CSCs cultures showed a decrease in the expression of stem cells markers and lost their tumorigenic potential.

BIODEGRADABLE TRIDIMENSIONAL SCAFFOLD-CELL INTERACTION FOR THE RECONSTRUCTION OF THE INTESTINE BY TISSUE ENGINEERING

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Introduction. Making the 3D-scaffolds for the future tissue and developing a biocompatible surface are one of the very important steps in developing complex structures of an organ. In

order to obtain a tissue-engineered intestine, scaffolds made from biodegradable materials (polylactic acid – PLA and polydioxanone – PDO) were produce by electrospinning method and functionalized with dopamine and biotine. NIH3T3 fibroblasts were cultured on engineered scaffold and affinity for substrate was measured. The functionalized scaffolds were compared with non-functionalized ones. Materials and methods: A composite scaffold was produced from PLA, deposited on woven PDO structure using electrospinning. Following the electrospinning the composite were functionalized with dopamine and biotin by dipping the matrix in solutions with mentioned additives. Samples of as-machined and functionalized matrixes were tested in vivo using NIH3T3 fibroblasts. Cells were cultured in Complete Dulbecco's Modified Eagle Medium (CDMEM) with 10 % foetal calf serum (FCS) and changed every two days. Time points were established at 3, 17 and 24, hours to assess morphology (electron microscopy), cytotoxicity, viability and proliferation (Alamar Blue). Results: The PLA form a nonwoven mechanical stable matrix made from 0.32–1.8 |jm random fibres with 80% porosity quotient. Affinity for substrate, measured by changes in morphology, was best demonstrated for substrates functionalized with dopamine. Cytotoxicity, viability and proliferation after 24 hours were best demonstrated again for substrates functionalised with dopamine, followed by non-functionalized samples. Conclusion: PLA-PDO composite functionalised scaffolds machined by electrospinning form 3D matrix that expose an increased interaction between substrate and cells. After 24 hours cell attachment and proliferation was better for substrates functionalised with dopamine. Acknowledgements: This research is an integrant part of GRANT CNMP 42-118 BIOINTTECH.

RECENT ADVANCES IN CELL FATE AND PHENOTYPE REPROGRAMMING

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The successful generation of induced pluripotent cells has represented a landmark in scientific research and offered the proof that major phenotypic changes of terminally differentiated cells are possible by genetic reprogramming. The rarity of reprogramming events appears to contradict the robustness with which the complex phenotype of stem cells can reliably be generated. This apparent paradox, however, is explained by the epigenetic factors which characterize “preprogrammed” attractor states that emerge from the dynamical constraints of the gene regulatory networks. Significant new questions in the field of cell fate reprogramming were raised by recent findings of fibroblast to neuron or cardiomiocyte phenotype reprogramming by defined transcription factors. The role of transcription factors in defining cell phenotype by specifically controlling gene expression is also suggested by findings showing the possibility of inducing liver specific gene expression in fibroblasts. Directing differentiation potential of stem cells towards a specific lineage by forced expression of transcription factors might provide in the future new means of generation of terminally differentiated cells for regenerative therapies.

STUDIES OF TWO LOCAL DELIVERY METHODS OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS TO ACUTE IATROGENIC CUTANEO-MUSCULAR WOUNDS IN RATS

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Background. The mesenchymal stem cells (MSCs) have previously been used in preclinical and clinical applications with the intent to accelerate cutaneous wound healing. Even with favorable results reported worldwide, the role of bone marrow-derived MSCs has not been yet thoroughly elucidated. There are on-going attempts to determine the optimum number and route of cell delivery to the wound.

Objectives. Our purposes were: (1) to investigate a method of in vitro bone marrow-derived MSCs marking that enables their in vivo tracking; (2) to compare the engraftment of MSCs and their impact on healing by using two routes of cell administration to acute iatrogenic cutaneo-muscular wounds in Wistar albino rats.

