do not express NeuGc, making difficult the development of appropriate preclinical tumor models. Our aim was to obtain B16 melanoma cells with transient expression of NeuGc by in vitro antigen incubation or by stable overexpression of CMP-NeuAc hydroxilase.
Materials and Methods: Transient expression was obtained by in vitro incubation with mucin, a NeuGc-rich compound. Stable expression were done by molecular techniques in order to isolate and amplify the murine CMP-NeuAc hydroxilase sequence from normal liver. The cloning and transfection were done using the invitrogen cloning TOPO system.
Results: Incubation of B16 cells with mucin induced the presence of this antigen in B16 cell membrane during 48 hours. Preincubation with mucin caused an enhancement in tumor cell adhesion on plastic surfaces. In vivo, mucin-incubated B16 cells showed a rapid subcutaneous primary tumor formation and an increase in the metastatic ability after endovenous injection in syngeneic C57Bl6 mice. Transfected B16 cells showed the presence of CMP-NeuAc hydroxilase mRNA and the presence of the NeuGc antigen in tumor cell membrane. We observed an increase of in vitro proliferation and cell adhesion in transfected cells as compared with control non-transfected B16 cells. Interestingly, stable NeuGc expression was associated with a weak tumorigenicity in syngeneic mice after subcutaneous implantation of transfected B16 cells and a decrease of lung metastasis.
Conclusions: Taken together, the results indicate that the presence of NeuGc modulates positively in vitro proliferation and adhesion of mouse melanoma cells, but stable expression of the antigen may induce a negative selection during tumor progression in immunocompetent mice.

362 POSTER

Pepsinogen C gene polymorphism and breast cancer: Influence on the overall survival

A. Pinto Correia[1], D. Pinto[1], D. Pereira[2], H. Rodrigues[2], J. Abreu de Sousa[3], H. Sousa[1], B. Sousa[2], S. Sousa[2], C. Lopes[1], R. Medeiros[1]. *[1]Portuguese Institute of Oncology, Molecular Oncology Unit, Porto, Portugal; [2]Portuguese Institute of Oncology, Medical Oncology Unit, Porto, Portugal; [3]Portuguese Institute of Oncology, Surgical Oncology Unit, Porto, Portugal*

Background: Pepsinogen C gene (PGC) has been associated with hormonal control, and therefore the role of its protein has been investigated in breast cancer. We have studied the influence of an insertion/deletion polymorphism in the Pepsinogen C (PGC) gene, in the clinical outcome of breast cancer patients.
Material and Methods: The study was performed with 172 blood samples of breast cancer patients. The 6 polymorphic alleles were amplified using PCR: allele 1 (510 bp), allele 2 (480 bp), allele 3/4 (450/460 bp), allele 5 (400 bp) and allele 6 (310 bp).
Results: Our results indicate that patients carrying the allele 6 present a higher 5-year survival mean (83.4% of 6 allele carriers were alive at 5 years versus only 68.6% of non-carriers, $p = 0.001$), suggesting a role for this polymorphism in the outcome of breast cancer patients.
Conclusions: We hypothesize that PGC polymorphism can be a predictive biomarker in breast cancer, contributing to an individual profile of great interest in clinical oncology.

363 POSTER

Genomic instability in non-small cell lung cancer assessed by arbitrarily primed polymerase chain reaction

J. Bankovic[1], J. Stojsic[2], S. Zunic[3], I. Selakovic[1], V. Paunovic[1], S. Ruzdijic[1], N. Tanic[1]. *[1]Institute for Biological Research "Sinisa Stankovic", Molecular Neurobiology, Belgrade, Serbia Montenegro; [2]Institute for Lung Diseases and Tuberculosis, Clinical Centre of Serbia, Belgrade, Serbia Montenegro; [3]Institute for Nuclear Medicine, Clinical Centre of Serbia, Belgrade, Serbia Montenegro*

