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CONFERENCES

DEVELOPMENT OF ONCOLYTIC VIRUSES AGAINST SOLID TUMORS

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Two major groups of anti-cancer agents, *i.e.* cytotoxic drugs and antibodies, are in clinical use at present. Yet, there is an urgent need to develop new modalities to improve the treatment. Onocolytic viruses, represent a new promising group of anti-cancer agents. Engineering of viruses to infect and kill specifically tumor cells sparing normal cells can be accomplished by various means and this approach is an active research area, both at the preclinical and clinical phases. In this presentation we describe the development of a novel ex vivo experimental system to evaluate the selective tropism, efficacy and toxicity of potential onclytic viruses. Normal and tumor tissues are obtained from the surgery room and maintained *ex vivo* as organ cultures. Selectivity of infection and spread of the virus in the tumor tissue as compared to the normal Tissue is evaluated using biochemical and histo- chemical methods. Using this *ex vivo* method, we have studied the molecular mechanisms of viral tropism and the oncolytic potential of two attenuated viruses, herpes simplex type 1 (HSV1) and New Castle disease virus (NDV). HSV1 has been shown to be selective to colon cancer tissue over normal colon due to differences in the extracellular matrix of the two tissues. NDV on the other hand, selectively kill advance melanoma tissue due to the over expression of a unique pro-apoptotic protein. Experiments in mouse models in vivo further exhibit the potential of these two viruses in the treatment of the respective tumors. A phase 1 clinical study has shown a safety profile for the oncolytic NDV.

OUT OF BREATH IS NOT OUT OF FUNCTION: HOW DO TUMORS COPE WITH HIPOXIA

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Hypoxia has been recognized as one of the fundamentally important features of solid tumors and plays a critical role in various cellular and physiologic events, including cell proliferation, survival, angiogenesis, immunosurveillance and metabolism. Rapidly growing tumors quickly outstrip the vascular supply and, thus, result in a poorly vascularised microenvironment characterized by

hypoxia, low pH, and nutrient starvation. Such hypoxic zones have been postulated to have a reduced response to radiotherapy due to a decrease in oxygen- free radicals required to produce DNA damage. In addition, cells residing in these regions are considered to be chemotherapy-resistant due to limited delivery of drugs via the circulation. To survive in this hypoxic microenvironment, tumor cells co-opt adaptive mechanisms to switch to a glycolytic metabolism, promote proliferation, become resistant to apoptosis, obtain unlimited replication potential and genomic instability, evade immune attack, induce angiogenesis, and migrate to less hypoxic areas of the body. The responses to hypoxia are orchestrated by activation of several transcription factors among which the hypoxia-inducible factors (HIFs). HIF-1, 2 and CREB are key regulators of the response of mammalian cells to oxygen deprivation and plays critical roles in the adaptation of tumor cells to hypoxic microenvironment. Hypoxia inducible factor, HIF-l, is overexpressed in various human cancers and has been recognized as one of the master regulators of oxygen homeostasis and mediates a wide variety of responses to hypoxia cue. In the presence of 02, HIF-1 is marked for degradation by hydroxylation catalyzed by prolyl hydroxylase, ubiquitylated and targeted to the proteasome for degradation. In the absence of oxygen, the oxygen sensor proteins are inactive because of the lack of available oxygen, HIF 1 then translocates to the nucleus and binds to the hypoxia-response elements (HREs) in the promoters of hypoxia-responsive genes. cAMP-responsive element binding protein, CREB, is a transcription factor that regulates diverse cellular responses, including proliferation, survival, and differentiation. CREB is activated by a variety of growth factors and inflammatory signals and subsequently mediates the transcription of genes containing a cAMP-responsive element (CRE).We showed that the DNA binding of CREB is regulated by its oxidation status. The reduction of two cysteine residues (Cys 300 and Cys 310) located in the DNA binding domain, enhances the binding efficiency of CREB to DNA and regulates CRE-mediated gene expression. The time frame and the duration of the decrease in oxygen are critical aspects in cellular responses and cell survival. We have revealed in tumor cells during hypoxia an increase in CREB concentration, relative to total cell proteins, mostly recruited to the nucleus and the mitochondria. The rescue of CREB from hypoxia induced degradation is also regulated by the redox state of the Cys residues 300 and 310. The nuclear accumulation of CREB at hypoxia results in activation of the repertoires of genes required for the responses of tumor cells to hypoxia and affects greatly the growth rate of tumors in mice models. Based on the crucial role of HIF1 and CREB in the survival of tumor cells at hypoxia we constructed a MLV based replicating recombinant virus to knock-down both transcription factors in tumors.

