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FOREWORD

Professor **Nicolae Cajal (1919–2004)** is considered between the founders of a new Romanian scientific school, a research institute of the Romanian Academy called the "St. Nicolau" Institute of Virology. Many renowned physicians, pioneers of national medical specialties, have been educated in this Institute or in the Chair of Virology at the Bucharest University of Medicine and Pharmacy. Among Cajal's most significant scientific contributions we will cite only a few, such as production and application of viral vaccines against poliomyelitis, influenza, and measles; important research programs in the domain of significant viral diseases as: AIDS, viral hepatitis, acute and chronic viral neuroinfections; the implication of certain viruses in oncogenesis and others. Cajal set high standards for him and other and shaped the field by training many young virologists all over the country and abroad. His experience indicates that successful projects adopt multidisciplinary approaches. Many of Cajal's students and their second-, and third- "descendants" are still major contributors to the field of virology, vaccinology and cell biology. In the last years Cajal Foundation created by his daughter, **Irina Cajal**, organized annual scientific sympozia that have been attended by scientists in microbiology, biochemistry, informatics etc. as well as practitioners in the field of infectious diseases and epidemiolgy. This year symposium hosted three sessions: virology, genomics and translational oncology.

TRANSLATIONAL ONCOLOGY SESSION

FROM BENCH TO BEDSIDE: NEW POSSIBLE BIOMARKERS IN EARLY AND LATE STAGES OF COLON CANCER - PRELIMINARY RESULTS

LILIANA LIVIA PASLARU[1], ANCA NASTASE[2], SIMONA DIMA[3] , VLAD HERLEA[3], MIHNEA IONESCU[3],CRISTIAN GHEORGHE[3], TRAIAN DUMITRASCU[3], SORIN ALEXANDRESCU[3], ANA MARIA EFTIMIE[2],ANA MARIA NICULESCU[2], VLADIMIR LAZAR[4] and IRINEL POPESCU[3]

[1] University of Medicine and Pharmacy „Carol Davila" Bucharest, Postgraduate Department of Biochemistry
[2] RNTECH Bucharest, Romania
[3] Fundeni Clinical Institute of Digestive Diseases and Liver Transplantation
[4] Institute of Cancerology Gustave Roussy, Villejuif, France

INTRODUCTION

Today, an important priority in cancer research is the detection of new clinically useful biomarkers whose expression could represent important tools for a better diagnostic, prognostic and for implementing personalized treatment of the disease.

MATERIAL AND METHODS

The trasnscriptomic study was realized on a cohort of 31 patients with primary colon tumors.

We used triplets represented by normal, adenoma and tumoral tissue collected from the same patient during surgery. All these samples were analyzed by microarray and validated by real time-PCR.

In accordance with national and international standards, informed written consent was obtained from all patients and research protocols were approved by the ethical committee.

RESULTS

The microarray study allowed us to assess the candidate gene expression differences between tumoral and normal tissue and between adenoma and normal tissue. Thus, we could identify important genes involved in colon cancer carcinogenesis and progression.

In order to validate mRNA differential expression, we analyzed by real-time PCR the expression of 10 genes (MMP-1, MMP-3, MMP-7, DEFA-1, DEFA-5, DEFA-6, IL-8, CXCL-1, SPP-1, CTHRC-1), identified as up-regulated by microarray, in adenoma and tumoral samples compared with matched normal tissues.

CONCLUSION

All analysed genes are involved in tumorigenesis and progression.

We remarked that DEFA5 and DEFA6 are key factors in adenoma formation while matrylisin 1 (MMP7) is very important in the onset and in the progression of the disease.

The pattern of expression for the studied cytokine shows that colon cancer begins and progress as an inflammatory condition. Thus, we found out that IL8 has a high mRNA level in adenomas and irrespective of tumor stage, in adenocarcinoma as well. The level of expression for SPP1 is correlated with tumor level.

Therefore within our study we underline the importance of using a panel of biomarkers for diseases evaluation and the implementation of the personalized treatment.

TUMOR-ASSOCIATED FIBROBLASTS INVOLVEMENT IN CANCER DEVELOPMENT

PAUNESCU V.[1], BOJIN FLORINA[1], TATU C.A.[1,2], GAVRILIUC OANA[1], ROSCA ADRIANA[2], GRUIA ALEXANDRA[2], CIUCULESCU FELICIA[1], TANASIE GABRIELA[1,2], TATU CARMEN[1,2] and BUNU CARMEN[1]

[1]"Victor Babes" University of Medicine and Pharmacy Timisoara, Romania
[2] Clinical Emergency County Hospital Timisoara, Romania

INTRODUCTION

The complex cellular tumor (micro) environment comprises immunocompetent and inflammatory cells, endothelial cells and fibroblasts. All of these cell types may critically influence the multi-step process of carcinogenesis and malignant phenotype. Fibroblasts are known to take part in immune reaction during tissue damage and injury by modulating local cellular and cytokine milieu, adjusting the kinetics and components of the inflammatory infiltrate, and by modulating the functional status of the immunocompetent cells.

Main purpose of our study was investigation of tumor-associated fibroblasts (TAFs) phenotype and function, and to what extent they become activated within tumor environment and secrete different paracrine and autocrine factors, which will further play a role in tumorigenesis.

MATERIAL AND METHODS

Human TAFs were isolated from breast cancer surgical pieces using enzymatic digestion, cultivated and expanded in vitro as monolayer cell culture. When cell population was pure, the trilineage differentiation potential (to adypocytes, chondrocytes, and osteoblasts) was assessed under appropriate differentiation medium. Supernatants of cell cultures at different passages were collected and presence of IL-4, IL-10, IL-13, TGF-β1, TNF-α, INF-γ and VEGF was detected by ELISA method. Specific mouse anti-human fluorochrome-conjugated antibodies identified in flow-cytometric analysis surface markers of TAFs, such as CD14, CD117, CD90, CD106, CD44, CD29, CD73, HLA-DR, CXCR4. Expression of cytoskeleton and extracellular matrix proteins was revealed by immunocytochemistry (vimentin, α-smooth muscle actin). Breast cancer isolated tumor cells and tumor cell lines (SK-BR3, MDA-MB231, and MDA-MB468) were tested for their ability to adhere to the TAFs substrate in flowchamber when the shear stress generated was increasing from 0.35 to 15 dyne/cm^2. Stimulation of TAFs used endogenous secretory factors for flowchamber assays.

RESULTS

Secretion of cytokines with direct immuno-suppressive effect, such as IL-10 and IL-13, and other cytokines, IL-4 and TNF-α, was increased in cultured TAFs at all passages. Enhanced production of TGF-β1 by TAFs may also be a critical factor in tumor homeostasis. Expression of MHC class II molecules (HLA-DR) on activated TAFs contribute to an additional level of immunosuppression and pro-tumoral effect. High level of VEGF production suggests that TAFs provide not only structural support, but also pro-angiogenetic molecules for tumor vascularization. α-smooth muscle actin expression in fibroblasts is variable and associated with an activated status. Adherence of tumor cells on TAFs substrate was increased when stimulated with VEGF for all values of shear stress, while IL-4 induced increased adherence only for shear stresses less than 8 dyne/cm^2.

CONCLUSION

The complex network of effects TAF transduced by enhanced expression of various immune cell deactivating/suppressing factors and neoangiogenesis molecules should stimulate consideration of TAFs-based therapeutic designs.

Key words: Tumor-associated fibroblasts, chemokines, VEGF, tumorigenesis

ACKNOWLEDGEMENTS

The study was supported by PNCD-II Grant ONCOSTEM no. 41-019/2007.

MOLECULAR MARKERS IN BCR/ABL NEGATIVE MYELOPROLIFERATIVE NEOPLASMS. FROM DETECTION TO SELECTIVE THERAPY

CARMEN C. DIACONU[1], LAURA DRAGOMIR NECULA[1], MIHAELA CHIVU[1], MIHAELA CLOSCA-GHEORGHIU[2], ANCA R. LUPU[2], AURORA ARGHIR[3], GEORGETA CARDOS[3], ANA NEAGU[1], IOANA ALDEA[1], LILIA MATEI[1], CORALIA BLEOTU[1], IRINA ALEXIU[1], DENISA DRAGU[1], MARIUS ATAMAN[1], COSMIN I. STANCU[1], DELIA MUT-POPESCU[2], AGRIPINA LUNGEANU[3], MIHAIL E. HINESCU[3] and STEFAN N. CONSTANTINESCU[4]

[1] Stefan S. Nicolau Institute of Virology, Bucharest, Romania
[2] "Coltea" Clinic Hospital Bucharest, Romania
[3] "Dr. Victor Babes" National Institute of Pathology, Bucharest, Romania
[4] Université Catholique de Louvain, Brussels, Christian de Duve Institute of Cellular Pathology, Ludwig Institute for Cancer Research, Belgium

INTRODUCTION

The main BCR/ABL negative myeloproliferative neoplasms (MPN) are today recognized as Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF). These conditions are clonal disorders of hematopoietic progenitor cells that are able to differentiate into excess numbers of mature cells. The recently discovered *JAK2* V617F mutation has become the biological marker for these conditions and promoted the development of selective therapy. *JAK2* V617F or other JAK2 JH2 region mutations lead to a lack of inhibition of the JH1 domain and enables constitutive tyrosine kinase activity. Recently, mutations in the thrombopoietin receptor (c-Mpl) that act by causing activating conformational changes in the receptor, have been found.

The more precise diagnosis of the patients will lead to advances of clinical investigations aimed at defining the pathophysiology and more effective treatment of these diseases.