Materials and methods. MSCs were harvested, isolated, preserved, and multiplied from rat strains. MSCs were characterized by light microscopy and flow cytometry (CD1 lb-, CD34-, CD73+, CD90+, CD105+). MSCs from Wistar rats were incubated with SP-DiOC18(3) and observed for several passages. The fluorescence of cultured MSCs from the GFP+ LEW-Tg(CAG-EGFP) 1 Ys rats was checked by light microscopy and flow cytometry. The sterility of cultured MSCs was tested by terminal restriction fragment length polymorphism (T-RFLP). The cytogenic characterization of MSCs harvested from Wistar rats was performed by analysing a set of 40 single-nucleotide- polymorphisms (SNPs) on 21 chromosomes. MSCs from GFP+ rats were used for in vivo studies. Two delivery routes of identical number of cells to the iatrogenic cutaneo-muscular wounds were used on Wistar albino rats: local injection of MSCs or application of collagen sponge embedded with MSCs. No immunosuppression was used.The animals were macroscopically observed and digital photos were taken every 48 hours until the complete wound healing. The wound area was assessed by planimetry using the demo version of PictZar software. Repeated biopsies of the regenerating tissues were performed and assessed by T-RFLP, histology, and immunofluorescence.

Results. Fluorescent dye staining of MSCs from Wistar rats was lost during passaging and thus precluded theirin vivo usage. MSCs from GFP+ rats expressed 81% fluorescence and were used for further studies. Cytogenic studies did not detect abnormalities. T-RFLP confirmed the sterility of cultured MSCs. Bacterial burden of regenerated tissues was insignificant. The healing time and engraftment of GFP+ were not significantly different between the two different routes of cell administration.

Conclusions. Culture and tracking of GFP+ MSCs administered for acute iatrogenic rat wounds were possible. The number of engrafted GFP+ cells in the regenerated tissues was similar in coherence with tight wound healing times for both MSCs administration routes.

POSTERS

IT POSSIBLE TO USE IL28B GENE POLYMORPHISM FOR PREDICTION OF EARLY VIROLOGICAL RESPONSE IN PATIENTS WITH RECURRENT HEPATITIS C FOLLOWING LIVER TRANSPLANTATION?

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Introduction. In patients with recurrent HCV infection after liver transplantation (LT), analyses of single nucleotide polymorphisms of IL28B in recipient and donor tissues proved to allow prediction of sustained virological response to PEG-lnterferon and ribavirin therapy. Aim: To investigate IL28B polymorphism in Romanian LT recipients with recurrent hepatitis C during antiviral therapy. Methods: Twelve LT recipient DNA samples were screened for rsl 2980275 single nucleotide polymorphism near the IL28B gene. Results: There were analyzed 2 females and 10 males with a mean age of 52.5±6.9 years at beginning of antiviral therapy and a mean time since LT of 16.3±11.6 months. Distribution of IL28B genotypes were: C/C -1 patient (8.3%), C/T -7 patients (58.3%), T/T -4 patients (33.4%). Nine out of 12 patients had early virological response (EVR). EVR was not associated with recipients IL28B genotype non-T/T. Aminotransferases were significantly higher in genotype T/T patients compared to C/T and C/C patients: AST =285.7±87.4 *vs* 139.5±20.2 (p=0.04) and ALT= 325.5±84.4 *vs* 149±28.1 (p=0.03). Although not statistically significant, baseline viral load, necroinflammation score >2 and fibrosis stage >2 (METAVIR classification) were higher in genotype T/T patients. Conclusions: Recipient IL28B genotype is not sufficient to predict EVR. Donor IL28B genotype should be also investigated. LT recipients with T/T genotype seem to have a more severe recurrent hepatitis C.

ISOLATION OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS FOR DEVELOPING CELL BASED THERAPIES

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Cell therapy holds great promise for use in regenerative medecine. The adipose tissue became in the last years, a successfully and important alternative source of postnatal Mesenchymal Stem Cells (MSC). The use of Adipose Derrived Stromal Stem Cells (ADSCs), unlike Embryonal Stem cells (ES) does not involve ethical conflicts. ADSCs have the same properties and express multiple CD marker antigens similar to bone marrow derived MSC; cloning studies have shown that ADSCs present multilineage differentiation potential (adipogenic, chondrogenic, osteogenic, myogenic, hepatogenic, neuronal etc.). ADSCs may be clinically used without cell expansion because of their large quantities but, it is of important value to culture and expand them for further developments of new cell-based therapies. Here we describe our experience in the isolation of ADSCs from human adipose tissue and present preliminary results of ADSCs utilisation in a wound healing model.

