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

and oligodendroglial lineages but the progenitor cell from which PA could arise is still unknown. These tumors affect preferentially the cerebellum and the optic pathway, especially the hypothalamo-chiasmatic (H/C) region. Cerebellar tumors have a benign clinical course whereas H/C PA display a worse prognosis. Understanding the molecular basis responsible for the agressive behavior of H/C PA is prerequisite to set up new molecular targeted therapies. Material and methods: We used microarray technique to compare the transcriptionnal profile of 5 H/C PA and 6 cerebellar PA. Validation of the microarray experiment and comparison of PA with normal developing tissue was done by quantitative RT-PCR and immuno-histochemistry. Finally, we undergone a morphological study of the H/C region in human to identify candidate cell populations at the origin of PA. Results: Cerebellar and H/C PA appeared as two genetically distinct entities as hierarchical clustering perfectly classified the tumors according to their location. Numerous genes involved in cell proliferation, adhesion, migration and brain development were upregulated in H/C PA. These genes were increased in the developing chiasm in comparison with developing cerebellum. The study of fetal H/C region allowed us to identify a unique population of vimentin and GFAP-positive cells highly suggestive of radial glial cells; these cells disappear after birth but a discrete population of vimentin-positive astrocyte-like cells persists just above the optic chiasm in children and adults. Conclusion: Our study provides new molecular and morphological evidences for the developmental origin of PA. We hypothesize that the precursor in the H/C location should be a specialized radial glia cell.

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ALK and RPTPβ/ζ mediate HARP TSR-I like domains anti tumour biological actions

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Heparin Affin Regulatory Peptide (HARP) is a 15 kDa growth factor expressed in various tissues and cell lines. HARP participates in multiple biological actions including the induction of cellular proliferation, migration and angiogenesis, and it is thought to be involved in carcinogenesis. Despite the fact that Anaplastic Lymphoma Kinase (ALK), Receptor Protein Tyrosine Phosphatase (RPTPβ/ζ), and N-syndecan have been characterized as HARP receptors, HARP signal transduction pathway remains unclear.

Recently, our laboratory identified and characterized several HARP proteolytic fragments with biological activities similar or opposite to that of HARP and proposed that the biological activity of this growth factor is mainly attributed to the two central domains of the molecule as well as its C-terminal region. In an attempt to understand the structure/function relationship of HARP, we investigated the biological actions of P13-39 and P65-97, two synthetic peptides that correspond to a part of the N-terminal and C-terminal TSR-I motif of HARP, respectively. Our results show that both P13-39 and P65-97 inhibit in vitro migration and anchorage-dependent and –independent proliferation in PC3 cells, a human cancer prostate cell line. In addition, P13-39 and P65-97 inhibit angiogenesis in vivo, as determined by the chicken embryo CAM assay. ALK and RPTPβ/ζ mediated P65-97 and P13-39 biological actions respectively, as demonstrated by selective knockdown of ALK and RPTPβ/ζ expression with shRNA. Investigation of the transduction mechanisms revealed that these peptides affect the activation of SRC-kinase, AKT, and ERK1/2.

In conclusion, it seems that HARP interacts with ALK and RPTPβ/ζ through its C-terminal and N-terminal TSR-I motif, respectively. Each receptor triggers a signal transduction pathway that leads to specific biological cell responses.

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In vitro analysis of population specific splicing variants of BRCA1 gene

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Background: The BRCA1 is involved in DNA repair and its gene mutations are responsible for the development of majority of hereditary breast and ovarian cancer cases in the Czech Republic. Approximately 65% of BRCA1 mutations occur in form of population specific alterations, however, the remaining represent rare or unique genetic changes. Moreover, during mutation screening were detected numerous splicing aberrations of unknown clinical significance in both heterozygotic and homozygotic form. To test their biological importance we developed in vitro system.