MATERIALS AND METHODS

254 patients diagnosed with BCR/ABL negative MPN were investigated for the presence of different JAK2 and c-Mpl mutations using allele-specific PCR, sequencing and TaqMan assays. Using mutational information and bicistronic retroviral vector transduction, we have developed whole-cell systems that express mutated JAK2 and/or c-mpl receptor and allow cellular profiling in parallel against molecular libraries.

RESULTS

JAK2 V617F was most common mutation found in patients with BCR/ABL negative MPN (in 79% PV, 50% TE and 62% of the PMF patients). Sequencing JH2 region of JAK2 lead to the identification of different other mutations (C616G, C618R, K539L). The MPL515 mutations were infrequent, but it is expected that further c-Mpl mutations will be discovered.

The whole-cell systems that were developed, simultaneously interrogate the activities of libraries of molecules against a panel of cellular assays in replicates, in dose–response format.

CONCLUSION

The discovery of JAK2 and c-Mpl mutations is leading to rapid advancements in understanding the pathophysiology and in the treatment of MPN.

MODIFICATIONS OF GP130 SIGNALLING IN GASTRIC EPITHELIUM DURING TUMORIGENESIS

CHIVU M[1], NECULA LG[1], DIMA SO[2], NASTASE A[3], BADEA L[4], BLEOTU C[1], GRANCEA C[1], STANCU IC[1], DRAGU D[1], MATEI L[1], TUDOR S[2], DUMITRU A[5], DUMITRU L[5], CONSTANTINESCU G[5], POPESCU I[5] and DIACONU CC[1]

[1] Stefan S. Nicolau Institute of Virology
[2] Fundeni Clinical Institute for Digestive Disease and Liver Transplantation
[3] RN-tech Romania; [4] National Institute for Research and Development in Informatics
[5] Floreasca Emergency Hospital

Signal transducers and activators of transcription (STAT) proteins are constitutively activated in various solid tumors and hematological malignancies. Recent data showed that STAT3 activated by IL11 through gp130 pathway, play a significant role in gastric adenocarcionoma. To understand the role of STAT signalling in gastric cancer progression, we conducted genomic and proteomic studies analyzing differential expressed genes from this pathway relating with TNM staging.

Gene expression profile was investigated by microarray on 10 pairs of gastric adenocarcinoma samples and normal tissue obtained from patients. Changes in the gp130/JAK/STAT signalling pathway were analyzed and verified on 30 pairs of samples through additional methods such as: relative quantification by real time RT-PCR and Western blot, for a sequence of genes / proteins such as JAK1, 2 and 3, SOCS3, SHP2, STAT3.

Microarray results showed up-regulation of IL11, IL6, STAT1 ($p = 0.03$) and STAT3 expression, as well as a down-regulation of SHP2 gene expression. A minor variation could be seen in relation to TNM staging, which was not statistically significant ($p > 0.05$). SOCS1 and SOCS3 expression were not modified compared with normal tissue. The results were confirmed at the mRNA and protein level. STAT3 activation by phosphorylation at Tyr705 increased gradually from primary stage to advanced carcinoma with lymph nodes metastasis. In contrast, phosphorylated form of SHP2 protein (Tyr580) decreased quantitatively compared with TNM staging, thus highlighting the negative reciprocal regulation of the two signaling cascades STAT1/3 and SHP2/ERK.

It can be concluded that IL11 and STAT3 can be used as molecular biomarkers for staging and prognosis in gastric adenocarcinoma. This approach is particularly useful because it brings additional information on changes in gene expression during carcinogenesis.

NEW POTENTIAL MOLECULAR MARKERS FOR CERVICAL CANCER PROGNOSTIC

ANCA BOTEZATU[1], ADRIANA PLESA[1], CRISTINA D. GOIA[1], DEMETRA SOCOLOV[2], IULIA V. IANCU[1], CARMEN UNGUREANU[2], IRINA HUICA[1] and GABRIELA ANTON[1]

1. Institute of Virology "Stefan S Nicolau", Bucharest, Romania,
2.Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania

Cervical cancer is one of the most frequent cancer affecting women in Romania. Infection

with human papilloma virus (HPV) is the main cause for cervical neoplasia. Accumulating evidence suggests that E3 ubiquitin ligases play important roles in cancer development, especially because of gene amplification phenomena in this disease. The overexpression of WWP1 gene in cancer leads to excessive degradation of WWP1 target proteins like KLF5 and TGFβ 1 receptors. Although Kruppel-like factor 5 (KLF5) is a transcription factor involved in pathways critical for carcinogenesis, controversy regarding its role persists: is it a tumor suppressor or an oncogene? The aim of this study was to identify new mechanisms for progression of cervical lesions, evaluating mRNA levels for these target genes.

The biological samples (50) were obtained from patient's age between 21-54 years with CINI, CINII, CINII histological diagnosis and cervical cancer. In order to quantify the WWP1, KLF5 and TGFβ1 mRNA levels we used TaqMan specific primers and probes. 10 mRNA specimens isolated from HPV negative patients that underwent surgical interventions for other pathologies were considerate like controls.

The WWP1 gene is overexpressed in squamous cell carcinomas (SCC), while TGFβ1 receptor is overexpressed in CSS and also in adenocarcinomas (AC). The KLF5 gene expression is considerably reduced in CIN III and in cervical cancers. KLF5 and WWP1 gene expression in higher in HPV negative samples, while TGFβ1 expression in lower in this category.

THE ETHICAL ISSUES HIGHLIGHTED BY EUROPEAN GROUP OF ETHICS ON HUMAN STEM CELL USE AS ADVANCED THERAPIES

BUNU CARMEN[1], MARUSCIAC LAURA[1], TANASIE GABRIELA[1] and ENACHE ALEXANDRA[1]

[1]University of Medicine and Pharmacy "Victor Babes" Timisoara

Directive 2004/23/EC sets standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. Regulation (EC) No 1394/2007 defines Advanced Therapy Medicinal Products (ATMP) and states the rules on how ATMP should be authorised, supervised, and monitored to ensure quality, safety, and efficacy.

The European Group of Ethics highlighted aspects concerning information and consent, donation, privacy and data protection, traceability, safety, priorities of access, research and clinical trials, and patents.

The EGE states that the donor should be clearly informed on the potential future uses and different options of consent should be proposed, and that the point where withdrawing of consent will not be possible should be made explicit. The consent should contain a clear option to refuse specific future uses of donated tissue.

Regarding donation, it states that ownership and the degree of individual freedom to decide on the future use of the donated tissue in relation with the common good are viewed differently in different cultures and nations.

The EGE recognizes that there may be conflicting interests between the donor and the recipient, so strict data protection rules are needed.

The EGE considers that the question of traceability of engineered tissues deserves a better reflection, in order to establish what is meant by traceability, how far should it go and how to include it in the consent form. To be efficient, traceability must be complete – the more precise the traceability requirements are, the better the safety will be.

A highlighted safety aspect was that tissues intended for transplantation to third parties or for the preparation of pharmaceutical specialities must undergo advanced testing to provide maximum health guarantees in accordance with the state of the art. Also, specific care is required for the use of genetically modified organisms in tissue engineering, as they can raise specific safety problems.

There is also a need for defined, transparent criteria for priority access to tissue products, based on an objective evaluation of medical needs, and considering the objectives for public health in Europe.

Clinical trials should be governed by the following ethical principles: free and informed consent, anonymity of the donation, protection of the health of persons involved in clinical trials, and a scientific evaluation, in collaboration with the EU Agency for the Evaluation of Medicinal Products of stem cell use for therapy. A risk-benefit assessment is crucial.

Regarding patents, the EGE highlights the fact that Article 3 of the Charter of Fundamental Rights prohibits making profits from the human body and its elements, but the issue is the profit obtained with an invention resulting from the use of donated tissues.

ACKNOWLEDGEMENTS

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CLINICAL PROGNOSIS AND ADJUSTMENT OF THERAPEUTIC MANAGEMENT RELATED TO JAK2 V617F MUTATIONAL STATUS IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

MIHAELA CLOSCA-GHEORGHIU[1], ANCA ROXANA LUPU[1], LAURA DRAGOMIR NECULA[2], MIHAELA CHIVU[2], DELIA MUT POPESCU[1] and CARMEN CRISTINA DIACONU[2]

[1] „Coltea Clinical Hospital, Bucharest
[2] „Stefan S. Nicolau" Virology Institute, Bucharest

INTRODUCTION

At present, JAK2 V617F mutation is one of the key criteria of diagnosis algorithms and mutational status assessment is a routine procedure when BCR/ABL negative myeloproliferative neoplasms are suspected. The purpose of this study was to evaluate the prognostic and clinical significance of JAK2 V617F status in patients diagnosed with Philadelphia (Ph) negative myeloproliferative neoplasms between 2006–2009. The clinical outcome of patients under different chemotherapy was reevaluated according to JAK2 V617F status and therapeutic approach required readjustment.

MATERIALS AND METHODS

The study included 210 patients diagnosed with Ph negative myeloproliferative neoplasms. There was a special interest in analyzing clinical aspects such as: thrombotic and haemorrhagic accidents, splenomegaly grading, the general signs of disease (itching, sweating, weight loss) which were examined in relation to mutational status. The prognostic value of JAK2 V617F mutational status was also evaluated. Three distinct subgroups of patients were analyzed: a subgroup treated with Anagrelide, one treated with Hydroxyurea and the third one treated with interferon-alpha (IFN-alpha). The subgroups of patients were analyzed for disease progression under treatment and the presence or absence of JAK2 V617F mutation.

