Material and Methods: This work analyzed how the tumour evolution could interfere on the proteolytic enzymes activities and the muscle level of 20S subunit (ubiquitin-proteasome pathway), therefore, we investigated the muscle protein degradation in adults rats bearing Walker 256 carcinoma, under different tumour growth conditions. Wistar rats were distributed into 3 groups: **CA** – control group; **IpA** – intraperitoneal tumour-implant; **ScA** – subcutaneous tumour-implant.
Results: The tumoural evolution showed that the **IpA** group survived only 7 days indicating that the tumoural growth is faster than the **ScA**, which survived longer (12−20 days). The body weight was decreased around 11% in both tumour groups. The spleen relative weight was increased especially in **ScA** (5.9% **IpA** and 129.5% **ScA**) while the adrenal relative weight was increased especially in **IpA** (57.6% **IpA** and 5.0% **ScA**). The gastrocnemius muscle weight decreased especially in **ScA** (2.5% **IpA** and 18.8% **ScA**) as well as the protein content (2.2% **IpA** and 8.5% **ScA**), parallel the muscle chymotrypsin-like activity (proteasome system) increased only in **ScA** (64.7%), while muscle lysosomal enzyme (cathepsin B) decreased around 75% in both groups; and the calcium dependent protease (calpain activity) remained unchanged. The 20S subunits expression enhanced in both groups (45% in **IpA** and 64% in **ScA**).
Conclusions: In both tumour-bearing groups, the muscle protein waste involved mainly the ubiquitin-proteasome activity (higher 20S expression), although the **ScA** group presented a higher decrease on muscle weight and protein content suggesting that the participative activity of proteasome-system could be the principal process rather than the other tumoural effects produced in intraperitoneal tumour-bearing rats.

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760 Relationship between NF-kappaB and different clinical-pathological standard factors

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Background: Breast cancer is the second leading cause of cancer related deaths among females worldwide. Actually the search for new markers which could improve individual treatments is one of the most important aims in breast cancer. The NF-kappaB transcription factor family seems to play a pivotal role in breast cancer progression and resistance to chemotherapy. The NF-kappaB family is composed by five subunits (p50, p65, p52, c-Rel and relB) which are inactived in the cytoplasm. When a cell receives any of a multitude of extracellular signals rapidly the subunits form a heterodimer or homodimer which enters to the nucleus and active gene expression. The aim of this study is to analyze each subunit of NF-KappaB and their influence in clinical evolution.
Material and Methods: We analyze frozen tumour samples from 400 patients by hematoxylin and eosin stain and we chose tissues with a tumour fraction higher than 50%. Later we determine the expression of the subunits p50, p65, p52, c-Rel and Rel-B of NF-kappaB by Western Blot and union of NF-KappaB to DNA by ELISA. We characterize the relationship between the different subunits of NF-kappaB and clinical-pathological standard factors.
Results: We have observed that higher activation of p50 is related with presence of the disease in patients who are diagnosing before 50 years old. At the same time we have found p50, p65 and p52 are increased in tumoural tissue against non-tumoural tissue. Also our preliminary data suggest strong relationship between the decrease of at least subunits of p65, p50 and p52 when neoadjuvant therapy is given.
Conclusions: Our preliminary data suggest that higher activation of the different subunits of NF-kappaB is related with higher malignancy in breast cancer and that neoadjuvant therapy could act inhibiting NF-kappaB.