GREEN LIGHT EFFECT ON SERUM ALBUMINS AND DNA

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The interaction of electromagnetic fields with biological structures is well studied. Nevertheless, the large majority of these studies have been concerned with linear effects, within the canonic UV-Vis resonant theory. This paper presents two new experimental results: the protective effect of green light (GL) on ultraviolet (UV) denaturation of proteins, and the effect of GL on protein macromolecular structures. The protective effect of GL was revealed on human serum albumins (HSA) and nucleic acids (DNA), and recorded by gel electrophoresis and UV spectroscopy. We used HSA obtained from human blood, screened for normal chemical parameters and commercial DNA (Sigma-Aldrich) in a 0.9% NaCI stock solution, 1 mg/ml, prepared under refrigeration for 24 h with gentle stirring. As compared with the control recording, strong denaturation of the albumin fractions under UV is observed, leading to a decrease of the albumin/globulin (A/G) ratio from 1.97 in the control sample to 0.43 in the UV irradiated one. GL irradiation provided protection, leading to a decrease in A/G ratio of 1.11. A mechanism for these phenomena is suggested, based on a double-photon absorption process. Since the described effects reveal nonlinear interactions between GL and biological systems, GL irradiation may induce transitions similar to the Raman and Rydberg phenomenology. This nonlinear effect may lead to generation of long-lived Rydberg macromolecular systems, capable of long- range interactions. These newly suggested systems, with macroscopic quantum coherence behaviors, may block the UV denaturation processes. Such a protective effect, particularly as revealed by GL irradiation of HSA, may have significant implications for the medical field.

SYNERGISTIC EFFECT OF GOLD NANOPARTICLES CONJUGATED WITH CISPLATIN/DOXOROBICIN/ CAPECITABINE PLUS RIBAVIRIN COMBINATION CHEMOTHERAPY ON HEPATIC CANCER STEM-LIKE CELLS

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The failure of existing treatments for liver cancer has recently been attributed to the existence of cancer stem cells because of their chemoresistant properties as well as their ability to stimulate neoangiogenesis. Gold nanoparticles (GNPs) could provide significant gains in medical care due to their easily preparation, anti-angiogenic properties and their binding capacity to different target molecules. The aim of the current study was to evaluate *in vitro* the antitumor efficacy of gold nanoparticles conjugated to conventional chemotherapy drugs for liver cancer, hoping to provide new therapy with minimal toxicity and increased efficacy profiles. GNPs

stabilized with a monolayer of L-aspartate-cytostatic drug were synthesized as a tumor targeted drug delivery carrier. The drug (doxorubicin, cisplatin and capecitabine) was noncovalent conjugated onto the hydrophilic shell of GNPs-L-Aspartate nanostructure. TEM, UV-Vis, FTIR, spectroscopy and NMR analysis were employed to characterize the morphological, optical and structural properties of these drug metallic nanostructures. We also tested the in vitro effects of ribavirin as this drug is an essential anti-viral used in the treatment of chronic liver disease due to hepatitis B or C infection. The chemical structures formed by GNPs with each cytostatic were added in cultures of liver cancer stem cells, normal liver stem cells and more differentiated tumor cell line HepG2. The sensitivity of each drug delivery system, GNPs-L-Aspartate-Drug, was tested during MTT proliferation assay test. As the tumor cell growth rates of the anticancer drugs binded to GN Ps is statistically significant lower in comparison with cytostatic drugs alone, we can clearly say that GNPs facilitated the anticancer drugs to reverse the resistance of cancer stem cells to cisplatin, doxorubicin and capecitabine plus ribavirin, offering a new chemotherapy strategy for patients diagnosed with unresectable hepatocellular carcinoma. The identification of new therapeutic targets can aid in the development of better approaches in the near future for patients diagnosed with hepato cellularcarcinoma.

