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).