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.

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

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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.

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

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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.

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

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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.

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

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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.