CORRELATION BETWEEN COLLECTED CORD BLOOD VOLUME, TOTAL NUCLEATED CELL AND CD34* CELL COUNT OF UMBILICAL CORD BLOOD DERIVED STEM CELLS UNITS

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Umbilical cord blood (UCB) represents an efficient alternative source of hematopoietic stem cells for use in allogeneic and autologous transplantation. Parameters commonly used to evaluate a umbilical cord blood derived stem cells unit and predict transplant outcomes have been: collected cord blood volume, total nucleated cell (TNC) and $CD34^+$ cell count.

Objective. The aim of this study is to evaluate if UCB volume can be used to evaluate any given sample prior to processing, and if the total nucleated cell count (TNCC) alone are sufficient for predicting the efficiency of cord blood units for transplant.

Methods. A total of 827 collected UCB samples were processed, characterized and banked in Stem-Health Unirea -cord blood bank. The viable $CD34^+$ cells and TNCC were quantify by flow citometry respectively hematological analyzer.

Results. A typical successful UCB collection volume was on average 60–80 ml within a range 15–120aml. These samples contain 4.72 - 388.25×10^7 TNC and 0.09- $35{,}89\times10^6$ CD34+ cells. It was observed that higher volume samples were correlated with higher amounts of TNC, $CD34^+$ cells, but also it was observed that the high volume units may have lower or equal amounts of TNC and $CD34^+$ cell than low volume units. Despite an apparent linear correlation between TNCC and $CD34^+$ cell number, it was demonstrated a high degree of variation in $CD34^+$ cell counts that could be between 0.1%–2.07% of TNCC.

Conclusion. Neither collected cord blood volume nor TNCC offer accurate informations about number of $CD34^+$ cell in the UCB derived stem cells unit. Thus, for UCB transplantation it is necessary to know the $CD34^+$ cell count in order to exclude the possibility to use a poor unit in hematopoietic stem cells.

PROTEOMIC ANALYSIS OF INSULINOMA TISSUE – PRELIMINARY DATA

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Introduction. The increasing need for early diagnosis of pancreatic dysfunction including diabetes, promoted us to develop new strategies to identify molecular markers that will allow rapid and suitable clinical decision.

The aim of the project was to develop comparative high resolution two-dimensional electrophoresis of the pancreatic 3 cells harvested from human patients with known pathologies and healthy donor as control.

Materials and methods. Pancreatic samples were collected during abdominal surgery from one healthy donor (PUC) and two patients with insulinoma, histological diagnosed further as neuroendocrine carcinoma (PUI1) and benign neuroendocrine tumor (PUI2). Insulinomas are rare pancreatic endocrine tumors developed from 3 cells island, characterized by hypersecretion of insulin. The tissues were either immediately frozen or processed for biochemical analysis. Semithin sections were stained with toluidine blue for histological examination or with appropriate antibodies to evidence different heat shock proteins (HSP). Equivalent amounts of total protein extracted from each sample were separated by uni- and two-dimensional gel electrophoresis. The resulting gels were either stained with silver nitrate to evaluate the proteomic profile or transferred onto nitrocellulose membrane for immunoblotting experiments.

Results. The histological toluidine blue staining evidenced the local area of modified tissue in comparison with the healthy pancreatic island. Gel electrophoresis of selective extraction of cytosolic and nuclear fraction from the total tissue homogenate revealed a different protein distribution in tumor compared to normal tissue. Western blot experiments performed with specific antibodies demonstrated the overexpression of the stress proteins HSP60, HSP70 and HSP90 in insulinoma tissue. *Conclusions.* The preliminary results showed that, the proteomic analysis proposed is a promising strategy that will allow the evaluation of pathological proteomic differences in the pancreatic insulinoma. The work was supported by the POSDRU project no 89/1.5/S/60746 and the ICBP "N.Simionescu" of the Romanian Academy.

