rectum. Among 329 patients with known family history, 132 (40.1%) had positive FH. The overall rate of abnormal immunostaining for hMLH1, hMSH2, hMSH6 and PMS2 were 4.4%, 7.2%, 3.5%and 4.9% respectively. Overall 44 patients (12.7%) had at least one abnormal MMRP staining. Abnormal MMRP were not significantly associated with histopathologic factors including T stage, N stage and grade. There was no difference in MMRP staining as a result of whether patients were male or female except PMS2 that was significantly more abnormal in male ($p = 0.083$). Abnormal staining of MMRP were seen further in colon than in rectum that was significant for hMLH1 ($p = 0.044$). Patients with family history of CRC had more abnormal staining that was significant for hMSH2 ($p = 0.061$). There was no difference in MMRP staining according to vital status.
Conclusion: Our results suggest that abnormal MMRP is associated with clinical factors such as family history of CRC but not with pathologic factors. Abnormal MMRP is more important pathway for carcinogenesis in colon than rectal cancer.

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Two pathways of carcinogenesis in patients with colorectal cancer less than 45 years old

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Background: Colorectal cancer (CRC) arises from a complex series of molecular changes that involve at least two different pathways. These include microsatellite instability (MSI) pathway and chromosomal instability (CIN) pathway. The aim of this study was the determination of predominant pathway involved in carcinogenesis of patients with CRC less than 45 years old with and without family history (FH) of CRC.
Materials and Methods: In our study surgical pathology specimens of 108 patients with CRC less than 45 years old were immunostained for DNA mismatch repair proteins (MMRP) including hMLH1, hMSH2, hMSH6 and PMS2. Beta-catenin and P53 were also examined for CIN pathway.
Results: Totally 108 patients with median age of 40 (20−45) were evaluated. Fifty seven patients were male and 51 were female. The site of tumor in 84 patients was colon and in 14 were rectum. Among 96 patients with known family history, 33 (34.4%) had positive FH. The overall rate of abnormal immunostaining were MLH1 8.3%, MSH2 18.5%, MSH6 8.3%, PMS2 11.1%, P53 74.1% and beta catenin 35.2%. Meanwhile abnormal staining for hMSH2 and hMSH6 were significantly more seen in patients with positive family history ($p = 0.008$ and $p = 0.032$ respectively). Patients with positive FH for CRC had significantly more abnormal MMRP (54.5% vs. 20.6%, $p = 0.001$) and less positive p53 (54.5%vs. 81%, $p = 0.006$) than patients with negative FH. Patients with early T, N stage tumor had at least one more abnormal MMRP than advance T, N stage ($P = 0.050$ for T and $P = 0.030$ for N stage). Among different factors abnormal hMSH2 had significant association with lower cancer related death ($P = 0.060$). Patients with rectal cancer had more abnormal MMRP than patients with colon cancer but not significantly (35.7% vs. 29.8%, $p = 0.655$) and positive p53 staining for rectal and colon cancer were 71.4% and 72.6% respectively. Both in colon and rectal cancer patients with negative family history had more prevalent positive p53 (80.4% vs. 56.7%, $p = 0.022$ for colon and 81.8% vs. 33.3%, $p = 0.099$ for rectal cancer).
Conclusion: Our study indicates that even in CRC less than 45 years old, the main pathway for carcinogenesis in patients with negative family history is CIN, but in positive family history MSI is as effective as CIN. However main pathway in both colon and rectal cancer is CIN.

