Material and Methods: Plasma Membranes (PM) of brain tissue of adult mice (C57BL/6) and of the human prostate cancer cell line DU-145 were isolated and tested for organelle-specific markers. Detergent Resistant Membranes (DRM) were extracted by incubating the PM with 1% Triton-X100 and centrifuging them in a discontinuous sucrose gradient. Nine fractions were obtained and analyzed for DRM markers as well as for EAG1.
Results: We can observe differences in the distribution pattern of the EAG1 channel in the PMs of the brain compared with those of the DU-145 cells.
Conclusions: Differences in the partitioning of the EAG1 channel within the different cells was demonstrated. This could explain its different behavior among cells, promoting proliferation in some but not in others.

764 Increased expression of NFY-C (Nuclear Factor Y, subunit C) and RORA (Retinoic acid receptor-related Orphan Receptor Alpha) in colorectal adenocarcinoma

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Background: NFY-C gene codes one of the three subunits of nuclear factor Y, a highly conserved transcription factor, which binds with high specificity to CCAAT motifs in the promoters of various genes, including cell cycle-related genes. RORA is a member of the NR1 subfamily of nuclear hormone receptors and plays a critical role in the development of the cerebellum. It can bind as a monomer or a homodimer to hormone response elements upstream of several genes to enhance their expression.
Material and Methods: mRNA levels of NFY-C and RORA were evaluated by quantitative RT-PCR in 81 neoplastic colorectal tissue specimens and 51 normal tissue specimens from patients with colorectal adenocarcinoma. All patients had undergone curative resections at University Hospital of Patras, between 1995 and 2005. mRNA levels were assessed using SYBR Green intercalation dye and specific primers for NFY-C and RORA. NFY-C and RORA mRNA levels were normalised to the Alu-Sq levels and were analysed in relation to clinicopathological parameters. Protein expression of NFY-C was assessed by immunochemistry in 60 malignant and 20 normal samples from patients with colorectal adenocarcinoma.
Results: There was a significant difference in the mRNA expression levels of NFY-C and RORA between normal and malignant tissues ($p < 0.001$ and $p = 0.015$, respectively). The mRNA levels of NFY-C and RORA were also related to the primary site of the tumour ($p = 0.05$ and $p = 0.03$, respectively). A 3-year survival benefit was also observed in patients with high expression levels of NFY-C ($p = 0.023$). There was no correlation between the mRNA levels of NFY-C or RORA and age, gender, grade, stage and relapse of the disease. However, mRNA levels of NFYC of stage B patients were significantly correlated with time to disease progression ($p = 0.035$). NFY-C protein was detected only in the cytoplasm both in malignant and normal tissues, with strong and weak intensity respectively. NFYC protein expression levels were correlated with mRNA expression levels of NFYC in malignant tissues ($p = 0.011$) and with the primary site of the tumour ($p < 0.001$).
Conclusions: NFY-C and RORA exhibited elevated levels in colon carcinomas compared to normal tissue samples indicating a possible role for these molecules in colon carcinogenesis. The role of NFY-C and RORA in colorectal cancer warrants further investigation.

765 An intron 8 polymorphism G/T of NFKB2 gene is associated with NSCLC

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Background: The members of the NFkB family are among the most important transcription factors in cancer. NFkB1 and the classical pathway have become objects of detailed research in the last years, although, little is known relating to the possible role of NFkB2 (alternative pathway of NFkB) in carcinogenesis. NFkB1 and NFkB2 are produced as precursor molecules, p105 and p100 respectively, after post-transcriptional modifications. NFkB2 (p100/p52) and other molecules of these pathway, such as RelB and Bcl3 are overexpressed in different cancer types. However, there is no data about the expression of these molecules in lung cancer and their implication in tumourigenesis and cancer progression. The aim of this study was to define the relation of the NFkB2 single nucleotide polymorphism rs7897947 with non small cell lung carcinoma (NSCLC).
Material and Methods: We used 37 blood specimens and 89 paraffin-embedded tissue specimens from patients with NSCLC. We also used 129 blood specimens from healthy donors. DNA isolation was performed using the Qiagen DNA blood kit (blood specimens) and the QIAamp DNA FFPE Tissue kit (tissue-specimens). Samples were genotyped using real-time PCR with SYBR Green intercalation dye and specific primers for each allele. The results were confirmed by DNA sequence analysis.
Results: Approximately half of the healthy donors (49.6%) were TT homozygotes, 11.6% were GG homozygotes and 38.8% were GT heterozygotes. The corresponding percentages for the patients were 69%, 24.6% and 6.4%. The difference in allele frequencies between healthy controls and patients was statistically significant ($p = 0.007$). No correlation was found between allele frequencies and age, sex, primary site, histological subtype, grade or maximum diameter. However, patients carrying a G allele had a lower frequency of positive lymph nodes in comparison with patients carrying a T allele.
Conclusions: The presence of the T allele seems to be associated with NSCLC development and might increase the possibility of lymph node metastatic spread. This study is ongoing and more patients and healthy control donors are currently being recruited to confirm these results.