TISSUE PROTEOMICS IN NODAL B CELL NON-HODGKIN LYMPHOMAS

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Malignant lymphomas are monoclonal lymphoid proliferations of lymphocytes, lymphocytes precursors and histiocytes. At this time are recognized two main categories: non-Hodgkin and Hodgkin lymphomas. Lymphomas can be classified in nodal and extra nodal lymphomas, the last type being primary or secondary. For the estimation of morphologic and immunohisto-chemical markers implied in nodal B cell non-Hodgkin lymphomas we studied a group of patients diagnosed with this type of malignancy in Clinical County Emergency Hospital Constanta.

The group comprises 56 patients and their ages are between 2 and 88 years. The histopathological type of lymphoma diagnosed in our patients group were: diffuse large B-cell lymphoma (40,6%), small lymphocytic lymphoma (24%), lymphoblastic lymphoma (18,5%) and marginal zone lymphoma (16,9%). In these types of lymphoma we determined the immunohisto-chemical expressions of CD3, CD10, CD11, CD 15, CD19, CD20, Ki67, BCL-2, p53. The pan B cell markers expressions were positive which confirmed the affiliation to B cell lymphomas. The p53 and Ki67 intense positive expressions in aggressive lymphoma are correlated in our study with BCL-2 negative expression. This paper is realized inside of PNCDIII project, No. 42-157/2008, "Identification of Genetic and Morphologic Markers Involved in Early Diagnosis and Prognosis of Lymph Node B Cell Non-Hodgkin Lymphoma".

THE BONE REMODELLING INBALANCE IN PATIENTS WITH PSORIATIC AND RHEUMATOID ARTHRITIS

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Lntroduction. The RANK, sRANKL and OPG constitute a complex mediator system involved in the regulation of the bone remodeling process in arthritis. Obiectives:The aim of this study was to determine serum levels of sRANKL and OPG, elevated in patients with arthritis.

Material and Method. The study was made on three cohorts of patients with psoriatis arthritis, rheumatoid arthritis and the control group (pathients without arthritis). Serum levels of the markers were measured by ELISA technique.

Results and discussions. Cohort 1: serum levels sRANKL were 51.98±10.74 pg/ml, serum levels OPG were 39.05±4.89 pg/ml. Cohort 2: serum levels sRANKL were 64.71 ±2.06 pg/ml, serum levels OPG were 39.2±5.78 pg/ml. Control group: serum levels sRANKL were 32.48±3.03 pg/ml, serum levels OPG 38.05±4.89 pg/ml.

Conclusions. The serum levels OPG in patient with arthritis were increased, demonstrating osteoblast activation, as compared to control group. The serum levels sRANKL are semnificantly higher in rheumatoid artritis as compared to psoriatic arthritis and the control group. The bone remodelling inbalance in patients with arthritis decrease in bone formation and increases bone resorption.

INFLAMMATION BIOMARKERS IN HIV SEROPOSITIVE PATIENTS UNDERGOING ANTIRETROVIRAL THERAPY

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Objective. Despite antiretroviral therapy, HIV infection maintains chronic inflammation leading to atherosclerosis, cardiovascular diseases, metabolic disturbances and premature aging, even at young age. Romanian HIV seropositive population is made mainly of young adults parenterally infected

before 1990. Our objective is to assess the level of inflammatory biomarkers in young adult HIV seropositive population under antiretroviral therapy (HAART) and their clinical consequences.

Methods. preliminary results of an ongoing research grant (PNCDI2 no.62077/2008) on HIV infected patients undergoing HAART, recruited in INBIMB, in 18 months. Tumor necrosis factor-alpha (TNF alpha), interleukin-6 (IL6), monocyte chemotactic protein 1 (MCP1), high-sensitivity C-reactive protein (hsPCR) were performed. According to International Diabetes Federation, metabolic syndrome includes alteration of body mass index/central obesity, triglycerides, HDL cholesterol, glycemia, blood pressure. The cardiovascular risk was quantified by means of Framingham score.