SOMATIC CHANGES IN INTRAHEPATIC MALIGNANT PRIMARY TUMORS IN ROMANIA

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Chronic liver diseases triggered by hepatitis viruses, alcohol abuse, or various toxic and metabolic conditions are known for decades as major Public Health concerns in Romania. Malignant liver tumors, the ultimate outcome of chronic hepatic injuries are, according to the World Health Organization, relatively common in Romania but were never subjected to any genetic characterization so far. Interestingly, primary liver cancer is an amazingly heterogeneous disease with regard to its risk factors, for which molecular analysis is known to provide clues for therapeutic options as well as Public health interventions. We analyzed 66 intrahepatic malignant primary tumors from patients who underwent surgery between 2007 and 2010 at the Institute of Digestive Diseases and Hepatic Transplantation Fundeni in Bucharest. Different histotypes were represented:

hepatocellular carcinoma (HCC, 72%) was the dominant form followed by cholangiocellular carcinoma (CCC, 19.5%), cystadenocarcinoma (3%), neuroendocrine tumors (3%), squamous cell carcinoma and leyomyosatcoma (1.5% each). Major risk factors for the present cohort were hepatitis C virus (36% of cases), hepatitis B virus (35%) and alcohol abuse (13.6%). Point mutations were searched in 28 exons (p53, a-catenin, axinl, H/K/N-RAS, BRAF, PTEN) frequently altered in epithelial tumors and chromosome instability explored on 14 chromosome arms commonly targeted in liver carcinomas. The gene encoding p53 was mutated in 9 cases of HCC (19%) whereas we found mutations of the n-catenin/axin axis in 17 HCC samples (36%). One mutation of p53 was characteristic of aflatoxin B1 exposure (Arg249Ser), an occurrence particularly unfrequent if ever in HCC from European patients. The biliary tumor Proc. Rom. Acad., Series B, **2011**, *1*, p. 23–29s were paucimutated with a single p53 mutation (7.7%). Chromosomal instability measured as the proportion of alleles lost per tumor sample was high both in hepatocellular and cholangiocellular carcinomas (mean values 2917 and 3220% respectively) suggesting that tumorigenic processes in Romania relies primarily on chromosomal lesions rather than on milder epigenetic mechanisms. Further analyses and extension of the cohort are now warranted to correlate genomic alterations and disease outcome.

IN VITRO SUPPRESSION OF TUMOR DEVELOPMENT

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Introduction. It has become clear that progression of carcinomas depend not only on alterations on epithelial cells, but also on changes of microenvironment. Solid tumors survival and development is often based on vascular network sustaining such an intense metabolic process. Main purpose of our study was investigation of pro-angiogenic factors (VEGF) secreted within tumor environment and inhibition of autocrine and paracrine effects acting on both tumor cells and tumor associated fibroblasts (TAF).

Material and methods. Bone marrow-derived MSCs, TAFs and tumor cell line SK-BR3 were used. Level of VEGF secretion was determined using ELISA method, while immunocytochemistry revealed positive staining in case of TAF and SK-BR3 cell line. Ouabain, Na^+/K^+-ATPase inhibitor, was used in concentrations ranging from 10 |jM to 1 nM and the amount of cellular utilization was determined by HPLC. MTT-based viability assay showed decreased proliferation rates for higher concentration of Ouabain. Flowcytometric analysis investigated expression of phenotypical markers, including CD106, CD90, CD44, CD29, CD117, CXCR4 for MSCs and TAFs, and Her2, CD44, VEGF-R and CD29 for SK-BR3 cells. Gene expression of al and pi subunits of Na^+/K^+ pump, as well as VEGF were determined in genuine cells and treated ones using qRT-PCR method. Immunocytochemistry investigated presence of a 1 and pi subunits of Na^+/K^+pump, VEGF and adhesion molecules.