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Activation of signalling pathways by increased expression of HC GP-39 in brain tumors

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Background: The aim of this research is determination and characterization of potential molecular markers for human brain tumors and their possible interaction with main signal pathways in eukaryotic cells. Such knowledge is necessary not only for understanding the tumorigenesis, but also the mechanisms of normal brain functioning.
Materials and Methods: Diffrentially expressed genes were determined by Serial Analysis of Gene Expression (SAGE); gene expression have been analysed by Northern hybridization.
Results: The comparison of 9 glioblastoma and 5 human adult normal brain SAGE-libraries revealed 129 genes with >5-fold differences, 44 of them met the criteria for genes overexpressed in tumors. The majority of these genes are related only to a few functional groups: genes encoding proteins involved in angiogenesis, immune response, extracellular matrix, drug-resistance, and several genes are related to the mitogen-activated protein kinase cascades: CD74, EGFR, CTGF, IGFBP5, IGFBP7, and IGFII. We found unusual processing of IGF-2 primary transcript in meningiomas and ependimomas, anomaly expression of IGF-II may contribute towards tumorigenesis. Increasing of IGF-I gene expression was not found in glioblastomas. It is possible to suppose that glial tumor development is activated by some other way. C hitinase 3-like 1 gene encoding human cartilage glycoprotein-39 (HC gp-39 or YKL-40) was among the most upregulated genes and as was shown recently, it initiates cellular responses very similar to those elicited by IGF-1: activates both extracellular signal-regulated kinase (ERK) – and protein kinase B (AKT)-mediated signalling cascades, which are associated with the control of mitogenesis. Both proteins act synergistically with respect to their growth-stimulating activity; both suppress the cytokine-induced secretion of MMPs.
Conclusions: Since deregulation of the IGF system and HC-gp39 is a frequent pattern in tumors, IGFs/IGFBPs/HC-gp39 should be included in the panel of tumors markers used for histopathological diagnosis and serological surveillance procedures in various malignancies. Novel antisense and iRNA strategies targeting components of IGF-axis and HC-gp39 may offer additional options for treatment of malignant gliomas.

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Role of hepatocyte growth factor/c-met signaling in regulating urokinase plasminogen activator on invasiveness in human hepatocellular carcinoma: a potential therapeutic target

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Background: Hepatocyte growth factor (HGF), its transmembrane tyrosine kinase receptor (c-Met) and urokinase type plasminogen activator (uPA) is a key protein in the plasminogen activation system, which plays a proteolytically important role in the invasion and metastasis of various types of cancers. However, the mechanisms by which HGF/c-Met signaling mediates cancer progression and metastasis are unclear.
Methods: this study was designed to investigate the roles of HGF/c-Met in tumor progression and metastasis in HepG2 and Hep3B hepatoma cell lines.
Results: Treatment with HGF increased c-Met phosphorylation in a dose-dependent manner. Activity of c-Met phosphorylation was peak at 1 to 3 minutes later after HGF treatment and then declined. HGF enhanced the protein level and the activity of uPA in HepG2 and Hep3B cells and also uPAR protein level increased in a HGF dose dependent manner. HGF increase cell invasion through matrigel. A monoclonal antibody against human uPA receptor, mAb 3936, inhibited HGF-mediated tumor cell invasion in a dose dependent manner. Down-regulation of uPA using uPA-shRNA induced a decrease in in vitro cell invasion in HepG2 cells.
Conclusions: These results suggest that HepG2 and Hep3B cells express functional c-Met, which may provide a target for a therapeutic basis to interfere with metastases of cancer cells by inhibiting uPA system-mediated proteolysis.

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Role of manganese superoxide dismutase on growth and invasive properties of human estrogen-independent breast cancer cells

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Background: Manganese superoxide dismutase (MnSOD) is known to play a role in cancer. MnSOD exerts a tumor suppressive effect in estrogen-dependent human breast cancer cells. In the present study we investigated the in vitro role of MnSOD in the growth of some aggressive and highly metastatic estrogen-independent breast cancer cells, i.e. MDA-MB231 and SKBR3 cells.
Experimental procedures: This in vitro study used estrogen-dependent and estrogen-independent breast cancer cell lines. Antisense RNA strategy was used to inhibit MnSOD expression and to study consequence on breast cancer cell growth and invasiveness.
Results: We show that estrogen-independent cells expressed a significantly higher basal MnSOD level compared to estrogen-dependent human breast cancer cell lines (MCF-7 and T47D). For MDA-MB231 cells, the high MnSOD level was accompanied by an overproduction of

