phoshate (db-cAMP) addition or by androgen withdrawal (FBSc) for 7 days in LNCaP cells. db-cAMPc is an extended stimulus able to cause NE differentiation in several cell types including LNCaP cells, while androgen withdrawal is the main treatment strategy to avoid prostate cancer growth. Citosolic superoxide dismutase (SOD1), superoxide dismutase mithocondrial (SOD2) and two neuroendocrine markers were detected by western blot. Inmunocitochemistry of culture cells was also used to confirm western blot results. Total SOD cellular activity was determined spectrophotometrically. We observed a rise in the amount of SOD2 protein in all NE cells as compared to control cells. Variations but no significant changes were observed in SOD1. Synaptophisine and Neuron Specific Enolase analysis indicated differential patterns of NE markers according to the NE inductor. By enzymatic assay we could observe that melatonin and FBSc-induced NE cells shared a significative increment in SOD activity. All together these data indicate a heterogeneity among the NE cells observed in prostate cancer. These results could shed some light on the controversy between prostate cancer progression and neuroendocrine differentiation. This work was supported by "Plan Regional de Investigacion (FICYT IB05-126)".

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Impact of portal triad clamping (Pringle Maneuver)on hepatic function in a hepatocellular carcinoma murine model

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Background: Intraoperative blood loss and consequent transfusion needs are major factors influencing morbidity and mortality following partial hepatectomy (PH). Hepatic vascular inflow occlusion by Pringle maneuver (PM) is often used to prevent bleeding during PH. However, PM itself causes ischemia and reperfusion injury. This experimental study aimed to estimate the impact of PM in hepatic cells function, viability as well as the longest safe duration of PM in a murine model with hepatocellular carcinoma (HCC).

Material and Methods: Three groups of male Wistar rats with HCC (4 months old; N-nitrosodiethylamine 0,5 gr/L of H2O during 2 months) were subjected to a total liver ischemia period for 60 min: group A (n=12) submitted to a continuous inflow occlusion; group B (n=11) underwent to an intermittent clamping (IC) for 30 min with 5 min of reperfusion; group C (n=12) underwent an IC for 15 min with 5 min of reperfusion. The group D (n=11) was not subjected to a PM. A hepatic biopsy was done at the end of surgery. The degree of tissue injury was evaluated using: 1) Blood markers [aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), alkaline-phosphatase (AF), gamma-glutamyl-transpeptidase (GGT), total-bilirubin (TB), lactic-acid-dehydrogenase (LDH)] and hepatic extraction fraction (HEF) by radioisotopic methods three days before laparotomy (BS) and after surgery (AS); 2) hematoxylin-eosine staining; 3) apoptosis, necrosis and oxidative stress were investigated after collagenase cell isolation from hepatectomy pieces by flow-cytometry using the followed probes: propidium-iodide, annexin-V, DCFH2-DA and JC-1. Statistical analysis was carried out by variance analysis and, if applicable, post-hoc comparisons by Tukey-test ($p<0.05$).

Results: 1) Mortality: Group A-60%, Group B-46%, Group C-8,3%, Group D-0%.

Conclusions: We didn't observe differences in cell viability with our model, however the PM duration bigger than 15 minutes must be avoided. We think that these results are related to tumoral cell resistance to ischemia.

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Analysis of TGFβ-Induced, as a novel gene targets in breast cancers in young women

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The aim of this study is to investigate the significance of TGFβI, identified by cDNA microarray as a promising novel marker of breast cancers in young women. Previous studies in our research group have shown that breast cancers show more aggressive features in younger than in older women (Walker et al., 1996) including a higher frequency of loss of heterozygosity (LOH) in the BRCA1, BRCA2 and p53 (Johnson et al., 2002). These findings raised the question as to whether differences are present at the molecular level and prompted us to pilot cDNA microarray to identify novel gene expression changes in sporadic breast cancers in young women.

Gene expression was investigated at the protein level using both Immunohistochemistry (IHC) and Western Blotting with clone against the pure human TGFβI protein (Proteintech Group, Inc). For IHC FFPE tissue was available from 55 breast cancer cases that were stratified by age and 12 healthy female controls. For Western Blotting, protein lysate was isolated from 6 breast cell lines (MCF-7, MDA-MB-231, HBL-100, MDA-MB-468, T47-D and ZR-75-1), tumour and normal breast cell populations (organoids) isolated by digestion of breast reduction tissue. There was stronger nuclear staining in the epithelial cells of normal breast tissue than in the cancer cases. 37 of the 55 (67.3%) breast cancer cases examined showed down-regulation of this protein. Moreover, Chi-squared analysis showed a significant difference between the grade of the tumour and the IHC protein staining distribution, and between cases aged ≤35 years and 36-49 years ($p<0.05$). These results were confirmed by western blotting, which showed the absence of TGFβI protein in 6 breast cancer cell lines compared to normal breast organoids. These data suggest that down-regulation of TGFβI might be an important step in the development of sporadic breast cancers in young women. Current studies are focussed on investigation of the molecular mechanisms that lead to this down-regulation.

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Characterization of zinc toxicity in PC12 cells - reactive oxygen species generation and role of voltage calcium channels

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Zinc is an essential trace element in mammalian cells and also a structural component used by many metalloenzymes and transcription factors. Recent studies indicate a possible correlation of zinc levels with the cancer risk. However, the exact role of zinc in cancer progression is unknown. The disturbance of zinc homeostasis features with a significant decrease of cellular zinc level, and excess extracellular zinc is toxic and causes death to central neurons. The mechanisms of zinc-induced cell death are still unclear. The knowledge of these mechanisms could contribute to understand the role of this metal in the different biological processes in which is implicated. In this work we used rat pheochromocytoma (PC12) cells as a model to study zinc toxicity.

In this work we characterized zinc toxicity in PC12 cells measuring cell viability by trypan blue exclusion and 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl-tetrazolium (MTT) reduction assays. Reactive oxygen species (ROS) generation was evaluated using flow cytometry and fluorescent labeling with 5-(y 6-)-carboxi-2´,7´-dichlorofluorescein diacetate (DCF-DA). Nuclear condensation was measured by DAPI labeling.

Zinc toxicity in PC12 cells shows a doses- and time-dependent pattern, with an EC50 value of 0.30 ± 0.05 mM and a t1/2 of 173 ± 27 min. Nuclear condensation data indicate that cell death takes place through a necrotic process, and a massive ROS generation is measured from 6 hours of zinc incubation. Differentiation of PC12 cells with neuronal growth factor (NGF) decreased two-fold EC50 value to 0.14 ± 0.02 mM. NMDA receptor blocker MK-801 (10 mM) and non-NMDA receptor blocker CNQX (10 mM) did not protect against zinc toxicity, indicating that glutamate receptors do not play a significant role. Depolarization experiments carried out with high potassium showing a synergic effect with zinc and the protection measured with nifedipine (1 mM) suggest that voltage calcium channels are implicated in zinc toxicity allowing the entry of zinc ions or alternatively through a zinc-mediated calcium entry.

In summary, zinc toxicity is mediated by a massive ROS generation and takes place mainly through a necrotic process. Voltage calcium channels have a main role in zinc toxicity, becoming an important therapeutic target. The uncovering of the molecular mechanisms underlying zinc toxicity is important to understand the different biological process where zinc plays a role.

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Specific molecular signature and developmental hypothesisfor pilocytic astrocytomas of the optic pathway

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Introduction: Pilocytic astrocytomas (PA) are common grade I gliomas that occur predominantly in childhood. They share features of both astroglial