Lung cancer is the most common cause of neoplasia-related death worldwide. One of the crucial early events in carcinogenesis could be the induction of the genomic instability phenotype. The high incidence of genomic instability in lung cancers has been well established, and in some cases it has been associated to prognosis. We investigated genomic instability in patients with non-small cell lung cancer (NSCLC). Instability was correlated with patients' age at diagnosis, gender, NSCLC subtype, histological grade and stage, tumor necrosis and lymph node invasion.
DNA from tumor and corresponding normal tissues of 30 patients with NSCLC was isolated and amplified with five arbitrary primers using arbitrarily primed polymerase chain reaction (AP-PCR).
Four out of five tested primers produced informative sequence alterations differentiating normal tissue from NSCLC. Comparing AP-PCR profiles of normal and tumor tissue we identified significant genomic instability in most cases. Two types of electrophoretic changes were detected, qualitative changes (structural DNA alterations) and quantitative changes (chromosomal gains and losses). Genomic instability was represented as the frequency of DNA alterations. Genomic instability resulting from the total number of DNA changes was significantly higher in patients older than 50 ($P < 0.05$). Frequency of DNA alterations calculated from qualitative changes was significantly different between patients with adenocarcinoma and patients with squamous cell carcinoma ($P < 0.05$). ANOVA revealed a significant correlation between the total number of DNA changes and histological grades ($P < 0.006$) as well as between quantitative changes alone and histological grades ($P < 0.016$). Post hoc comparisons showed significant difference between the frequencies of DNA alterations in grade groups 1 and 2 ($P < 0.05$) and in groups 1 and 3 ($P < 0.005$), as well as in grade groups 2 and 3 ($P < 0.05$). Most importantly, genomic instability decreased with increasing tumor grade.
Our results suggest that high frequency of genomic instability in early stages of cancer development may be involved in progression of NSCLC. Lower degree of genomic instability in advanced stages of NSCLC (histological grades 2 and 3) could be considered as a marker of poor prognosis. Our study shows that AP-PCR is an effective method for the identification and analyses of genomic instability in NSCLC and may provide insight into the molecular mechanism of lung carcinogenesis.

364 POSTER

Importance of the pro-apoptotic Bcl-2-like protein Bak for radiation- and hypoxia-induced apoptosis

J. Rudner, M. Weinmann, R. Boras, C. Belka, V. Jendrossek. *Radiooncology, Experimental Radiation Oncology, Tuebingen, Germany*

The disruption of mitochondrial homeostasis is the key event in DNA damage- and stress-induced apoptosis. It involves breakdown of the mitochondrial membrane potential and release of pro-apoptotic factors from the mitochondrial intermembrane space with subsequent activation of the caspase cascade and execution of apoptosis. The mitochondrial homeostasis is controlled by pro- and anti-apoptotic proteins of the Bcl-2 family that either antagonize (Bcl-2, Bcl-x_L) or activate (Bax, Bak) downstream signalling events.
To gain further insight into the mechanisms of radiation- and hypoxia-mediated cytotoxicity at the level of the mitochondria we tested in how far crucial pro-apoptotic Bcl-2 proteins, namely Bak and Bax, are involved in apoptosis-induction using Jurkat T-lymphoma cell clones being either negative for Bax but expressing Bak (Jurkat Bak positive), or being negative for both, Bax and Bak (Jurkat Bak negative) Induction of apoptosis by hypoxia and irradiation was determined in Jurkat Bak positive and Jurkat Bak negative cells by flow cytometry (breakdown of the mitochondrial membrane potential, nuclear fragmentation), fluorescence microscopy (nuclear condensation), and Western blotting (activation of caspase-9, caspase-3, caspase-8 and cleavage of the caspase-substrate PARP).
Our results provide evidence for Bak-dependent pro-apoptotic effects of hypoxia and irradiation at the level of the mitochondria. While lack of Bax was not sufficient to inhibit radiation- and hypoxia-induced apoptosis in Jurkat cells expressing Bak, absence of Bak strongly reduced mitochondrial alterations compared to Bak-positive cells and completely abrogated treatment-induced caspase activation.
From these data we conclude that the pro-apoptotic Bcl-2 homologue Bak is essential for radiation- and hypoxia-induced apoptosis in Bax-deficient Jurkat T-lymphoma cells.

365 POSTER

Origin of 5-ALA-induced PpIX at brain tissues surrounding tumor (in vitro photometrical study)

T. Masubuchi, N. Nonoguchi, S. Kawabata, Y. Kajimoto, S. Miyatake, T. Kuroiwa. *Osaka medical college, Neurosurgery, Takatsuki, Japan*

Background: In surgical treatment of malignant glioma, we experienced the fluorescence of 5-aminolevulinic acid (5-ALA)-induced protoporphyrin IX (PpIX) at a non-tumor department, brain without blood-brain barrier (BBB), edematous brain tissues surrounding tumor and so on.
Materials and Methods: In vitro, we cultured several kinds of brain tumor cell lines (C6 rat glioma, U87delta human glioma, U251 human glioma and IOMM-Lee human malignant meningioma) and exposed to different condition of 5-ALA including culture medium. After this, fluorescent degree of the medium and cells were each measured by means of photometrical assay, and analized quantitifically with fixed-quantity of intracellular and extracellular PpIX.
Results: Comparing the fluorescence degree of a cell, C6 and U87delta had a peak in the vicinity of 0.5mM 5-ALA, but U251 and IOMM-LEE had not the peak. In addition, comparing the fluorescence degree of a nutrient medium, we recognized a peak in the vicinity of 0.5mM 5-ALA entirely.