intracellular hydrogen peroxide (H2O2) and by a low expression of the major H2O2-detoxifying enzymes, catalase and peroxiredoxin 3, compared to MCF-7 cells. Suppression of MnSOD expression by antisense RNA was associated with a decrease of H2O2 content and caused a stimulation of growth with a reduced cell doubling time but induced a decrease of colony formation. Furthermore, treatment of MDA-MB231 cells with H2O2 scavengers markedly reduced tumor cell growth and colony formation. In addition, MnSOD suppression or treatment with H2O2 scavengers reduced the invasive properties of MDA-MB231 cells up to 43%, with a concomitant decrease of metalloproteinase-9 activity.
Conclusions: We conclude that MnSOD plays a role in regulating tumor cell growth and invasive properties of estrogen-independent metastatic breast cancer cells. These action are mediated by MnSOD-dependent H2O2 production. In addition, these results suggest that MnSOD up-regulation may be one mechanism that contributes to the development of metastatic breast cancers.

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Synergic antiproliferative effect of Hsp90 inhibitor in combination with cisplatin in gastric carcinoma cell lines

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Background: 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) is a new anticancer agent currently in clinical trials. The ability of 17-DMAG to abrogate the function of heat-shock protein Hsp90 and modulate cellular sensitivity to anticancer agents has prompted recent research to use this compound in drug combination therapy. In this study, we determined whether 17-DMAG potentiates the cytotoxic effect of cisplatin (CDDP) on gastric cancer lines and investigate the mechanism underlying this enhancement of CDDP-induced cytotoxicity by 17-DMAG.
Methods: In a panel of four gastric cancer cell lines (MKN-1, MKN-7, MKN-45, and NUGC-4) we investigated the antiproliferative and cytotoxic effects of 17-DMAG, CDDP alone or a simultaneous combination of two drugs using in vitro tetrazolium-based colorimetric assay (MTT). The combination treatment was evaluated for synergism, additivity, or antagonism with a quantitative method based on the median-effect principle of Chou and Talalay. Cell cycle alterations were analysed by flow cytometry, while apoptosis was assessed by the occurrence of DNA internucleosomal fragmentation. Along with Western blotting experiments were performed to determine whether this synergistic combination of two drugs has significant effect on MAPK signaling and biochemical markers of apoptosis.
Results: In all four gastric cancer cells, 17-DMAG synergistically potentiated the inhibitory effect of CDDP on cell growth. Western blot analysis showed that phosphorylation of JNK1 and c-Jun induced by CDDP was down-regulated by the combination treatment. Mechanistic studies showed enhanced accumulations of the sub-G1 phase population in cells treated by the combination, which indicate the induction of apoptosis. Moreover, this correlated with enhanced activation of caspases 3, 8 and 9 and poly (ADP-ribose) polymerase cleavage. Evidence of synergy was formally demonstrated and occurred across a wide range of drug concentrations.
Conclusion: 17-DMAG synergistically augments the growth inhibition inserted by CDDP in gastric cancer cells. The synergistic effect was mediated through inhibition of activation of JNK1-cJun and induction of apoptosis. These studies provide the basis for potential clinical evaluation of this combination treatment for gastric cancer patients.

