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

Z. Diamantopoulou[1], J. Delbé[2], J. Courty[2], P. Katsoris[1]
[1]University of Patras, Biology, Patras, Greece; [2] Université Paris XII, CNRS UMR 7149, Paris, France

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

J. Sevcik[1], E. Vondruskova[1], P. Pohlreich[1], F. Lhota[1], E. Matouskova[1], E. Bursikova[1], Z. Kleibl[1]
[1]Charles University in Praque, Biochemistry & Experimental Oncology, Prague, Czech Republic

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

V. Yasasever[1], H. Oguz Soydinc[1], H. Camlica[2], D. Duranyildiz[1], N. Dalay[1]
[1]Istanbul University Oncology Institute, Basic Oncology, Istanbul, Turkey; [2] Istanbul University Oncology Institute, Preventive Oncology Biostatistics and Epidemiology, Istanbul, Turkey

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

Y. Choi[1], C. Jin[1], C. Park[1], T. Kwon[2]
[1]Dongeui University College of Oriental Medicine, Department of Biochemistry, Busan, South Korea; [2] Keimyung University School of Medicine, Department of Immunology, Taegu, South Korea

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

resistance, suggesting a novel strategy for increasing NGEN sensitivity in Bcl-2 overexpressing human leukemia cells.

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Cancer inhibition by normal differentiated cells

F.C. Martins[1], M.F. Botelho[2], A.M. Cabrita[3], C.F. de Oliveira[1]
[1]Hospitais da Universidade de Coimbra, Serviço de Ginecologia - CIMAGO, Coimbra, Portugal; [2] IBILI - Faculdade de Medicina da Universidade de Coimbra, Instituto de Biofísica e Biomatemática - CIMAGO, Coimbra, Portugal; [3] Faculdade de Medicina da Universidade de Coimbra, Instituto de Patologia Experimental - CIMAGO, Coimbra, Portugal

Background: The stem cell theory of cancer states that tumor development is originated in a mutated stem or progenitor cell. Stem cells are susceptible of inhibition when there is no need to proliferate and have the same regulatory pathways as tumor cells. These facts allow us to hypothesize that tumor as well as stem cells could be inhibited by normal differentiated cells.

Materials and methods: In our study, we used Balb-c nude mice and MCF-7 breast cancer cells. The nude mice were divided in two groups (n=10), the control group (CG) and the test group (TG), whose mice were submitted to an epithelial removal. The animals of both groups were subcutaneously injected with β-estradiol and progesterone, every day, for three weeks, to simulate the pregnancy full differentiation of the mammary gland. Afterwards, 2 million of MCF-7 cells were injected in the mammary gland (CG) and in the cleared mammary fatpad (TG), in the respective group. Five weeks later, the tumors were removed and their volumes evaluated.

Results: The median volume of the tumors in the TG (64,6mm^3) was superior to the median volume in the CG (5,9mm^3) with a statistical significance (p = 0,003), using the Mann-Whitney test.

Conclusions: Our results demonstrate that there is an inhibition of tumor development by normal mammary epithelial cells, when we use the MCF-7 tumor cell line. They also strengthen our previous hypothesis about the existence of an inhibitory stimulus of normal cells in the carcinogenesis process and may elucidate different unexplained mechanisms, namely the protective role of pregnancy in breast cancer or the graft-versus-leukemia effect in haematological malignancies.

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Detection of deleted malignant brain tumors 1 and runt-related transcription factor 3 gene expressions in bladder carcinoma

Y. Dodurga[1], C. Biray Avci[1], N.L. Satiroglu-Tufan[2], C. Tataroglu[3], Z. Kesen[4], Z.O. Dogan[1], S. Yilmaz[1], S. Numanoglu[1], C. Gunduz[1]
[1]Ege University Faculty of Medicine, Medical Biology, Izmir, Turkey; [2] Pamukkale University Faculty of Medicine, Medical Biology, Denizli, Turkey; [3] Adnan Menderes University Faculty of Medicine, Pathology, Aydin, Turkey; [4] Denizli Government Hospital, Pathology, Denizli, Turkey

Background: Bladder cancer, comprises 3% of cancer among women and 7% of men, is the second most common malignancy of the genitourinary system is the fourth most common cause of death from cancer in men and eighth most common in women. Deleted in Malignant Brain Tumors 1 (DMBT1) gene, located at chromosome 10q25.3-q26.1 is highly expressed in alveolar and macrophage tissues. Some alterations in DMBT1 gene are caused in gliomas. Despite, a loss or reduction of DMBT1 expression in various cancers including gastric, colorectal, brain, lung and esophageal cancers, it has not been reported in bladder cancers. Runt-related transcription factor 3 (RUNX3) is a candidate tumor suppressor gene, a Runt domain transcription factor involved in TGF-β signaling. It is localized on the chromosomal region 1p36. RUNX3 gene expression in bladder carcinogenesis is particularly unknown. We aimed to evaluate DMBT1 and RUNX3 gene expression profiles in bladder cancer and how their expressions could be related to carcinogenesis in the bladder and their correlation with clinicopathological parameters.