RESULTS

The presence of JAK2 V617F is associated with faster progression of the disease, older age at diagnosis, higher number of leukocytes and higher level of haemoglobin and cardiovascular events. Patients with JAK2 V617F mutation had a mild response to Anagrelide then to Hydroxyurea, while the patients treated with IFN-alpha showed the best response.

CONCLUSIONS

JAK2 V617F mutational status evaluation in BCR/ABL negative myeloproliferative neoplasms improved considerably the therapeutic approach of these diseases.

VIROLOGY SESSION II

VIROLOGICAL SURVEILLANCE OF A (H1N1) – 2009 PANDEMIC IN ROMANIA

VIOREL ALEXANDRESCU , EMILIA LUPULESCU , ALINA BAETEL , EMIL IONITA , MARIA ELENA MIHAI, CRISTINA TECU and GHEORGHE NECULA

National Institute for Research and Development for Microbiology and Immunology "Cantacuzino" Bucharest

Influenza pandemic represents a major threat for public health at national, subregional and international level. National Influenza Center (NIC) from National Institute for Research and Development for Microbiology and Immunology "Cantacuzino" Bucharest (N.I.R.D.M.I) .

INTRODUCTION

N.I.R.D.M.I. "Cantacuzino" was recognized by WHO in 1969. In 1995 NIC – CI was admitted in WHO network (Flunet) and in GISN (Global

Influenza Surveillance Network). In 2000, NIC-CI was accepted in the European network (EISS) after a 5 years evaluation period in EUROGROG network. In present NIC-CI participates in EUROFLU system who replaces EISS system. After the NIC-CI response in the 2005–2006 avian influenza outbreak, WHO proposes the development of NIC-CI into a Subregional Influenza Center with the aim to coordinate influenza surveillance in eight neighborhood countries (Albania, Bulgaria, Bosnia Herzegovina, Croatia, Macedonia, Montenegro, Serbia, and Republic of Moldova). NIC-CI development into a Subregional Centre took place in 2009 after three evaluation sesions by WHO (2005, 2006 and 2007).

The influenza sentinel surveillance system in Romania was created in 1995 and developed till now by NIC-CI. The surveillance system was evaluated by WHO and EISS and was recommended like a model for the countries coordinated by the Subregional Center. The last EURO-WHO and CDC – Atlanta visits from 9-11 March 2010 confirmed the NIC-CI quality as a Subregional Centre. The 2009 A/H1N1pandemic in April 2009 assessed the Ministry of Health (MoH) to make the National Influenza Coordination Committee. NIC-CI was appointed by the MoH to coordinate the virological surveillance and the laboratory diagnosis at the national level. In addition WHO confirmed in June 2009 that NIC-CI was the only laboratory at that time that could perform the pandemic H1N1 2009 laboratory diagnosis in Romania.

In the following period, in order to improve the influenza H1N1-2009 pandemic virus circulation surveillance and especially to increase the accessibility to laboratory diagnosis of hospitalized cases, MoH considers opportun reactivation of three Regional Centers in Iasi, Constanta, Timisoara and the accreditation of a molecular diagnosis laboratory from National Institute for Infectious Diseases “Matei Bals”.

In April – Septeber2009 influenza evolution in Romania was related to imported cases from countries/zones with pandemic influenza outbreaks and some contacts. In Iasi and Brasov were detected two collectivity outbreaks with imported origin. There was not registered local sustained transmission.

Starting with the second half of October 2009 the H1N1 pandemic virus circulated active (increase in the detections by RT-PCR number) according to the boost in the number of reported ARI, ILI, and SARI cases. In the following period the detections and clinical cases number continued to increase, maximum being in weeks 47, 48 and 49. Decline was observed in weeks 1, 2 and 3 in 2010, attending the normal seasonal values in week 4-2010.

METHODS

Diagnosis and surveillance influenza methodology used in NIC-IC included: cell culture and embrionated eggs isolation followed by antigenic characterization by HI assay, Real-time RT- PCR detection, sequencing of isolated strains, antiviral sensitivity testing.

RESULTS

1. 15,465 samples were tested by Real-Time RT-PCR between April 2009 – March 2010 (week 11) of which 5,554 were positive for A (H1N1) pandemic 2009 (35, 91%), 13 subtype A/H3 (0,08%), 3 seasonal subtype A/H1 (0,0 1%), 38 type B (0,24 %).

2. 23 influenza viruses were isolated in MDCK cell line of which 20 similar to A/California /07/09 (pandemic virus), 1 similar to A/Brisbane/10/07 and 2 similar to B /Brisbane/60/08

3. 23 strains were sequenced in which were found the following mutations: 23 strains with S203T substitution, 3 strains with D222G substitution, 1 strain with K163G substitution and 1 strain with G140E substitution.

4. 7 strains of pandemic influenza virus H1N1 – 2009 were tested for sensitivity to oseltamivir. All strains were sensitive.

293 samples were further tested in tissue cultures of which: 102 were positive for A/H1N1 – 2009, 3 positive for adenoviruses, 5 positive for RSV, 8 positive for hMPV (parainfluenza), 3 positive for Coronavirus, 4 positive for parainfluenza virus type 1 and, 7 positive for parainfluenza type 3.

CONCLUSION

In October 2009 – February 2010 in Romania was the first pandemic wave (A/H1N1 – 2009 pandemic virus).

NON-CLINICAL STUDY OF H1N1 PANDEMIC VACCINE

CRINA STAVARU, EMILIA LUPULESCU, CRISTIN COMAN , ORHAN RASID, ADINA DANIELA IANCU, ALINA BAETEL, GHEORGHE NECULA, EMIL IONITA, LAURENTIU BERBECILA, E. VLASE,VIOREL ALEXANDRESCU, MARGARETA MAZILU, DOREL LUCIAN RADU and ADRIAN ONU

National Institute for Research and Development for Microbiology and Immunology "Cantacuzino" Bucharest.

INTRODUCTION

Influenza infection continues to be a major public health problem and influenza vaccination is the most effective method of protection and prevention. The aim of this study was to evaluate the H1N1 A California/7/2009 (reassortant strain X179A) influenza vaccine immunogenicity, in terms of the 2009 epidemic outbreak with pandemic A influenza virus (H1N1).

MATERIALS AND METHODS

BALB /c mice, 6–8 weeks, and ferrets (common European line *Mustella putorius*) 4–8 months, were used. Immunization was performed by intramuscular (IM) administration of two doses from A H1N1 California/7/2009 (reassortant strain X179A), whole inactivated or split virus; IVR 148 (A / Brisbane/57/09- like) whole inactivated or split virus and trivalent seasonal flu vaccine. The level of the specific antibodies was detected by haemagglutination inhibition assay (HI) from blood samples collected before the first inoculation and, on day 20 and 42, calculated from the first inoculation. The health of animals during the experiment was evaluated by monitoring clinical signs and body weight.

RESULTS

In this non-clinical study of immunogenicity conducted on two animal species (BALB/c mice and ferrets) the detected HI titer showed seroprotection and seroconversion after a single administration of 15 microg HA per dose of inactivated whole virus or split virus of H1N1 A California/7/2009 (reassortant strain X179A). After one dose of seasonal trivalent flu vaccine the results showed that seroconversion and seroprotection depends on the vaccine strain. A second dose of pandemic vaccine does not enhance the antibodies level.

CONCLUSION

All these results led to the optimal choice of the vaccination scheme on human subjects and the recommendation is for a 15 microg HA per dose, split virus, monovalent pandemic influenza vaccine formulation CANTGRIP. This study was conducted within the MS-VGP01 contract funded by Ministry of Health.

IMPACT OF DETECTABLE CYTOMEGALOVIRUS VIRAEMIA ON PROGRESSION OF HIV INFECTION IN PATIENTS UNDERGOING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

MIHAILESCU R.*, ARAMA V.**, STREINU-CERCEL A.**, OTELEA D.*, PARASCHIV S.*, TUDOR ANA-MARIA*, CHIOTAN C.**, OLARU I.*, MUNTEANU D.*, HRISTEA A.*, NEAGA E.* and RADULESCU M.*

*"Prof. Dr. Matei Bals" National Institute of Infectious Diseases (INBIMB), Bucharest
**"Carol Davila" University of Medicine and Pharmacy, Bucharest

OBJECTIVE

To evaluate the impact of detectable cytomegalovirus (CMV) viraemia on progression of HIV infection to AIDS events and death in patients undergoing HAART.

METHODS

We conducted a prospective Romanian research grant (CNCSIS 848/2006) on newly diagnosed HIV-infected patients, seropositives for anti-CMV IgG antibodies, in INBIMB, between June 2006-June 2008. Clinical, immunological (CD4) and virological (HIV and CMV viraemia) screening was performed every 3 months. CMV viraemia was quantified by means of RoboGene Human Cytomegalovirus (HCMV) Quantification kit (aj Roboscreen) and randomly retested with the commercial test CMV PCR kit (Qiagen Diagnostics). Both PCR reactions were performed on ABI Prism 7000 (Applied Biosystems).

RESULTS

We included 105 HIV-CMV co-infected subjects with average age of 30 years and M:F=1:1. Average follow-up was 18 months. Clinical CDC stage was A in 15% of cases, B in 40% and C in 45%. Median CD4 cell count was 164.5/mmc. The

results of the 2 molecular techniques were widely the same. CMV viraemia was found detectable in 20 cases at enrollment, 6 new cases at second visit and 1 new patient at 4th visit. All patients with detectable CMV viraemia received HAART, but only 2 patients with CMV symptomatic disease (ophthalmological and digestive) got specific anti-CMV therapy. In the multivariate model, the risk of progression of HIV infection to AIDS events and death was 4.93 times higher in people with detectable CMV viraemia in their history than in patients with constant undetectable viraemia (p=0.001; IC 95% 1.86–13.06). Detectable CMV viraemia is a risk factor for progression of HIV infection, regardless CD4 count.