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Increased expression of EphA2 correlates with adverse outcome in primary and recurrent glioblastoma multiforme patients

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Background: Glioblastoma multiforme (GBM) is the most aggressive form of brain tumor characterized by exuberant angiogenesis. The dismal prognosis of patients with GBM warrants the development of new targeting therapies based on novel molecular markers. The EphA2 receptor tyrosine kinase plays a pivotal role in tumor angiogenesis and increased expression in glioma patients has been recently reported. In this study, we investigated the expression of EphA2 in primary and recurrent GBM and correlated it with clinical pathological parameters and patient's outcome.
Materials: The immunohistochemical expression of EphA2 receptor tyrosine kinase was analysed in a series of 32 formalin-fixed, paraffin embedded primary and recurrent GBM previously treated with surgery and radiation therapy. In addition, tumor microvascular density (MVD) was quantified by immunostaining for endothelial cell marker, von Willebrand factor (vWF). The correlation between expression of EphA2 and MVD as well as the prognostic relevance of EphA2 and MVD for long-term survival were investigated using the Kaplan-Meier statistical test.
Results: Different intensity of membranous and cytoplasmic expression of EphA2 were observed in GBM samples analysed while a strong expression of EphA2 was demonstrated in 24 (60%) of these primary and recurrent GBM. Additionally, no strong association between EphA2 expression and MVD was found ($P > 0.05$). No close correlation was noted between the expression levels of EphA2 or MVD and clinical pathological parameters such as age, and gender of patients. Increased expression of EphA2 protein was significantly associated with adverse outcome of GBM patients ($p < 0.01$ for overall survival) but was not prognostic for disease-free survival ($P > 0.05$).
Conclusions: The data presented in this study define for the first time the expression pattern of EphA2 in primary and recurrent glioblastoma and suggest the involvement of EphA2 in the development of GBM. The EphA2 might be used as surrogate marker to screen patients for tyrosine kinase inhibitor therapy.

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Effects of magnetic field exposure in the mammary gland tissue of female F344 rats and the impact of amylase

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Epidemiological data have raised concerns about the relationship between exposure to power frequency magnetic fields (MFs) and breast cancer. We have shown previously in in vivo animal experiments that the effect of MF exposure on the rat mammary gland differs depending on the different rat strain or substrain that is used. Comparison of different rat strains indicated that the genetic background plays a pivotal role in the MF effects. Among several rat strains, only Fischer 344 (F344) rats showed an enhanced proliferative activity in the mammary epithelium exposed to MF for 2 weeks. Prolonged MF exposure significantly increased tumor development and growth in the dimethylbenz[a]anthracene (DMBA) breast cancer model in F344 rats. These results indicate that the F344 inbred rat serves as a MF-sensitive rat strain.
Recently, we investigated the gene expression in the breast tissue of F344 rats and compared the results with Lewis rats (Lew) that are considered MF-insensitive. Unexpectedly, the most striking result was a marked decrease of amylase gene expression in MF-exposed F344, but not in Lew. Because of this finding, we now determined amylase enzyme activity in the breast tissue of juvenile F344 rats that were exposed to MFs or to the synthetic estrogen diethylstilbestrol (DES). F344 were MF-exposed over different periods, the insensitive Lew rats only over 2 weeks. DES was administered to F344 rats at different dosages and time points. Enzyme activity was measured colorimetrically with Starch Azure as a substrate. The grade of differentiation of the breast tissue was checked up by whole mount analysis.
DES application increased the appearance of more differentiated structures in the breast tissue in a dose-dependent manner. No alteration was observed in MF-exposed whole mounts of mammary glands. DES significantly increased amylase activity at the highest dosage (30 μg, 6 times). MF exposure also significantly increased enzyme activity in F344 rats (2 or 4 weeks of exposure) and in Lewis rats (2 weeks).
These data demonstrate that MF exposure and DES altered amylase activity in the rat mammary gland tissue. In literature, few associations between amylase and tumor development are described, but the underlying mechanisms are not known. Future cell culture experiments from breast tissue and breast tumors might be able to reveal the amylase effect in the tissue.
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Papaverine derivatives – new telomerase inhibitors

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One of the cancer cell growth inhibiting strategy is application of drugs poisoning or inhibiting activity of enzymes engaged in DNA processing including topoisomerases and telomerase. The effectiveness of these enzymes inhibition results from the ability of drugs to intercalate DNA