Methods: Using pSUPER and pcDNA 3.1 vectors the stable clones of breast adenocarcinoma cell line MCF-7 expressing population-specific BRCA1 splicing variants, anti wtBRCA1 shRNA or combination of these were generated by puromycine-selection following calcium phosphate transfection. The shRNA sequences were targeted to sequences missing in splicing variants. The up and down-regulation of BRCA1 gene was scored by qPCR on the mRNA level and by Western blotting on the protein level. At least two different clones for each BRCA1 splicing variant were analyzed in triplicates. The recovery of cell growth following gamma irradiation was tested by MTT and flow cytometry.

Results: We successfully established stable clones expressing BRCA1 splicing variants (Del e14-15; Del e14-18; Del e17-19) affecting BRCA1 phosphorylation sites or BRCT domains, stable pSUPER clones down regulating BRCA1 to 10-15% of control cells wtBRCA1 mRNA expression and clones stably expressing splicing variant BRCA1 exons 14-18 del with down regulated wtBRCA1. The cells expressing the BRCA1 splicing variants showed prolonged population-doubling time (~1.5-times), morphological changes and higher viability comparing to controls. The growing patterns of MCF-7 cell with down-regulated wt BRCA1 were not changed, comparing to mock. The growth properties examined in relation to gamma-irradiation induced DNA damage show that while the control cells reacted to gDNA damage by decreasing their growth rate and plating efficiency, growth properties of cells expressing BRCA1 exon 14-18Δ splicing variant with down-regulated wt BRCA1 remained almost unaffected.

Discussion: Our current in vitro results shows that splicing variants of BRCA1 may alter BRCA1 DNA repair capacity, however the molecular mechanism of these changes are currently under investigation.

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Angiogenic factors in breast cancer

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Background: Tumor angiogenesis, the formation of new blood vessels, is one of the most important biologic features that are related to tumor growth and metastasis. In this study, we analyzed the circulating serum levels of potent angiogenic factors, including vascular endothelial growth factor (VEGF), angiogenin and transforming growth factor-beta 1 (TGF-beta1) in breast cancer patients. Materials and methods: The study group consisted of 90 breast cancer patients consecutively presenting to Istanbul University Oncology Institute in a 10-month period and 75 healthy controls. The median age of patients was 49 (24 – 71) years and it was 43 (28 – 69) years for healthy controls. Serum VEGF, angiogenin and TGF-beta1 levels were measured by enyzme -linked immunosorbent assay (ELISA). Data analysis was performed by using SPSS 11. Results: There was no significant difference in the serum VEGF (p=0.156), angiogenin (=0.976) and TGF-beta1 (=0.215) levels between breast cancer patients and the controls. There is a significant correlation between VEGF and angiogenin levels in patients (p<0.001). Significant correlations were observed between the parameters of angiogenin - TGF-beta1 (p<0.001) and TGF-beta1 - VEGF (p<0.001) in the healthy controls. Conclusions: In conclusion, we did not observe significant differences in angiogenic factors between breast cancer patients and controls. Vascular metastasis may be seen earlier in the patients who have increased VEGF and angiogenin values in sera than the others.

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Naringenin-induced apoptosis is attenuated by Bcl-2 but restored by the small molecule Bcl-2 inhibitor, HA 14-1, in human leukemia U937 cells

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Naringenin (NGEN), a flavonoid, has shown cytotoxicity in various human cancer cell lines and inhibitory effects on tumor growth. We determined the effect of ectopic Bcl-2 expression on NGEN-induced apoptosis and whether the small molecule Bcl-2 inhibitor, HA14-1, could increase NGEN sensitivity. Bcl-2 overexpression markedly protected U937 cells from time- and dose-dependent induction of apoptosis by NGEN, as did caspase-3 or caspase-9 inhibitors, and increased their cell survival. Bcl-2 attenuated NGEN induced Bax translocation and cytosolic release of cytochrome c. Co-administration of HA14-1 and NGEN increased apoptosis in U937/Bcl-2 cells by restoring loss of MMP and activation of caspase-3, -9 and cleavage of poly (ADP-ribose) polymerase (PARP). This result indicates Bcl-2 confers apoptosis resistance to NGEN by inhibiting a mitochondrial amplification step in U937 cells. HA14-1 reversed Bcl-2-mediated NGEN