CONCLUSIONS

Detectable CMV load identifies patients with a poor prognosis, despite prescription of HAART.

CORRELATION BETWEEN DIAGNOSTIC TESTS ON IMPLICATION OF HEPATITIS VIRUSES IN CHRONIC LYMPHOPROLIFERATION - PREVALENCE OF INFECTIONS AND IDENTIFICATION OF MOLECULAR MECHANISMS INVOLVED IN ONCOGENESIS

(Research Grant LIMFOVIR – Results of the 3rd phase)

R. MIHĂILESCU[2], V. ARAMĂ[1, 2], A. STREINU CERCEL [1, 2], S.S. ARAMĂ[1], A.M. VLĂDĂREANU[1,3], C. DOBREA[4], V. MOLAGIC[1], D. OTELEA[2], M. PARASCHIV[2], D.A.ION[1], A. RAFILA[1, 2], M. OLARIU[2], C. TILIŞCAN[1], D. MUNTEANU[2], M. RADULESCU[2], H.BUMBEA[3], C. CIUFU[1,3], S. COSTOIU[1] and M. MARZA[2].

[1]University of Medicine and Pharmacy " Carol Davila", Bucharest, Romania
[2]National Institute of Infectious Diseases "Prof. Dr. Matei Bals", Bucharest, Romania
[3]Emergency University Hospital, Department of Hematology, Bucharest, Romania
[4]National Institute of Research in Pathology and Biomedical Sciences "Victor Babes", Bucharest, Romania

OBJECTIVE

To evaluate the impact of hepatitis B, C, D viruses on chronic lymphoproliferations (CL) evolution.

METHODS

We present the preliminary results of a prospective multidisciplinary grant PNCDI-II 41-012/3/2007 LIMFO-VIR on the molecular mecanisms involved in the etiopathogeny of CL in HBV, HCV infected patients. CL was diagnosed by osteomedullary/ lymph node biopsy and immunophenotyping tests, viral hepatitis by serological tests ELISA; viremia was detected by Real-time PCR COBAS TaqMan (Roche Diagnostics). We studied the immunophenotypic and prognosis markers.

RESULTS

The preliminary results of the ongoing grant showed 43 subjects, F/M=1.5/1, median age of 63 years (30–84). Hepatitis B was found in 14/43 cases (32.5%), hepatitis C in 25/43 cases (58.2%) and double/triple co-infection – 4/43 (9.3%). Hepatitis B was mostly associated with large B-cell NHL, while hepatitis B+D and hepatitis C – with small marginal zone B-cell NHL. Aggressive forms of CLD were noticed in two thirds of B hepatitis cases, but in one third of C hepatitis cases. We found an increased expression of the immunophenotypic markers of B-cells-CD19 (Md 95/92), CD20 (Md 90/39), CD79b (Md 58/31), CD23 (Md 67/37) and of the prognosis markers- CD38 (Md 49/24), bcl-2 (Md 46/5), cyclin D1 (Md 11/0.5), but no changes in ZAP-70 expression.

CONCLUSIONS

Among our patients diagnosed with CLD, hepatitis B has a lower prevalence than hepatitis C. But agressive CLD forms are linked more frequently with hepatitis B than with hepatitis C. When CLD are associated with viral hepatitis, immunophenotypic and prognosis markers suggest atypical and progressive types of B-cell chronic lymphoproliferations.

JAK-STAT PATHWAY AND HEMATOPOIETIC STEM CELL MALIGNANCIES

STEFAN N. CONSTANTINESCU

Ludwig Institute for Cancer Research Ltd.de Duve Institute,Université Catholique de Louvain Brussels, Belgium

Over the past four years, the Philadelphia chromosome negative myeloproliferative neoplasms

(MPNs) have seen major progress – the identification of the unique acquired somatic V617F mutation in JAK2 (Janus kinase 2) in >95% of Polycytemia Vera (PV) and >50% of Essential Thrombocytemia (ET) and Primary Myelofibrosis (PMF) patients, the discovery of activating mutations in thrombopoietin receptor (TpoR, c-Mpl) in 3–10% of ET and PMF patients negative for JAK2 V617F, and the initiation of clinical trials with JAK2 inhibitors for myelofibrosis patients. Our group and several others have established that JAK2 V617F has become as common as the assessment of BCR-ABL fusion product for chronic myeloid leukemia. The rare PV patients that do not harbor JAK2 V617F were subsequently identified to harbor activating mutations in exon 12 of JAK2, in a region around K539 residue. Overall, it has become clear that blocking JAK2 tyrosine kinase emerged as a major goal for therapy in MPNs, as available evidence argue for general disfunction of JAK2-STAT5/STAT3 pathway in MPNs.

Both the BCR-ABL negative and positive (CML, chronic myeloid leukemia) are known to be due to acquisition of mutations at the hematopoietic stem cell (HSC) level.

The mechanisms of mutation acquisition are unknown. Transplantation of mutated and non-mutated HSCs from MPNs into immuno-compromised mice showed that apparently the mutated HSCs do not exhibit a proliferative advantage, at least in this setting.

A clinical case of bone morrow transplantation of HSCs with low level positivity for JAK2 V617F from patient with past tromboembolic events led to long term reconstitution in the receipient, with documented presence of JAK2 V617F clone eight years after transplantation, but not overt disease. Thus, either the homing process during adoptive transfer masks the proliferative effects of JAK2 V617F in HSCs or other events, besides acquisition of JAK2 V617F (or, similarly of TpoR mutants) are required for clonal dominance and disease phenotype. We present several approaches based on inducible retroviral transduction in mouse and human HSCs that we undertake in order to determine which signaling pathway cooperates with constitutive JAK-STAT activation in order to induce expansion of mutated HSCs and establish the MPN phenotype *in vivo*.

ANTIANGIOGENIC THERAPY FOR CANCER: FROM CONCEPT CONFIRMATION TOWARDS INDIVIDUALIZED TREATMENT

G. DUDA

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

No validated biological markers (or biomarkers) currently exist for appropriately selecting patients with cancer for antiangiogenic therapy. Nor are there biomarkers identifying escape pathways that should be targeted after tumors develop resistance to a given antiangiogenic agent. A number of potential systemic, circulating, tissue and imaging biomarkers have emerged recently completed phase I–III studies. Some of these are measured during baseline (for example VEGF gene polymorphisms or soluble VEGFR1), others are measured during treatment (such as hypertension, MRI-measured Ktrans, circulating angiogenic molecules or collagen IV), and all are mecha-nistically based. Some of these biomarkers might be pharmacodynamic (for example, increase in circulating VEGF, placental growth factor) while others have potential for predicting clinical benefit or identifying the escape pathways (for example, stromal-derived factor 1 alpha, interleukin-6). Most biomarkers are disease or/and agent specific and all of them need to be validated prospectively. I will discuss the current challenges in establishing biomarkers of antiangiogenic therapy, define systemic, circulating, tissue and imaging biomarkers and their advantages and disadvantages, and comment on the future opportunities for validating biomarkers for individualized antiangiogenic therapy.

DIRECT CONTACT OF HUMAN UMBILICAL CORD BLOOD-DERIVED ENDOTHELIAL PROGENITOR CELLS WITH LIVING CARDIAC TISSUE IS A PREREQUISITE FOR GENERATION OF VASCULAR TUBE-LIKE STRUCTURES

MARILENA LUPU[1], MAYA SIMIONESCU[1], MARKUS KHALIL[2,3], JÜRGEN HESCHELER[2] and HORIA MANIU[1]

[1]Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
[2] Institute for Neurophysiology, University of Cologne, Cologne, Germany
[3] Pediatric Cardiology Department, University of Cologne, Cologne, Germany

The umbilical cord blood derived Endothelial Progenitor Cells (EPCs) contribute to vascular

regeneration in experimental models of ischemia. However, their ability to form Vascular Tube-like Structures and thus participate to cardiovascular tissue restoration has not been elucidated, yet. We employed a novel transplantation model to investigate whether human EPCs have the capacity to integrate into living and ischemic murine embryonic ventricular slices, and to participate to neovascularization. EPCs were cocultured with either living or ischemic murine embryonic ventricular slices in the presence or absence of a pro-angiogenic growth factor cocktail consisting of VEGF, IGF 1, EGF, and bFGF. Tracking of EPCs within the cocultures was done by cell transfection with green fluorescent protein or by immunostaining using antibodies against human vWF, CD 31, nuclei and mitochondria. The results shoved that EPCs generated Vascular Tube-like Structures when the cells were in direct contact with living ventricular slice preparations. Furthermore, the pro-angiogenic growth factor cocktail reduced significantly the tubes formation. Cocultures of EPCs separately from the living ventricular slices (in transwell system) did not lead to Vascular Tube-like Structures formation, demonstrating that the direct contact is necessary and that soluble factors secreted by the living slices were not sufficient for their induction. No vascular tubes were formed when EPCs were cocultured with ischemic ventricular slices, even in the presence of the pro-angiogenic cocktail.

In conclusion, EPCs from Vascular Tube-like Structures in contact with living cardiac tissue and the direct cell-to-cell interaction is a prerequisite for their induction. Understanding the cardiac niche and microenvironmental interactions that regulate EPCs integration and neovascularization are essential for applying these cells to cardiovascular regeneration.