Results. Our population included 106 patients, gender balanced with mode age of 20 years; median CD4 cell count 492/mmc; HIV viremia undetectable in 70% of cases. Subjects with undetectable *versus* detectable HIV viremia had median values for TNF alpha, IL6, MCP1, hsPCR as follows: 9.5 pg/ml *versus* 12.6 pg/ml, 27.4 pg/ml *versus* 25.4 pg/ml, 278.4 pg/ml *versus* 304.9 pg/ml, 1.9 ng/ml *versus* 2.3 ng/ml, respectively, without statistically significant differences. Cardiovascular risk was not associated with inflammation biomarkers in that phase of analysis. But metabolic syndrome was significantly correlated with high values of MCP1 ($p<0.05$).

Conclusions. Even if HIV replication was controlled, we registered chronic inflammation in these young patients and a correlation with the presence of metabolic syndrome. Anti- cytokine medication should be added to HAART, with consequent benefits on preventing premature atherosclerosis and metabolic diseases.

E6, E7, LCRHPV16 VIRAL VARIANTS IN CERVICAL CANCER

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Introduction. Human papilloma virus (HPV) represents the etiologic factor of malign genital neoplasia. Epidemiologic studies revealed that different HPV16 viral variants have been found to show different geographic distribution with a varied oncogenic potential. Although a number of sequence variations have been reported in HPV 16 E6, E7 and LI gene as well as in LCR in cervical cancer, the most interesting are those of E6 and E7 oncogenes. E6 viral oncogene has been found to show more variations compared to E7, which is relatively conserved.

Materials and methods. *Paraffin embedded tissues* were collected from patients with histopathologicall diagnosis of HPV infection. The HPV 16 genotype presence in biological samples was confirmed by viral testing and genotyping. *E6HPV16, as well as* E7, LCR variants were determined by sequencing performed with ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems) using ABI PRISM BigDye Terminator v3.1 kit.

Results and discussions. The most frequent mutations found in E6 gene were *missense* and *silent:* C279G in 6/8 (75%) samples, T350G in 4/8 (50%) samples, A305C in 3/8 (37%) samples. The variants number differs with histopathological diagnosis. Most mutations were found in CIN3 lesions (7 missense mutations) - A279G (66%), A305T (50%) and T350G (50%). In the investigated samples, we discovered two new E6 variants: A305T and A279G variants, which were present in 37% and 75% respectively of analyzed samples.

Conclusions. We found a significant correlation between mutations and cervical lesions severity ($p=0.0227$, CI (95%) =0.01028-0.8546). In CIN2 lesions the average value for mutations (0.411) was smaller than in CIN3 and SCC.

THE CYTOKINE PROFILE IN CERVICAL DISEASE HPV INDUCED

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Introduction. As inter-individual variations of immune response in genital infection with human papilloma virus (HPV) are determinant factors of persistent viral infection and in progression of cervical lesions to cancer, *the aim* of our study was to investigate cytokine levels in persistent lesions of the uterine cervix.

Materials and Methods. 184 high-risk HPV DNA positive patients (confirmed by viral testing with Linear Array, Roche), age 17-48 years, were monitored between 2008-2010. HPV positive patients with cytological interpretation of ASCUS orLGSIL, presenting HPV persistent infection were investigated for cytokine levels detection. The persistent infections were confirmed by the presence of the same HPV DNA type in consecutive testing. The control group consisted of 10 high-risk HPV- negative women. Using exfoliated cells collected by cervical cytology brush, we assessed pro- and antiinflammatory cytokine expression [interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor a (TNF-a), and interferon y (IFN-y)] in RT-real-time PCR (Taqman).

Results. Persistence was significantly greater by oncogenic HPV types 16,18 and 51 alone or in association with low/medium risk types. By compare with control group, we found that IFN-y levels was increased in ASCIIS and seems to be associated with low viral oncogene expression. The pro-inflammatory cytokines expression levels (TNF-a, IL-6, and IL-10) were higher in LGSIL lesions. While IL-4 levels were higher in LSIL lesions, especially in HPV 16 and 18 positive cases, IL-2 levels were slightly increased in patients presenting ASCUS and HPV 18 positive infection. No correlation with other oncogenic types (31, 33 and 45) was observed.

Conclusions. Among HPV-persistent infected women, pro-inflammatory cytokine expression levels are increased and that may be considered as a prognostic marker for oncogenic potential of high-risk HPV.