Results. Secretion of VEGF was significantly reduced in Ouabain-treated SK-BR3 and TAFs. Although level of expression for a 1 catalytic subunit was increased in SK-BR3, we could not find presence of corresponding protein, reflected also in high levels of Ouabain detected in cellular supernatant. Expression of adhesion molecules was decreased, and we found profound changes in phenotypic profile of both TAFs and SK- BR3 cells.

Conclusion. Based on their anti-angiogenic and anti-proliferative activity, Na^+/K^+ pump inhibitors could open novel anti-tumoral therapeutic strategies.

INVOLVEMENT OF MICRORNAS TO CANCER GENESIS AND HOMEOSTATIS

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Background and aims. MicroRNAs (miRs) recently emerged as prominent regulators of homeostasis in both normal and cancer cells. In the current study, we aimed at 1. Identifying and characterizing miR species dysregulated in human cholangiocarcinoma (CCA) and 2. Elucidating regulatory pathways and mechanisms through which the miR species chosen for this study, miR-494, participates in CCA homeostasis.

Methods. miR-494 was identified as downregulated in CCA based on miR arrays. Its expression was verified with quantitative real time RT-PCR (qRT-PCR). Ingenuity pathway analysis coupled with mRNA arrays was used to identify putative molecules and pathways downstream of miR-494. For the purpose of enforcing the miR expression, we employed both transfection methods, as well as a retroviral construct to stably overexpress miR- 494.

Results. Enforcing the expression of miR-494 in cancer cells decreased growth, consistent with a functional role in cancer. mRNA arrays of cells treated with miR-494, followed by pathway analysis, suggested that miR-494 impacts cell cycle regulation. Cell cycle analyses demonstrated that miR-494 induces a significant Gl/S checkpoint reinforcement. Further analyses demonstrated that miR-494 downregulates multiple molecules involved in the regulation of the Gl/S transition checkpoint. Last, xenograft experiments demonstrated that miR- 494 induces a significant cancer growth retardation *in-vivo.*

Conclusions. Our findings are consistent with the model that miRs exert their action through a moderate, but highly coordinated effect on multiple molecules along the same pathway with a significant phenotypic end result. miR-494 appears to function as a rheostat of a canonical pathway through simultaneous and convergent effects at parallel and consecutive levels, rather than as an on-off switch of one target gene. These findings support the paradigm that miRs are salient cellular signaling pathway modulators, and thus represent attractive therapeutic targets.

NS2 PROTEIN OF HEPATITIS C VIRUS COORDINATES THE ASSEMBLY PROCESS OF THE VIRION

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Hepatitis C virus (HCV) represents a major health problem worldwide. Understanding the major steps of the life cycle of the virus is essential to develop new and more efficient antiviral molecules. Virus assembly is the least understood step of the HCV life cycle. Our picture of HCV assembly began to get shape recently when a cellular system which recapitulates the whole viral cycle was developed (HCVcc). Since then, it has been shown that both structural and non-structural proteins are involved in the assembly process. NS2 is a non-structural protein which was reported to be essential for the assembly process by an unknown mechanism. Using FRET-FLIM technique and immunoprecipitation, we showed that NS2 interacts with p7 protein in recombinant systems. In the HCVcc, we showed that NS2 interacts with E2 protein and this interaction is affected by mutations in NS2 transmembrane region. We further investigated the NS2 subcellular localization in

HCVcc replicating cells by confocal microscopy. Thus, NS2 is localized in the endoplasmic reticulum and also accumulates in dotted structures which are positive for El, E2, NS5A and NS3 proteins. NS2 dot-like structures are juxtaposed to the core protein and the lipid droplet (LD). NS5A positive NS2 dots formation is independent of the core recruitment to the LD. Moreover, NS5A positive NS2 dots formation is positively and negatively modulated by envelope proteins, p7, NS5A and the transmembrane region of NS2. We discuss an assembly model involving three functional units: core. El E2p7NS2 and the replication complexwhich crosstalk towards the virion assembly.