GENERATION OF HEPATOCYTE-LIKE CELLS FROM STEM CELL SOURCES

RAZVAN IACOB

University of Medicine and Pharmacy „Carol Davila” Bucharest

Many of the regenerative technologies for liver diseases include cellular components which are either transferred into the target organs or utilized in extracorporeal devices. The primary hepatocytes which can be isolated from adult liver organs are still the more important resources in clinical situations in which specific liver functions need to be replaced. Due to the impossibility of maintaining hepatocytes in culture or to expand hepatocytes in vitro, the search for alternative sources, which can either be expanded in cell culture or can be easily harvested from the body in large quantities, has been stimulated. It has been proposed that subpopulations of adult hematopoietic stem cells (HSC), mezenchymal stromal cells (MSC) and cord blood stem cells (CBSC) can transdifferentiate into hepatocytes after transplantation but the efficacy by which these cells form hepatocytes and liver tissue in animal experiments is still questionable. As an alternative concept HSC, MSC and CBSC are being transplanted in patients with chronic liver diseases with the therapeutic aim to induce liver regeneration and remodelling. High expectations have been attributed to embryonic stem cells (ES) and more recently to induced pluripotent stem cells (iPS). These cells can be maintained in a state of pluripotency for long periods of time, grown in large quantities. Hepatocytes derived from ES cells may serve as unlimited cell source unlike primary hepatocytes isolated from donor livers. In order to generate hepatocytes-like cells in vitro. The various differentiation protocols usually mimic the events occurring during embryonic development of the liver. Accordingly, the protocols usually mimic the events occurring during embryonic development of the liver. Accordingly, the pluripotent ES cells are differentiated into the hepatocytes state by formation of embryoid bodies, followed by the induction of definitive endoderm using Activin A. The endoderm cell populations can be further induced toward the hepatocytes lineage by exposure to bone morphometric protein (BMP) 4 and fibroblast growth factor (FGF) 2, which represent important signals from the cardiac mesoderm in early embryogenesis. Last differentiation steps use hepatocytes growth factor (HGF) and oncostatin M. To date, most published ESC differentiation protocols generate hepatocytes-like cells, but not the fully functional, mature, transplantable equivalents of hepatocytes that are isolated from adult liver. Recently several research groups have tried to improve the hepatocytes differentiation protocols by ectopic expression of liver enriched transcription factors. Hepatocytes-

like cells with a mature phenotype could be differentiated from murin adult liver derived progenitor cells by ectopic expression of three transcription factors.

IN VITRO INDUCTION OF BONE MARROW DERIVED MESENCHYMAL STEM CELLS IN EPITHELIAL-LIKE CELLS

TANASIE GABRIELA[1,2], BOJIN F.[1], TATU C.A.[1,2], GAVRILIUC O.[1], TATU R.F.[1], BUNU C.[1], ANGHEL S.[1,2], CRISTEA M.[1] and PAUNESCU V.[1,2]

[1] University of Medicine and Pharmacy "Victor Babes" Timisoara
[2]Clinical County Hospital Timisoara

Adult mesenchymal stem cells (MSCs) are extremely attractive for studying the regeneration and reparation features in various types of tissues. The concept of plasticity means the properties of stem cells to differentiate in a distinct cell line apart from the original tissue. The in vitro differentiation techniques are based on using differentiation agent, coculture with specific cells or structures and modification in some gene expression. The purpose of this study was to evaluate the effect of some biochemical inductors to differentiate adult MSCs into epithelial like cells.

MATERIAL AND METHODS

The experiment was developed after obtaining the agreement of The Ethics Committee of the University of Medicine and Pharmacy Victor Babes Timisoara. The bone morrow samples were harvest from 4 patients suffering surgical hip replacement. The procedure used for MSCs isolation was based on plastic adherence. For induction of epithelial like cells, MSCs at 3rd passage were used. The media was supplemented with cytokines and growth factors used either alone or in combinations. To evaluate the presence of some specific markers after 14 days the cells were fixed with 10% formalin and stained with ant-Vimentin and anti Pancytokeratin antibodies. RNA extraction and RT-PCR analysis of gene expression for Cytokeratin 19 and E-cadherin have also been performed. The surface markers of epithelial induced cells were analyzed by flow cytometry and the secretory profile of the cells was evaluated by ELISA.

RESULTS

The better results were obtained using media supplemented with epidermal growth factor (EGF), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF) and insulin like growth factor II (IGF 2). A specific marker for MSCs, vimentin, had a weaker expression and the expression of pancytokeratin was stronger in this culture. Also, the cytokeratin 19 and E-cadherin were very well expressed in these cells. The secretory profile of epithelial induced cells was characterized by detectable levels of interleukin 4 and 10, vascular endothelial growth factor and tumor growth factor 1.

DISCUSSION AND CONCLUSION

The experiments revealed that MSCs differentiation toward the cells expressing epithelial markers is relatively easily to obtain in vitro, using combination of biochemical inductors, without genetic manipulation of the cells. The cells expressed specific surface markers and seem to have immune properties.

ACKNOWLEDGEMENTS

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MICRO RNA INVOLVEMENT IN THE PATHOGENESIS AND DIAGNOSIS OF BILE DUCT CANCERS

FLORIN SELARU

Gastroenterology and Hepatology, Johns Hopkins University

INTRODUCTION

Cholangiocarcinomas (CCA) are aggressive cancers with high mortality and poor survival rate. Only radical surgery offers patients any hope of cure; however most patients are not surgical candidates because current diagnostic methods detect this cancer late. Micro RNAs (miRs) were shown to be involved in every cancer studied thus far, but they have not been evaluated in primary human tissue CCA.

MATERIALS

All specimens were obtained at surgeries performed at Johns Hopkins University. Prior informed consent was obtained from all patients. Histologically confirmed normal bile duct specimens were collected at surgery performed for pancreatic cancer. Histologically normal liver specimens were collected at surgery for CCA. Micro RNA arrays were performed using Agilent platform. Differentially expressed miRs were identified by Significance Analysis of Microrrays (SAM).

RESULTS

Five primary CCAs and five normal bile duct specimens (NBDs) were analysed. Twenty miRs were over- and 112 were underexpressed in CCAs vs. NBDs. The top 5 miRs in each category are listed in the Table. MiRs expression was verified by quantitative RT-PCR (qRT-PCR). To evaluate the ability of miR-21 to diagnose CCA, qRT-PCR was performed on 15 additional primary CCA and 9 normal liver (NL) specimens. NLs displayed uniformly low levels of miR-21, with standard deviation (SD) of 1.05. In contrast, CCAs displayed more variable expression level (SD=8.64). The fold difference between CCA and NL was 6.21. Receiver operating characteristic (ROC) curve analyses showed that miR-21 was 95% sensitive and 100% specific in distinguishing between CCA and normal tissues, with an area under the ROC curve (AUC) of o.992.

CONCLUSION

MiR-21 was found overexpressed in a variety of solid cancers, but this is the first report of its expression in human CCAs. miR-21 was found to have a consistently higher expression in CCAs compared with normal hepatobiliary tissues and shows promise as a biomarker of this cancer. Furthermore, a panel combining miR-21 along with other differentially expressed miRs in CCAs found in this study; deserve further consideration to elucidate their role in the CCA carcinogenesis.

TUMOR-ASSOCIATED FIBROBLASTS INVOLVEMENT IN CANCER DEVELOPMENT

PAUNESCU V.[1], BOJIN FLORINA[1], TATU C.A.[1,2], GAVRILIUC OANA[1], ROSCA ADRIANA[2], GRUIA ALEXANDRA[2], CIUCULESCU FELICIA[1], TANASIE GABRIELA[1,2], TATU CARMEN[1,2] and BUNU CARMEN[1]

[1]"Victor Babes" University of Medicine and Pharmacy Timisoara, Romania
[2] Clinical Emergency County Hospital Timisoara, Romania

INTRODUCTION

The complex cellular tumor microenvironment comprises immunocompetent and inflammatory cells, endothelial cells and fibroblasts. All of these cell types may critically influence the multi-step process of carcinogenesis and malignant phenotype. Fibroblasts are known to take part in immune reaction during tissue damage and injury by modulating local cellular and cytokine millieu, adjusting kinetics and components of the inflammatory infiltrate and, by modulating the functional status of the immunocompetent cells. Main purpose of our study was investigation of tumor associated fibroblasts (TAFs) phenotype and function, and to what extent they become activated within tumor environment and secrete different paracrine and autocrine factors which will further play a role in tumorigenesis.

MATERIAL AND METHODS

Human TAFs were isolated from breast cancer surgical pieces using enzymatic digestion, cultivated and expanded in vitro as monolayer cell culture. When cell population was pure, the trilineage differentiation potential (to adypocytes, chondrocytes, and osteoblasts) was assessed under appropiate differentiation medium. Supenatants of cell cultures at different passages were collected and presence of IL-4, IL-10, IL-13, TGF-beta 1, TNF alpha, IFN-gamma, and VEFG was detected by ELISA method. Specific mouse anti-human fluorochrome conjugated antibodies identified in flow cytometric analysis surface markers of TAFs, such as CD4, CD117, CD90, CD106, CD44, CD29, CD73, HLA-DR, CXCR4. Expression of cytoskeleton and extracellular matrix proteins was revealed by immunohistochemistry (vimentin, alpha-smooth muscle actin). Breast cancer isolated tumor cells and tumor cell lines (SK-BR3, MDA-MB231, and MDA-MB468) were tested for their ability to adhere to the TAFs substrate in flowchamber when the shear stress generated was increasing from 0.35 to 15 dyne/cm. Stimulation of TAFs used endogenous secretory factors for flowchamber assays.