On the other hand, the peak of fluorescence degree of a nutrient medium showed 2−10 times higher when compared to the fluorescence degree of a nutrient medium with a cell. We observed the existence of PpIX in the culture medium, however degree was different. We think that 5-ALA-induced PpIX formed by the brain tumor cells leaks out to the outside of the tumor cells.
Conclusions: Each brain tumor cell generated PpIX by the 5-ALA, and 5-ALA-induced PpIX was leaked out to the outside of brain tumor cell.

366 POSTER

Mechanism of the initiation of DNA methylation de novo by small RNA

V. Halytskiy. *Palladin Institute of Biochemistry of NAS Ukraine, Molecular Immunology Department, Kiev, Ukraine*

DNA methylation is an important epigenetic mechanism that assigns and maintains gene expression profile and thus enables cell differentiation, allelic exclusion and other key phenomena. We investigate possible role of small interfering RNA and microRNA in the DNA methylation de novo.
Human, mouse and rat sequences of siRNA – in all 599 sequences – were extracted from database "siRNA Database and Resources for RNA Interference Studies", http://www.rnainterference.org/Sequences.html. Human, mouse and rat sequences of mature miRNA – in all 1083 sequences – were extracted from database miRBase, http://microrna.sanger.ac.uk/.
We discover only 14.36% siRNA sequences and 20.68% mature miRNA sequences, containing none of 5′-CG-3′ dinucleotides or 5′-CNG-3′ trinucleotides. 5′-CG-3′ frequency amounts to 2.89% in siRNA sequences and 2.39% in mature miRNA sequences. This level exceeds more than twice the average genomic frequency of 5′-CG-3′ dinucleotides, that makes up 1% in human or mouse genome, and 1.2% in rat genome. 5′-CNG-3′ frequency amounts to 6.29% in siRNA sequences and 6.49% in mature miRNA sequences. Nevertheless, the 5′-CNG-3′ or 5′-CG-3′ frequency should theoretically make only 4.41% in random human DNA sequence, though this frequency appeares to be in reality much less as a result of 5-methylcytosine hypermutability.
Thus, 5′-CG-3′ and 5′-CNG-3′ sites are discovered in siRNA and miRNA sequences more often than they should be found in random sequence. This circumstance is evidence of an important biological purpose of 5′-CG-3′ dinucleotides and 5′-CNG-3′ trinucleotides in siRNA and miRNA sequences.
In our opinion, complexes of small RNA and Argonaute protein scan nucleotide sequence of DNA strands while RNA polymerase II is untwisting DNA molecule during the transcription. Recognition and binding of complementary site in DNA by siRNA leads to recruiting of DNA methyltransferases that methylate de novo cytosine in 5′-CG-3′ dinucleotides and 5′-CNG-3′ trinucleotides of DNA, which appeared to be bound with similar sites in the siRNA sequence. Histone deacetylase and histone methyltransferase are also attracted to DNA site, which was recognized by small RNA. They delete active chromatin marks. Several genes can be switched off simultaneously when they contain the motif, which is recognized by small RNA. We suppose that gene modules (elementary units of cell differentiation network) contain miRNA genes, which are activated in certain moments for the purpose of stable epigenetic repression of other gene modules that complete their mission in course of cell specialization or are responsible for other differentiation directions.

367 POSTER

Statistical correlations around the transcription initiation site in the DNA sequences of human promoters

G. Mahon[1], M. Dicato[2]. *[1]Centre Hospitalier, Foundation for Research on Cancer and Blood Diseases, Luxembourg, Luxembourg; [2]Centre Hospitalier, Foundation for Research on Cancer and Blood Diseases – Department of Hematology-Oncology, Luxembourg, Luxembourg*

Background: Genetic control sites, such as promoters, generally have a characteristic consensus sequence. We have been interested in variation about the consensus sequence, especially correlations, i.e. the tendency of a particular base at one position to be associated with a particular base at another. This work addresses correlations in the region of the transcription initiation site, and extends our analysis of correlations upstream of that site in the region of the TATA motif (ESMO 2006, abstract 113P).
Methods: A dataset of 1975 promoters recognised by human RNA polymerase II was assembled from the Eukaryotic Promoter Database. Many of these promoters are of interest in oncology and the dataset includes sequences for the promoters of genes for growth factors (e.g. GM-CSF, erythropoietin, various interleukins) oncogenes and tumour viruses among others. For the 30-base sub-sequences from positions −19 to +10 relative to the transcription start, the consensus sequence was derived. The sequences were coded numerically and a correlation analysis performed. A principal components analysis enabled those promoters with the most similar sequences to be grouped taking account of the correlations.
Results: The consensus sequence was observed to be ggggg gc(c/g)cg ggggg cggca ttgcg gccgg. There were numerous statistically significant correlations, and 51 of these were greater in absolute value than 0.103 and thus very highly significant ($P < 0.000005$). As many as 38 of these correlations were positive and the rest negative. Almost half the correlations concerned bases in the range −2 to +6, i.e. at the initiation site or the first few transcribed bases. For example, a purine (an A or a G) at position −1 was associated with a purine at position 0, an A or a T at position 1 was associated with a C or a G at position 2. Almost all the highly significant correlations concerned bases separated by a few positions at most.
Conclusion: We have already shown significant correlations in the DNA sequences of human promoters associated with the TATA box; we now show comparable correlations at the transcription initiation site. Thus the variation among the sequences is seen not to be random. Principal components analysis allows groups of promoters with similar sequences to be defined, which may have similar functional properties.