THE ER HOMEOSTASY REQUIRES A FINE TUNE OF EDEM PROTEINS REGULATION

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To maintain protein homeostasis in secretory compartments, eukaryotic cells harbor a quality control system by which misfolded proteins or those that have failed to become post-translationally modified are discarded by ER-association degradation (ERAD). Failure of any step within ERAD process result in accumulation of toxic protein within ER which trigger ER stress, ER storage diseases, and ultimately, apoptosis. It is known that ER degradation- enhancing □-mannosidase-like proteins 1, 2, & 3 (EDEM1-3) are essential for recognition and disposal of the terminally misfolded glycoproteins. However, their role in a context of whole multicellular organism has not yet been addressed, and therefore we are investigating the role and redundancy of the EDEMs using the model organism *Caenorhabditis elegans.* Similar to their mammalian orthologues, C. *elegans* has different requirements for EDEMs, judging by examination of their mRNA levels and tissue expression pattern. Although inactivation of all three EDEMs activated the Unfolded Protein Response (UPR), we found that single inactivation of *edem* genes in the absence of ER stress not only that did not have a dramatic effect on worm viability, but simultaneous depletion of EDEM-1 and EDEM-2 extended the lifespan of the worm suggesting a synergistic role of EDEM-1 and EDEM-2. Moreover, our genetic interactions suggested a tight regulation between EDEM 1-3 functions to maintain ER homeostasy during development in ER stress conditions.

SAME CHALLENGES, SAME POPULATION, NEW SOLUTIONS: ASSESSMENT OF A NEW PANEL OF GENES IN COLON ADENOMAS AND TUMORS

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As shown in our previous communication the need of new biomarkers for early detection, progression and prognostic in colon cancer is imperative. The aim of our study is to asses by qPCR the level of expression for some candidate genes involved in colon carcinogenesis and progression for identifying a new panel of biomarkers for this disease.

Material and methods. All used tissues were considered operatory waste and were obtained with a written informed consent. qPCR was realised on triplets of colon mucosa (normal, adenoma, tumoral tissue).

Results and disscusions. A list of 23 up-regulated genes in adenoma and tumoral samples compared with matched normal tissues was validated by qPCR. Our results show that DEFA5, DEFA6, TCN1 and LCN2 are key factors in adenoma formation while MMP7 is important in the transition from a benign to a malignant status. We find from the expression of the studied cytokine that inflammation plays an important role in colon cancer onset and progression. IL8 has a high mRNA level in adenomas and irrespective of tumor stage, in adenocarcinoma as well. The mRNA level of SPP1 is correlated with tumor level. KLK10, CLDN1, ASCL2 and INHBA are genes involved in the progression of colon cancer.

Conclusion. DEFA5, DEFA6, TCN1 and LCN2 are potential biomarkers for early diagnostic, high levels of IL8 and MMP7 could emphasize the transition from adenoma to adenocarcinoma while high levels of KLK10, CLDN1, ASCL2 and INHBA could be characteristic for late stages of colon cancer.

DIFFERENTIATION OF MESENCHYMAL STEM CELLS ON BIOMATERIALS

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In this work we tested the biocompatibility of B-type carbonated hydroxylapatite (B-CHA) thin films obtained by radio frequency magnetron sputtering (RF-MS). Human mesenchymal stem cells (hMSCs), in vitro differentiated osteoblasts, and explanted bone cells were grown over the surface of CHA coatings for periods between a few hours and 21 days. Bone marrow aspirates were the source for hMSCs, which were isolated by ficoll gradient centrifugation. Osteoblasts were either obtained by differentiation with induction factors from hMSC or derived from trabecular bone fragments. Osteoprogenitor cells maintained viability and characteristic morphology after adhesion on CHA coatings. Ki67-positive osteoblasts were the evidence of cell proliferation events. Cells showed positive staining for markers of osteoblast phenotype such as collagen type I, bone sialoprotein and osteonectin. Our data showed the formation of mineralized foci by differentiation of hMSCs to human primary osteoblasts after cultivation in osteogenic media on RF-sputtered films. The results demonstrate the capacity of B-type CHA coating to support MSCs adhesion and osteogenic differentiation ability.

ASSOCIATION OF G72/G30 GENE WITH PHENOTYPIC TRAITS OF BIPOLAR I DISORDER IN THE ROMANIAN POPULATION

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The *G72/G30* gene is one of the common loci shared both by schizophrenia and bipolar disorder. Studies accumulating since the discovery of this gene complex produced controversial results in both disorders in different populations.

Objective. We investigated the association between *G72/G30* gene and bipolar I disorder (BPI) in the Romanian population paying special attention to the association of *G72/G30* with lifetime psychosis in BPI patients.