RESULTS

Secretion of cytokines with direct immuno-suppressive effect such as IL-10 and IL-13, and other cytokines IL-4 and TNF alpha was increased in cultured TAFs at all passages. Enhanced production of TGF-beta1 by TAFs may also be a critical factor in tumor homeostasis. Expression of MHC class II molecules (HLA-DR) on activated TAFs contribute to an additional level ofimmuno-suppression and pro-tumoral effect. Hihg level of VEGF production suggests that TAFs provide not only structural support, but also pro-angiogenic molecules for tumor vascularization. Alpha smooth muscle actin expression in fibroblasts is variable and associated with an activated status. Adherence of tumor cells on TAFs substrate was increased when stimulated with VEGF for all values of shear stress, while IL-4 induced increased adherence only for shear stressed less than 8 dyne/cm.

CONCLUSION

The complex network of effects TAF transduced by enhanced expression of various immune cell deactivating/suppressing factors and neoangiogensis molecules should stimulate consideration of TAFs-based therapeutic designs.

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DIABETES MELLITUS BETWEEN BETA-CELL DYSFUNCTION AND A PRESUMED INSULIN RESISTANCE

CONSTANTIN IONESCU-TIRGOVISTE

National Institute of Diabetes, Nutrition and Metabolic Diseases, NC Paulescu, Bucharest, Romania

Diabetes is a syndrome which results from the alteration of the energy homeostasis of the human body. Several available clinical and experimental data can sustain the existence of the common pathogenetic mechanism operating in all diabetic phenotypes related with the β-cell mass/function. This defect is a *sine qua non* condition for the decompensation of blood glucose regulation. Classically, the pathogenesis of type 2 diabetes results for the contribution of two different mechanisms: a presumed *peripheral insulin resistance* and a *β secretory defect*. The first mechanism (insulin resistance) is a hypothetical disturbance witch, in fact, is a consequence of overweigh /obesity. The genetic studies carried out in the last years didn't succeed to identify any insulin resistance gene.

The main β-cell defect could be located somewhere in the process of formation, storage and exocytosis of the β-cell secretory vesicles. About 100 specific proteins encoded by the same number of genes take part in this process. Ours dates and some published by various authors suggest the endoplasmic reticulum as the main player in the β-cell defect.

It is worthy of note that hyperglycemia, considered as the only marker of diabetes and unique diabetes diagnosis criterion, appears only when more than 50% of beta cell mass/function is irreversibly or altered.

For this reason, hyperglycemia could be a valid marker only for the late phase of diabetes. In our view, diabetes has two separates stages: one pre-hyperglycemic and the other, hyperglycemic one.

According to our experience the pro-insulin level is early increased, not only in diabetes but also in its related disorders, obesity and metabolic syndrome. For that reason we propose as an early diagnosis of diabetes the determination of proinsulin/insulin ratio. These two parameters could be determinate in one fasting sample of plasma, in witch glucose could be also determined.

USING ADULT TISSUES FOR GENERATING NEW ORGANS: AUTOLOGOUS CELL REPLACEMENT THERAPY FOR DIABETES.

SARAH FERBER, KEREN RON, TAMAR SAPIR, VERED AVIV, ITZICK RACHMUT, KFIR MOLAKANDOV, DANA ZEITUNI and IRIT MEIVAR-LEVY

The Stem Cells and Regenerative Medicine Institute at Sheba Medical Ctr. Tel-Hashomer, Sackler School of Medicine, Tel-Aviv University, Israel
DRI, Miami University School of Medicine, Miami, FL, USA

It is becoming accepted that islet cell implantation, as a treatment for diabetic patients will be widely available only when new sources of islets or beta cells are found. Our working hypothesis is that adult cells retain a considerable level of plasticity which allows them to switch their original developmental fate in a process dictated by transcription factors functioning as master regulators of organogenesis.

We present the efficacy of inducing functional endocrine pancreas in adult human liver cells by nuclear reprogramming, using ectopic expression of pancreatic transcription factors, primarily Pdx-1. These developmentally shifted human liver cells produce insulin, process the hormone and secrete it in a glucose regulated manner. The cells ameliorate diabetes when implanted in immunodefficient SCID-NOD mice for long periods. The mechanism that underlies the transcription factors' controlled developmental switch from liver to pancreas is not completely understood. It has been documented that hepatic dedifferntiation is obligatory but insufficient for the activation of the alternate pancreatic repertoire. Moreover, Pdx-1 plays a dual role in the reprogramming process; while dictating

the activation of the pancreatic lineage it also induces hepatic dediffrentiation by actively repressing CEBP beta. Several soluble factors have been suggested to both increase the number of pancreatic progenitor cells in human liver and to increase the maturation of the transdifferentiated liver cells along the pancreatic lineage and beta-cell-like function.

Importantly, developmentally altered cells in liver not only resist autoimmune diabetes, but also induce immune modulation which halts the autoimmune attack in diabetic NOD mice.

THERAPEUTIC SIGNIFICANCE

Generating new functional tissues from adult organs is a fundamental concept in regenerative medicine. The activation of the pancreatic lineage and function in adult human liver cells allows the diabetic patient to be also the donor of his own insulin producing tissue. Using liver to replace ablated insulin production overcomes both the limited supply of tissues from cadaveric donors and the need for anti-rejection treatment.

CELL THERAPY FOR LIVER DISEASES

MICHAEL OTT

TWINCORE Centre for Experimental and Clinical Infection Research, Hannover, and Dept. of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover

Cell therapy can be defined as “the use of living cells to restore, maintain or enhance the function of tissues and organs”. Such strategy has emerged as an achievable therapeutic approach for inherited metabolic liver diseases in the past decade. Primary hepatocytes isolated from donated organs have been transplanted in a small series of patients with urea cycle defects by our group. A controlled clinical trial currently investigates the role of liver cell therapies as a “bridging” or “definitive” therapy in patients with urea cycle defects. The therapeutic results up to now and future strategies to improve the therapeutic benefits of liver cell therapies for metabolic liver diseases will be presented. Only modest results have emerged so far from ongoing clinical trials and animal experiments herein putative stem/progenitor cells were grafted in the setting of liver diseases.

Efficient hepatic differentiation protocols for adult and embrionic stem sources have been developed but, so far, low efficacies of engraftment and liver repopulation in transplantation experiments have been achieved. Much of the confusion in the field originates from a variety of experimental procedures jeopardizing interpretation of the results and comparison of different published reports. We will report on a novel standardized animal model, which allows experimental comparison of stem cell and hepatocyte liver repopulation capacities and we will discuss the consequences for future clinical trials.

THERAPEUTIC VACCINE FOR β CELL MALIGNANCY

MIRCEA C POPESCU, L.W. KWAK[2]; S.S. NEELAPU[2], R.J.ROBB[3] and others

[1]Plainboro, New Jersey
[2]MD Anderson Cancer Center, Houston, TX
[3]XEME Biopharma, Inc. Plainsboro, NJ

The established field of prophylactic vaccines is now enlarged with the area of therapeutic vaccines (theravaccines) for the treatment of cancer and other chronic diseases. Such active immunotherapy is adding a new less toxic modality of cancer treatment in addition to surgery, radiation, chemotherapy and biotherapy (mAbs and cytokines). Here we briefly describe our attempt to produce a theravaccine prototype for non-Hodgkin’s lymphoma (NHL), chronic lymphocytic leukemia (CLL), multiple myeloma and other B cell malignancies. The vaccine is composed of a single synthetic phospholipid (DMPC), interleukin 2 (IL-2) and tumor antigen (s), such as immunoglobulin idiotype (IgId), found in tumor cell membrane.

Material and methods used in this research are described in our previous publications; Kwak L.W. *et al.* J Immunol 1998, 160, 3637-3641; Neville M.E. *et al.* Cytokine (2000), 12, 1702-1711 and 1691-1701; Popescu M.C. *et al.* Blood 2007, 109, 5407-5410 etc.

We discovered that the hydrophilic moiety of IL-2 interacts with the carbonyl group of DMPC in small unilamellar vesicles (SUV, 30nm) leading to coalescence and fusion of membranes into a larger, new kind of liposome: the multi-lamellar coalescent vesicles (MLCV). The vaccine was obtained by the addition of the IgId or the tumor membrane containing the Id and other costimulatory proteins at the beginning of the

process MLCV formation. A particle of the vaccine contained IL-2 and antigen both in the interior volume and in the membrane of DMPC at the surface. Both were available for slow release and for direct interaction with relevant cellular receptors. Mouse studies revealed that the vaccine induced strong Th1 and Th2 immune responses and protected animals against a lethal tumor challenge. In a first phase I clinical trial conducted at the NCI, the vaccine was minimally reactogenic and nontoxic based on 5 years observation. All 10 patients mounted a strong cellular anti-tumor immune response and 8/10 had specific ant-Id antibody responses. The time to disease progression was more than doubled. A second phase I trial in Grade 3 and 4 patients revealed that the vaccine can reverse tumor-induced immuno-suppression in the majority of the subjects and confirmed safety results. This vaccine is now considered for further clinical research.

THE SIGNIFICANCE OF HIV RNA LEVELS IN CSF OF HIV-1 INFECTED PATIENTS WITH CENTRAL NERVOS SYSTEM COMPLICATIONS.

DUICULESCU D[1], ENE L.[1], TARDEI G[1], MINDU N[1], RUTA S.M.[2,] and CEAUSU E[1]

[1]*'Dr. Victor Babes' Hospital for Infectious and Tropical Diseases, AIDS Department, Bucharest, Romania,*
[2] "Stefan S. Nicolau" Institute of Virology and „Carol Davila" University of Medicine, Bucharest.