AN EUROPEAN NETWORK ON CERVICAL CANCER SURVEILLANCE AND CONTROL IN THE NEW MEMBER STATES: AURORA PROJECT PRESENTATION

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AURORA project aims to identify a common and feasible strategy on how to promote Cervical Cancer Screening in the New EU Member States targeting women in the reproductive age (30–69 years old) and ensuring the coverage of the hard to reach groups, assist the New EU MemberStates in the implementation of evidence-based screening for cervical cancer and promote a European exchange of information and expertise on the development and implementation of good practices in Cervical Cancer Prevention and Advocacy. Knowledge acquired through AURORA will be disseminated in the EU, particularly to EU 12 MemberStates: Latvia, Bulgaria, Romania, Czech Republic, Cyprus, Slovakia, Slovenia, Hungary and Poland. AURORA is structured in several macro-tasks. Firstly, the project partners will carry out the analysis of the local context which will be useful to the identification and analysis of good practices and strategies in the fight against Cervical Cancer on how to promote the Cervical Cancer Screening among the project target groups. Then, in line with the COUNCIL RECOMMENDATION of 2 December 2003 on Cancer Screening stating that "*adequate training of personnel is a prerequisite for high quality screening*" and with the European guidelines for quality assurance in cervical cancer screening –

Second Edition, stating that the function of advocacy groups in cancer screening is increasingly essential, AURORA will organize a training course for healthcare professionals and a training course for advocacy leaders. Finally, thanks to the mapping and analysis of all the prevention and training actions implemented in the participating countries, an E-Learning environment will be developed to serve all the users of the participating countries interested to be trained on the project issues.

H-CUBE: HBV-HCV-HIV: THREE DIFFERENT AND SERIOUS THREATS FOR EUROPEAN YOUNG PEOPLE, A NETWORK TO STUDY AND TO FACE THESE CHALLENGES IN EU 2009-2011

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H-CUBE project is aimed to analyse the huge number of training courses and prevention campaigns about HBV, HCV and HIV implemented in Europe in the last years. Thanks to this activity, H-CUBE consortium will be able to identify the best practices about these topics and to share them with other EU subjects not directly involved in the project. Knowledge acquired through AURORA will be disseminated in the EU, particularly to EU Member States: Lithuania, Bulgaria, Romania, Czech Republic, Greece, Cyprus, Slovakia, Slovenia, Hungary, and Poland. According to this framework H-CUBE project is focused on ways for the exchange and dissemination of good practices on STDs training and prevention programmes and identification of innovative strategies to promote safer sex among adolescents, including access to targeted services and improving awareness of STDs. Due to development of the digital platform H-CUBE is aimed to deliver an e-Learning training course targeted on needs of young people, and individual regional background. The courses will be organised in various sections, including information about HBV, HCV and HIV/AIDS, the impact of HBV, HCV and HIV/AIDS epidemic and the global response, leader preparation to identify worries and concerns about HBV, HCV and HIV/AIDS. The training courses prepare professionals and parents to teach on the STDs subject but also to use the e-learning methodology. The direct beneficiaries of the project outcomes will be, in the first phase, the community involved in the consortium. The dissemination activities will be thus primarily addressed to these targeted users such as: Public Administrations, Associations involved in the fight to HBV, HCV and HIV, Clinical associations, Healthcare professionals and Parents Movements.

THE ROLE OF LABORATORY ANIMALS IN TESTING AND DEVELOPEMENT OF BIOLOGICAL PRODUCTS

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The administration of biological products (*e.g.* vaccines and immune-modulators) to man and animals described from the product characteristics point of view have been made since a century ago

starting with the first vaccines and serums prepared by Pasteur and other microbiologists.

Presently, laboratory animals are used in safety tests for developing and testing vaccines and immune-modulators that are made in compliance of GLP / GMP regulations and pharmacopoeia monographs. The number of “in vivo” tests declared valid for testing biological product safety is higher than “in vitro”. “In vitro” methods are not considered to be capable of assessing all the relationships between the human/animal body and test product.