Method. Fourteen G72-SNPs were genotyped in a Romanian sample of 198 BPI patients and 180 controls screened for psychiatric disorders. Statistical analysis was performed with FAMHAP and Haploview-v3.32. The significance level of the results was corrected through permutations in 100,000 simulations.

Results. None of the fourteen SNPs was associated with the global diagnosis of BPI in our total patient sample or with the psychotic BPI subtype. But four SNPs reached nominal significance in the non-psychotic BPI subgroup [rs3916965 (Ml2) (P=0.044), rs 1935057 (P=0.037), rs3916967 (M14) (P=0.043), rs2391191 (M15, non-synonymous) (P=0.043)]. After correction through permutations, the haploblockGA including Ml4 and Ml 5 showed a trend to association with BPI (P=0.0524; OR=l .82) in the non-psychotic BPI subgroup.

Conclusion. We report a potential association of some SNPs and of a haplotype (M14-M15) in the G72/G30 gene with non-psychotic BPI disorder. Some of our findings replicate for the first time previous results reported for the British population (Williams *et al.*, 2006), where G72/G30 gene with non-psychotic BPI disorder. Some of our findings replicate for the first time previous results reported for the British population (Williams *et al.*, 2006), where G72/G30-SNPs were associated with non-psychotic major mood episodes.

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INVESTIGATION OF IL28B GENE POLYMORPHISM IN ROMANIAN CHRONIC HEPATITIS C PATIENTS TREATED WITH PEGYLATED INTERFERON AND RIBAVIRIN

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Introduction. Approximately 50% of chronic hepatitis C genotype 1 patients do not respond to antiviral therapy. Genome-wide association studies showed a link between certain host genetic determinants and hepatitis C virus persistence.

Aim. To investigate IL28B polymorphism in Romanian hepatitis C patients that underwent pegylated interferon and ribavirin therapy and its associations with treatment outcomes.

Results. There were included 65 patients (38.5% females and 61.5% males) with a mean age of 49.1±10.1 years at beginning of antiviral therapy.

Distribution of IL28B genotypes were: C/C - 23.3% of patients, C/T - 63.3%, T/T -13.4%. C/C genotype was associated with presence of early virological response (p=0.03) and sustained virological response (SVR) (p=0.01). Genotype non-CC was associated with nonresponder status (p=0.009). In the C/T genotype subgroup of patients, SVR was significantly higher in female patients (p=0.03) and with mild hepatitis (F0-1 METAVIR) (p=0.0006). In this case, mild hepatitis was the independent predictor of SVR (p=0.008). Conclusions: Host IL28B genotype is useful for prediction of antiviral therapy response. The majority of Romanian hepatitis C patients are heterozygous for IL28B polymorphism and fibrosis stage 0-1 is the independent predictor of response in these patients.

THE GENETIC BIOMARKERS AS PREDICTIVE FACTORS IN RECTAL CANCER

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Different genes included in apoptosis may be involved in tumor biology and identify specific groups of patients with individual therapy.

Aim. To evaluate the prognostic value of some apoptosis markers in conjunction with pathological factors in operable rectal cancer patients.

Methodology. Tumorsamples from 87 patients with rectal adenocarcinoma treated using surgical approach followed by adjuvant treatment were analysed retrospectively. The immunohistochemistry from "tissue array" for the expression of p53, p21, Bcl-2 and cycline D1 was performed. These data combined with pathological features were correlated with the survival data. The postoperative follow-up period was 48.65±35.93 months.

Results. Five-year cancer-specific survival was 44.9%. Protein p53, p21 and Cyclin D1 were positive in more than half of the patients. On multivariate analysis, the independent prognostic factors for cancer-specific survival were p53 and p21 protein, whereas for cancer-free survival p21 was a positive independent factor. When pathological factors were also introduced in the analysis, the positive prognostic role on 5-year cancer-specific survival was obtained for p21 and lymphatic invasion; the 5- year cancer-free survival was positively influenced by the presence of p21 protein and lymphatic invasion. For patients with pNO, p21 protein was an independent predictive markerfor cancer-specific survival and cancer-free survival.

Conclusion. Molecular markers of apoptosis, such as p53 and p21, had a significant prognostic role in rectal cancer patients, together with lymphatic invasion. The presence of p21 protein in pNO patient outcome may influence management, but needs further evaluation.