OBJECTIVE

We aimed to compare cerebrospinal fluid (CSF) and plasma HIV RNA levels (VL) in children and adolescents with and without neurological complications in order to establish the usefulness of CSF HIV RNA quantification.

PATIENTS AND METHODS

This is a cross-sectional study on HIV RNA levels in paired CSF-plasma samples (measured by Amplicor, LCX and Taqman) on a group of 102 HIV-1 infected patients. Patients assigned according to their neurological status: 54 with AIDS-defining neurological opportunistic infections (OI) and HIV encephalopathy (HIVE), 24 with non AIDS neurological diseases (11 of them diagnosed with subacute measles encephalytis –SME) and 24 without neurological impairment. Comparison between variables was performed using t-test if normaly distributed and Wilcoxon if non-normal distributed, and the correlation between CSF and other plasma parameters (CD4, albumin, and pleocytosis) was used.

RESULTS

Overall median plasma VL (5.01 log10 c/ml) was higher compared with CSF levels (2.71 log10 c/ml). However 23 patients had higher CSF HIV RNA levels, most of them with HIVE (13) and criptococcal meningitis (3). Patients on HAART with virological failure had significantly lower ($p<0.05$) CSF VL compared with naïve patients and with patients who stopped HAART for more than one month before evaluation, regardless of their neurological condition. There was a lack of correlation between CSF and plasma VL in naïve patients and those who stopped their antiretrovirals. CSF HIV RNA levels were positively correlated with albumin levels (rho=0.22; $p=0.05$) and with CSF pleocytosis (rho=0.51; $p<0.001$). Out of 22 patients with HIVE, 13 have higher CSF compared with plasma HIV RNA levels (5.5 *vs*.4.7 log10 c/ml, $p=0.005$). Patients with PML and SME had lower CSF VL levels compared with plasma (3.1 *vs*. 4.8 log10 c/ml; $p=0.001$ and 2.4 *vs*. 4.3 log10 c/ml; $p<0.001$ respectively). 10 of 11 patients with SME have CSF HIV RNA <400 c/ml, despite the fact that most of them have detectable plasma HIV RNA levels.

CONCLUSION

CSF HIV VL correlates with CSF albumin and pleocytosis. The lack of correlation between CSF and plasma HIV RNA levels observed in patients not undergoing HAART suggests the existence of compartimentalisation of HIV infection in CSF. Assessment of HIV VL in CSF could be a useful marker in diagnosis of CNS diseases (high levels in HIVE, low levels in PML and SME).

GUIDELINE FOR MANAGEMENT OF HEALTHCARE WORKERS WHO ARE INFECTED WITH HEPATITIS VIRUSES AND HIV

COSTIN CERNESCU

"St. S. Nicolau" Institute of Virology, Bucharest, Romania

Societal views of patients' rights are tough, and most patients believe that they have a right to know if their physician or other healthcare worker is infected with a potentially transmissible bloodborne pathogen (irrespective of the magnitude of risk). Case law has generally concluded that informed

consent includes disclosure of risks but physician confession would very likely require to abandon or substantially modify his or her practice–an unwarranted outcome in light of our current understanding of the risks for provider-to-patient transmission of bloodborne pathogens. On the basis of the substantial changes in the risk profile since the previous guideline was published (eg, new safety devices, new infection control strategies, better techniques for monitoring diseases, effective postexposure management, and effective therapy), the Society for Healthcare Epidemiology of America (SHEA) emphasizes that infected healthcare providers should not be totally prohibited from participating in patient-care activities solely on the basis of a bloodborne pathogen infection. The guidelines assessed three types of procedures as associated with an increased risk for provider-to-patient transmission of hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV: procedures with de "minimis risk"; with risk theoretically possible but unlikely, and with definite risk or "exposure-prone". For each type of procedure specific infection control interventions and appropriate safety devices must be implemented in order to ensure patient "zero-risk" posture. This lecture is a comment to updated recommendations of the SHEA and of similar European societies regarding the management of healthcare providers who are infected with hepatitis viruses and, HIV.

DETECTION OF NUCLEOTIDE SUBSTITUTIONS WHICH CONFER ANTIRETROVIRAL RESISTANCE IN ANTIRETROVIRAL TREATMENT NAÏVE HIV- POSITIVE PATIENTS

AURA TEMEREANCA[1,2], LUMINITA ENE[3], CODRUTA VAGU[2], CAMELIA SULTANA[1,2], LOREDANA MANOLESCU[1,2], CAMELIA GRANCEA[2], PETRUTA MIHAILA[2], DAN DUICULESCU[3] and SIMONA RUTA[1,2]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]"Stefan S. Nicolau" Virology Institute, Bucharest, Romania
[3]"Dr. Victor Babes" Hospital for Infectious and Tropical Diseases, Bucharest, Romania

INTRODUCTION

Drug resistance mutations are more and more frequently detected in antiretroviral-naive HIV positive patients. In this context, the transmitted resistance becomes a critical problem in the natural evolution of the HIV/AIDS epidemic.

OBJECTIVES

Considering the fact that data on non-B subtypes is limited, and subtype F is predominant in Romania, our goal is to analyze resistance mutations in the pol gene of HIV-1 isolates from drug-naive patients.

METHODS

The study included 12 HIV-1 infected, untreated patients, 6 newly diagnosed and 6 chronically infected, all with detectable HIV RNA viral load. Drug resistance genotyping was performed using the TruGene HIV-1 Genotyping Assay (Bayer Diagnostics). HIV subtype was determined using the Stanford database algorithm.

RESULTS

7/12 (58.3%) strains belong to the F subtype, 1/12 (8.3%) to the G subtype, and the rest (33.3%) of the studied strains appear to be K/F (2/12), K/B (1/12) and F/B (1/12) inter-subtype recombinant forms. In our study, 6/12 (50%) drug-naïve patients carried HIV strains with at least one resistance-related mutation. Newly diagnosed patients harbored resistant variants more often than did chronically infected patients (4/6; 66.6% *vs*. 2/6; 33.3%).

Resistance to 2 drug classes was present in 2/12 (16.6%) patients, and resistance to all three drug classes was observed in 2/12 (16.6%) patients. Major mutations associated with NRTI resistance were identified in 6/12 (50%) patients: M41L (2/12), K70R (2/12), K219Q (2/12), D67N (1/12), T215F (1/12), M184V (1/12), M184I (1/12). Major mutations associated with NNRTI resistance were present in 2/12 (16.6%) patients: K103N (1/12) and Y181C (1/12). Major mutations associated with PI resistance were observed in 3/12 (25%) patients: M46I, I47V, I54V, V82A, V82F, V82S, I84L. All viral strains had minor mutations in the protease gene.

CONCLUSIONS

The prevalence of major mutations associated with NRTI, NNRTI and PI resistance in the studied group was 50% (6/12 patients), 16.6% (2/12) and

25% (3/12), respectively, major mutations being more frequently identified among newly diagnosed patients compared to chronically infected patients. These data supports the use of genotypic resistance testing in treatment-naive HIV positive patients, especially in newly diagnosed ones in order to guide the selection of the first line of antiretroviral treatment.

PRELIMINARY SURVEY ON THE HEPATITIS C VIRUS INFECTION AMONG INJECTION DRUG USERS FROM BUCHAREST

CODRUTA VAGU[2], CAMELIA SULTANA[1,2], LOREDANA MANOLESCU[1,2], CAMELIA GRANCEA[2], AURA TEMEREANCA[1,2], JOSEFINA SLOBOZEANU and SIMONA RUTA[1,2]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]"Stefan S. Nicolau" Virology Institute, Bucharest, Romania
[3]CAIA Pantelimon

INTRODUCTION

The epidemic of hepatitis C (HCV) infection in Europe is continuously evolving and the epidemiological parameters (prevalence, incidence, disease transmission patterns and genotype distribution) have changed during the last 15 years. Intravenous drug use has become the main risk factor for HCV transmission in certain European countries, although in Romania this route is less studied.

OBJECTIVES

A preliminary analysis of the HCV prevalence and of the HCV genotypes circulating among injecting drug users from Bucharest.

PATIENTS AND METHODS

The study group included 45 patients with history of intravenous drug use evaluated at a methadone substitution center in Bucharest. HBV, HCV and HIV markers were determined by third generation commercial ELISA assays. The diagnosis of hepatitis C was based on the presence of anti-HCV antibody and was confirmed by means of HCV-RNA testing (RT-PCR-Cobas Amplicor HCV Monitor v2.0). HCV genotype was determined by a line probe hybridization assay (LIPA HCV, Innogenetics, Belgium).

RESULTS

86,6% of patients had anti-HCV antibodies, 55% of them presented total anti-HBc antibodies as a marker of past hepatitis B virus infection; in addition 55% of them had markers of HCV/HBV coinfection. Less than 12% of the previously HBV infected patients were HBsAg carrier and only one patient had active viral replication (HBeAg present). 64% of patients infected with HCV had viral load detectable and values were reported between 10^4 UI/ml and 10^6 UI/ml. The majority of patients (48%) were infected with HCV genotype 1b, 22% with genotype 1a, 8% with genotype 1(undetermined subtype), genotype 4 was found in 13% of patients and only 8% presented genotype 3a.

CONCLUSIONS

The high seroprevalence of HCV infection among a intravenous drug users raises a major public health concern. As intravenous drug use has become the main risk factor for HCV transmission in the entire Europe, a close follow up of the circulating HCV genotypes in this vulnerable group became compulsory, as it may decisively influence the genotype distribution in the general population and may generate recombinants with more effectively transmission among non-IVDU.