766 Is there a role for RAD51 genetic variants in cervical cancer development?

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Background: Cervical cancer is the second most common cancer in women worldwide, with approximately 500 000 women developing the disease each year. It is known that specific types of human papilloma virus (HPV) are the principal etiologic agents for both cervical cancer and its precursors. However, alterations in oncogenes and tumour suppression genes may play additional roles in carcinogenesis of cervical cancer. The importance of the study of low-penetrance genes, such as genes involved in DNA repair, has become clear in recent years. The *RAD51* gene is a tumour suppressor gene, the protein is required for mitotic and meiotic recombination and is crucial in the repair of DNA lesions.
In this work we developed a case-control study with the objective of analyzing the frequencies of the *G135C* polymorphism in the *RAD51* gene in a group of individuals without cancer and a group of patients with cervical cancer and to assess the influence of the studied polymorphism in the genetic susceptibility to this tumour. We also evaluated the role of this genetic variation in the overall survival and therapy response of cervical cancer patients.
Material and Methods: We analysed the *G135C RAD51* polymorphism by PCR-RFLP in the genomic DNA isolated from peripheral blood of 652 individuals, including 311 cases with cervical cancer and 341 healthy individuals. Statistical analysis was performed using the computer software SPSS for Windows (version 11.5). Chi-square analysis was used to compare categorical variables and a 5% level of significance was used in the analysis. The odds ratio (OR) and its 95% confidence interval (CI) were calculated as a measurement of the association between *RAD51* genotypes and cervical cancer risk. The associations between *RAD51* polymorphism and survival were estimated using Kaplan-Meier analysis.
Results: The results suggested that the *RAD51* G135C polymorphism is not associated with cervical carcinogenesis ($P = 0.299$). Regarding the analysis of overall survival of cervical cancer patients, the results shown no statistical significant associations between *RAD51* genetic variants and overall survival time in these patients ($P = 0.891$).
Conclusions: These results may contribute to a better understanding of the role and influence of *G135C* polymorphism in the *RAD51* gene in the development of cervical and treatment response in these patients.

767 Cks2 overexpression leads to an increase of gammaH2AX

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Background: Using a knockout mouse model, we have begun studies in cultured cells to determine the molecular functions of Cks2 whose overexpression has been associated with more aggressive forms of cancer.
Materials and Methods: Cks2 homozygous knockout (KO) mice were created by inserting an artificial splicing cassette in intron 1 that causes loss of exon 2 and exon 3. Mouse embryonic fibroblasts (MEFs) were derived from Cks2 knockout mice and immortalized with an shRNA against p53. For localization of Cks2, these cells were transfected with a vector expressing CKS2 fused to mCherry under the control of the CKS2 promoter. To determine division time, cells were infected with H2B-EGFP.
Results: We filmed wildtype (WT) and Cks2 KO cells containing an H2B-EGFP fusion protein to measure the completion of one cell cycle, from one anaphase to the next. One cycle took more than 23 hours in WT whereas Cks2 KO cells divided in just over 20 hours. This accelerated cell cycle in the absence of Cks2 may lead to an increase in DNA damage. Using γH2AX as an indicator of DNA damage in immunofluorescence experiments, it was found that a larger number of Cks2 KO cells were positive for γH2AX than WT cells.