761 Reverse-phase protein arrays as a tool to discover mutation-associated alterations in cell signaling pathways

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Cancer is a result of an accumulation of various factors promoting tumour growth and metastasis. Somatic mutations are major molecular determinants underlying tumour development and progression. However, the complex patterns of genetic alterations within tumours provide a considerable challenge to the understanding of changes in cell signaling pathways that promote cell growth and proliferation.
The effect of known gene mutations on the complex signaling network can be analyzed in a cellular system by using applications for quantitative proteomics. A site-specific recombination system for rapid generation of highly standardized, isogenic cancer cell lines was established to generate a library of cell lines stably over expressing mutated genes. After induction of gene expression, cell viability was determined and the corresponding protein lysates were analyzed by reverse-phase protein array (RPPA) technology. This technique offers an excellent possibility to trace the differences in cell signaling pathways between normal and breast cancer cells. It allows for studying key proteins and their phosphorylation status in several hundreds of samples in parallel. For quantitative protein measurements by RPPA, a protein lysate (i.e. the equivalent of the protein content of few cells) from cell cultures or tissues is immobilized directly on a surface layer of a coated microscope slide. Specific primary antibodies are then used to detect the proteins of interest. A secondary, near-infrared-labeled antibody binds to the primary antibody and signals can be detected using an appropriate scanner.
A cell viability assay served as a selection tool to identify potential candidate proteins and led to a group of key signaling components (i.e. PI3K, PTEN, KRAS, HRAS and B-Raf) which were chosen for time course experiments. Abnormalities in protein expression were detected by RPPA and gave novel insights into cell signal transduction pathways in breast cancer cells.
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762 PALB2: a new inactivating mutation in a breast cancer family

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Background: *PALB2* (partner and localizer of BRCA2) encodes a protein implicated in BRCA2 nuclear localization and stability. Biallelic PALB2 mutations are responsible for N-subgroup of Fanconi Anemia. Monoallelic mutation in *PALB2* are rare and confer an intermediate risk of breast cancer. To further investigate if monoallelic PALB2 mutations confer susceptibility to breast cancer we have sequenced the gene in 95 individuals with familial breast cancer tested negative for BRCA1/2mutations.
Results: The mutational analysis of PALB2 gene identified a frameshift mutation (c.1517delG) that generates a premature stop codon (L451X). The proband was diagnosed at 52 year of age with an infiltrating ductal breast carcinoma of grade 3, expressing both Estrogen Receptor (ER) and Progesterone Receptor (PgR). The mother was also affected by breast cancer at 39 years of age and one out of 3 proband's sisters was affected by basocellular carcinoma and addominal melanoma. To verify the inactivation of the wt allele on proband tumour tissue we performed LOH analysis and we did not find any LOH event. This mutation was also identified in two proband's sisters. One was healthy at 48 years and the other was affected by melanoma at 51 years and basocellular carcinoma at 56 years. We identified 7 missense variants in seven different patients: 5 previously described (T1100T; Q559R; E672Q; P864S; G998E) (frequency >1%), and 2 not previously described (Y334C;L1143H) (frequency respectively 0.5% and 1%). Two variants in the 5′ UTR (−159 G>C; −47 G>A) and an intronic variant (IVS3−57 A>C) have been also identified. The two novel missense variants were tested in 50 healthy controls and the Y334C was found once (frequency 1%). The pathogenicity of these variants is analyzed with two software: SIFT and PolyPhen and these variants result both tolerated for SIFT while for PolyPhen the L1143H results "possibly damaging".
Conclusions: The frameshift mutation c.1517delG generates a truncated protein lacking the WD40 domain essential for the interaction BRCA2/PALB2. Preliminary results indicate that the mutated allele is expressed in peripheral blood lymphocyte of the patient. If coimmunoprecipitation experiments will allow to demonstrate the lack of interaction between the 2 proteins we will add evidence that the interaction between them is essential for BRCA2 function in DNA Double Strand Break Repair.

763 Altered partitioning of the EAG1 potassium channel in the plasma membrane of cancer cells

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Background: The role of the voltage-gated potassium channel EAG1 in the genesis of several tumours has been shown in the last decade. We are interested in determining the distribution pattern and interacting partners of EAG1 within the plasma membrane domains of cells that express EAG1 under physiological conditions (brain) and compare it with their pathological counterpart (tumours).

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.