EVALUATION OF ANTIRETROVIRAL RESISTANCE MUTATION PROFILE AND OF PHYLOGENETIC PARTICULARITIES OF F CLADE IN A GROUP OF NAIVE CHILDREN AND ADOLESCENTS

ENE L.[1], DUICULESCU D.[1], RADOI R.[1], BUCKTON A.[2], RUTA S.M.[3] and SMITH D.[4]

[1]'Dr. Victor Babes' Hospital for Infectious and Tropical Diseases, AIDS Department, Bucharest , Romania ,
[2]Centre for Infections, Health Protection Agency, Virus Reference Department, London, United Kingdom
[3] "Stefan S. Nicolau" Institute of Virology and „Carol Davila" University of Medicine ,Bucharest , Romania
[4] University of California at San Diego Department of Medicine, La Jolla, California , USA

OBJECTIVES

We aimed to evaluate the drug resistance mutations in the genome of HIV-1 F subtype from strains collected from Romanian ART-naïve children and adolescents and to assess the phylogenetic relationship of VBH strains with other HIV-F strains.

METHOD

Samples were collected between September 2005 and September 2008 from 28 ART-naive patients (median age 17.8 range 0.1-20.5 years): 24 had parenterally acquired HIV infection during their early childhood and four by mother-to-child transmission (MTCT). Drug resistance genotyping was performed using an in-house assay and Sequencer DNA analysis software version 4.8. Drug resistance interpretation was undertaken using the Stanford University HIVdb (http://hiv.db.stanford.edu/).

Mutations associated with transmitted drug resistance (TDR) were identified using the WHO 2009 list. All clade F, F1, F2 and recombinant HIV-1 pol sequences were downloaded from the HIV LANL database (http://www.hiv.lanl.gov/content/sequence). These sequences were aligned with the Romanian sequences using Clustal W and manually edited Neighbor-joining trees were then generated under the HKY model in Geneious program and re-sampled via bootstrapping 1,000 times

RESULTS

CD4 count and HIV RNA viral load at collection were 141 (range 2-2478) lf/mmc and 5.48 (range 4.7-6.7) log10 cp/ml, respectively. Major RT resistance mutations were identified in 3/24 (12.5%) of samples from adolescents: K103N (2 patients), K219Q (1 patient). Four patients had other RT mutations at codons including V179DV, E138G and E138AE, (described as being associated with a decreased response to Etravirine). No patient had any major resistance mutations to the PI class. Children with MTCT HIV-infection had no major or minor resistance mutations. Sequences from the study group clustered together regardless the HIV-infection route and the TDR pattern. The Romanian sequences segregated together with sequences from Angola and Cameroon, and separate from South American sequences.

CONCLUSIONS

We found major RT resistance mutations among 12.5% ART-naïve, parenterally-infected adolescents who have been living with HIV for up to two decades. As initial infection with TDR is doubtful, occurrence of primary resistance mutations might be explained by superinfection with another HIV strain. The clinical significance of the presence of subtype F polymorphism mutations which affect response to Etravirine has to be further evaluated. Phylogenetic analysis showed a distinct cluster of HIV-F clade in Romanian studied population that is closer to Angola and Cameroon sequences.

THE SIGNIFICANCE OF HIV RNA LEVELS IN CSF OF HIV-1 INFECTED PATIENTS WITH CENTRAL NERVOS SYSTEM COMPLICATIONS

DUICULESCU D.[1], ENE L.[1], TARDEI G.[1], MINDU N., RUTA S.M.[2] and CEUASU E.[1]

[1]"Dr. Victor Babes' Hospital for Infectious and Tropical Diseases, AIDS Department, Bucharest, Romania,
[2] "Stefan S. Nicolau" Institute of Virology and „Carol Davila" University of Medicine, Bucharest , Romania

OBJECTIVES

We aimed to compare cerebrospinal fluid (CSF) and plasma HIV RNA levels (VL) in children and adolescents with and without neurological complications in order to establish the usefulness of CSF HIV RNA cuantification

PATIENTS AND METHODS

This is a cross-sectional study on HIV RNA levels in paired CSF-plasma samples (measured by Amplicor, LCX and Taqman) on a group of 102 HIV-infected patients. Patients assigned according to their neurological status: 54 with AIDS-defining neurological opportunistic infections (OI) and HIV encephalopathy (HIVE), 24 with non-AIDS neurological diseases (11 of them diagnosed with subacute measles encephalitis – SME) and 24 without neurological impairment. Comparison between variables was performed using the t-test if normaly distributed and Wilcoxon if non-normal distributed, and the correlation between CSF and other parameters (CD4, albumin, pleocytosis) was used.

RESULTS

Overal median plasma VL (5.01 log10 c/ml) was higher compared with CSF levels (2,71 log10 c/ml). However 23 patients had higher CSF HIV RNA, most of them with HIVE (13) and criptococcal meningitis (3).

Patients on HAART with virological failure had signifficantly lower ($p<0,05$) CSF VL's compared with naive patients and with patients who stopped HAART for more than 1 month before evaluation, regardless their neurological condition. There was a lack of correlation between CSF and plasma VL in naive patients and those who stopped their antiretrovirals. CSF HIV RNA levels were positively corelated with albumin levels (rho=0.22, $p=0,05$) and with CSF pleocytosis (rho=0,51, $p<0,001$). Out of 22 patients with HIVE, 13 had higher CSF compared with plasma HIV RNA (5,5 vs 4,7 log10 c/ml, $p=0,005$). Patients with PML

and SME had lower CSF VL levels compared to plasma (3,1 *vs* 4,8 log10 c/ml, p=0,001 and 2,4 *vs* 4,3 log10 c/ml, p<0,001 respectively). 10 of 11 patients with SME had CSF ARN HIV <400 c/ml, despite the fact that most of them 8 had detectable HIV VL's. **Conclusions**: CSF HIV VL correlate with CSF albumin and pleocytosis. The lack of correlation between CSF and plasma HIV RNA observed in patients not undergoing HAART sugests the existence of compartmentalisation of HIV infection in CSF. Assessment of HIV VL in CSF could be a useful marker in diagnosis of CNS diseases (high levels in HIVE, low levels in PML and SME).

VIROLOGICAL CRITERIA FOR ANTIRETROVIRAL THERAPY MONITORING

SIMONA RUTA

"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
"Stefan S. Nicolau" Virology Institute, Bucharest, Romania

Although the antiretroviral treatment efficiency is evaluated through the long-term reduction of the morbidity and mortality associated with HIV infection, the earliest predictor factor is virological success, expressed by the decrease in viral load. The current therapeutic strategies aim to maximize suppression of viral replication using increasingly complex combinations of antiretroviral drugs, which target different steps in the virus life cycle (adsorption-coreceptor inhibitors; internalisation-fusion inhibitors; reverse transcription- NRTI and NNRTI, integration- integrase inhibitors; maturation of viral proteins- protease inhibitors). HIV viral load monitoring is essential for the prognosis of disease evolution, determining the indications for initiation/ changing of antiretroviral therapy, assesing the response to treatment and early detection of resistance development. Low initial levels of plasma HIV RNA are predictive factors of complete suppression of viral replication, although an undetectable viral load cannot always be correlated with a good immune response or a low rate of virological failure.

The immune response is crucial in establishing a basic level of HIV replication ("set point") and may contribute further to a slow progression of the disease. Highly dynamic active HIV replication facilitates selection from pre-existing viral population of resistant strains which gain a replicative advantage on the wild virus, becoming the dominant quasispecies and contributing to the treatment failure. Other important factors in the ART failure are the emergence of rapidly growing syncitia-inducing viral strains, with inadequate drug penetration into cellular reservoirs, which determine the decrease or even loss of drug activity and a low patient compliance to treatment. All these factors should be closely monitored in order to to have a better understanding of the virus -host immune response or virus – antiretroviral therapy relationship. New anticellular, immunomodulating therapies that aim to diminish the chronic activation and inflammatory processes that contribute to the HIV induced pathogenesis are under development.

EPIGENETIC ASPECTS IN HPV-INDUCED GENITAL ONCOGENESIS

GABRIELA ANTON[1], SIMONA O. DIMA[2], ANCA BOTEZATU[1], ANCA NASTASE[2], DEMETRA SOCOLOV[3], CRISTINA D. GOIA[1], IULIA V. IANCU[1], and I. POPESCU[2]

[1] Stefan S. Nicolau Virology Institute
[2] Clinical Institute for Digestive Diseases and Liver Transplant Fundeni,
[3] UMF – Grigore T Popa – Iasi

Disruption of epigenetic processes can lead to altered gene function and malignant cellular transformation. The initiation and progression of cancer is recognized to involve epigenetic abnormalities correlated with genetic alterations. Recent data in field of cancer epigenetics have shown extensive reprogramming of every component of the epigenetic machinery including DNA methylation, histone modifications, microRNA expression. Microarray technology was used for investigating alterations in the epigenetic landscape that occur in cervix cancer. Global DNA methylation as well as specific DNMTs expression levels were analyzed.

Unlike DNA methylation, histone modifications can lead to either activation or repression depending upon which residues are modified and the type of modifications present. Active and repressive histone modifications (H3K4me3 and H3K9me3) have been investigated by flow-citometry. Taking into account that miRNAs can also modulate epigenetic regulatory mechanisms by targeting enzymes responsible for DNA methylation, DNMT3A, DNMT3B and EZH2 expression were determined in Western-blot Our findings support the hypothesis that epigenetics play a central role in neoplasia being the key perpetuators of cancer.