368 POSTER

Genetic instability at 9p21 and its significance as prognostic indicators in liver fluke related cholangiocarcinoma

T. Limpaiboon[1], P. Chinnasri[1], P. Jearanaikoon[1], C. Pairojkul[2], B. Sripa[2], V. Bhudhisawasdi[2]. *[1]Khon Kaen University, Centre for Research and Development of Medical Diagnostic Laboratories, Khonkaen, Thailand; [2]Khon Kaen University, Liver Fluke and Cholangiocarcinoma Research Center, Khonkaen, Thailand*

Cholangiocarcinoma (CCA) is the highest incidence cancer in Northeast Thailand. CCA is caused by liver fluke, Opisthorchis viverrrini, infection resulting in genetic alterations. Loss of heterozygosity (LOH) and microsatellite instability (MSI) are the phenotypes of genetic instability caused by the abnormalities of tumor suppressor and DNA mismatch repair (MMR) genes. We investigated LOH and MSI on the chromosomal region 9p21-pter in 94 CCA patients using 6 microsatellite markers and determined the association between microsatellite alterations and clinicopathological parameters. A total of 59 out of 94 cases (62.8%) showed LOH in one or more loci. LOH was found most frequently at D9S157 (36.1%), D9S286 (34.2%) and D9S1752 (34%). MSI was found in 50 of 94 cases (53.2%) at one or more loci. Fine mapping at 9p21-pter showed a distinctive region of common loss, a region between D9S157 and D9S1752, indicating the existence of putative tumor suppressor genes that is likely to play important roles in the development of CCA. Tumor suppressor genes located at 9p21 are cyclin-dependent kinase inhibitor 2A (CDKN2A)/p16INK4A, CDKN2A/p14ARF, CDKN4B/p15INK4B, MTAP and interferon beta-1 (IFNB1). Nuclear factor 1 (NF1B) and endophilin-1 are located at D9S286 and D9S157 of chromosomal regions 9p24 and 9p22, respectively. Patients with LOH at D9S288 ($P = 0.022$) and D9S286 ($P = 0.043$) showed more blood vessel invasion while patients with LOH at D9S161 exhibited more lymphatic invasion than those without ($P = 0.015$). Moreover, patients who demonstrated LOH at D9S171 showed a poor prognosis ($P = 0.0296$). Our studies suggest that genetic alterations of tumor suppressor genes and DNA mismatch repair genes are involved in carcinogenesis and pathogenesis of liver fluke related CCA and genetic instability of 9p21 is of value as prognostic indicators in this cancer.

369 POSTER

Mismatch repair proteins and clinicopathologic factors in colorectal cancer

M. Molaei[1], A. Motlagh[2]. *[1]Shahid Beheshti Medical University, Research Center for Gastrointestinal and Liver Disease (RCGLD), Tehran, Iran; [2]Shahid Beheshti Medical University, Cancer Research Center (CRC), Tehran, Iran*

Background: Microsatellite instability due to defective mismatch repair proteins (MMRP) is one of the major pathways for carcinogenesis in colorectal cancer (CRC). The impact of these proteins in prognosis is not well defined. The aims of this study were the evaluation of abnormal MMRP prevalence and its relationship with some clinical and pathologic factors.
Materials and Methods: In our study 350 patients with CRC were immunostained for DNA mismatch repair proteins (MMRP) including hMLH1, hMSH2, hMSH6 and PMS2. Patients with at least one abnormal above factors considered in abnormal MMRP group. Clinical factors such as sex, tumor site (colon or rectum), family history of CRC and vital status (alive or dead) is considerd. Pathologic factors including grade, T and N stage in tumor specimen were examined.
Results: Totally 350 patients with median age of 51 (20 to 94) were evaluated. One hundred ninety five patients were male and 151 were female. The site of tumor in 270 patients was colon and in 68 were