Material and Methods: Fifty-six paraffin embedded specimens of transitional cell carcinoma of the urinary bladder were used in the study. Total RNA was extracted from bladder specimens and cDNA was synthesized. The quantification of DMBT1 and RUNX3 mRNAs were succeeded according to the manufacturers' instructions by using Lightcycler instrument.

Results: DMBT1 and RUNX3 gene expressions were identified in 100% of bladder carcinoma samples. No significant association was found in these genes expression levels when compared to sex and age. RUNX3 gene expression was decreased non-significantly in high-grade tumors. When DMBT1 gene expression was compared to tumor grades, a significant decrease was detected between grade I and III (p=0,028). We compared the expression results between patients' sex, age, pathologic degree and grades. We found that DMBT1 gene expression was decreased when grade was increased in this research.

Conclusion: A correlation was found between the DMBT1 gene expression and tumor grades. Expressions of tumor suppressors like DMBT1 and RUNX3 genes could be used as diagnostic markers in early detection and prognosis of the bladder cancer. Furthermore, detailed studies including these genes should be performed in protein levels in a large scale study.

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Expression of calreticulin in breast and cervical cancer in relation to clinical outcome

A. Eric[1], Z. Juranic[1], Z. Milovanovic[2], D. Jovanovic[2], D. Gavrilovic[3], N. Borojevic[4], V. Plesinac[4], I. Markovic[5], M. Inic[5]
[1]Institute for Oncology and Radiology of Serbia, Experimental Oncology, Belgrade, Serbia; [2] Institute for Oncology and Radiology of Serbia, Pathology, Belgrade, Serbia; [3] Institute for Oncology and Radiology of Serbia, DATA Center, Belgrade, Serbia; [4] Institute for Oncology and Radiology of Serbia, Radiology, Belgrade, Serbia; [5] Institute for Oncology and Radiology of Serbia, Surgery, Belgrade, Serbia

Calreticulin is chaperone protein of endoplasmic reticulum, found in the cytotoxic granules of lymphocytes and natural killer cells. It is released with granzymes and perforin upon recognition of target cells. Contrary to its previous defined function in efficient interaction between cytotoxic and target cells, it is shown in recent studies that tumor spread could be influenced by calreticulin, which is overexpressed in some cancer cell lines and in some tumors. It is also shown that calreticulin may induce tumor progression, because at the concentration of 2,2 x 10-7 M it completely blocks perforin-mediated lysis, by stabilizing membranes preventing polyperforin pore formation. The purpose of this study was to investigate whether the expression of calreticuiln in breast and cervical cancer exists and, if so, whether that expression is related to clinical outcome and tumor progression. In this study 33 patients with breast cancer and 25 patients with cervical cancer were included. Patients with breast cancer underwent surgery, while cervical cancer patients were treated by radiotherapy. Clinical outcome was evaluated for two years for breast cancer patients and for one year for cervical cancer patients. Expression of calreticulin was determined prior to clinical treatment, by immunohistochemistry, using rabbit anti-calreticulin polyclonal antibody, according to manufacturer recommendation. Among 22/33 breast cancer patients who had expression of calreticulin, three of them developed distant metastases. On the other side, among 12/25 cervical cancer patients with calreticulin expression, four of them had progressive disease. It has to be noticed that those progressive cancer patients with calreticulin positivity were also the only patients with progressive disease in both observed groups of patients. Namely, all patients with progression of malignant disease expressed tumor positivity for calreticulin. Our findings support the state that calreticulin can regulate lytic and cytotoxic function. These preliminary results indicate the need for further investigation related to the role of calreticulin in malignant behavior.

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Over-expression of PRKAR1A in hepatic progenitor cells during cholangiocarcinogenesis induced by liver fluke (Opisthorchis viverrini)

P. Yongvanit[1], N. Namwat[1], W. Loilome[1], R. Thanan[1], E. Phanomsri[2], C. Pairojkul[2]
[1]Khonkaen University, Biochemistry, Khonkaen, Thailand; [2] Khonkaen University, Pathology, Khonkaen, Thailand

Background: PRKAR1A, a regulatory subunit of protein kinase A type I (PKA I) which plays a crucial role in cell proliferation and differentiation was found to be overexpressed in cholangiocarcinoma (CCA). To clarify the role of PRKAR1A in cholangiocarcinogenesis, we have studied the expression of PRKAR1A in Opisthorchis viverrini (Ov) and N-nitrosodimethylamine (NDMA) induced CCA in hamster model. Materials and Methods: Syrian golden hamsters were treated with Ov and NDMA to induce CCA and were sacrificed on weeks 1, 4, 12 and 24. The immunofluorescence technique was used for localizing PRKAR1A, PCNA and glypican-3 in liver tissues.

Results: PRKAR1A positive staining was markedly increased in hyper-proliferating bile duct epithelial cells indicated by a proliferating marker PCNA observed for liver tissues that belonged to hamsters induced from weeks 12 to 24. PRKAR1A were prominently positive at week 24 as tumor developed in tumor cells. Interestingly, the liver progenitor cell marker, glypican-3 was coexpressed in PRKAR1A positive tumor cells.

Conclusions: Our result indicates that PRKAR1A may regulate cellular hyper-proliferation triggered by the liver fluke and plays role in cholangiocarcinogenesis by induced the aberrant proliferation and differentiation of hepatic progenitor cells.