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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377 POSTER

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

structures and to stabilize them. The very promising strategy of studies focuses on telomerase, which is responsible for cancer cells longevity. It was shown that telomerase is detected in over 90% of cancer cells while it is generally inactive in most normal cells.
The aim of the study was to estimate the influence of two ligands (1 and 2), papaverine oxidation products, on cells viability and DNA-quadruplexes stabilization and thus, inhibition of telomerase activity. The two ligands were shown to have high affinity to guanine quadruplexes (G-4 DNA) in vitro, which suggests that they could be able to block DNA-telomerase interactions.
The cytotoxicity of ligands was measured in Cell Proliferation MTT Kit and the influence of the compounds on Telomerase activity was assessed by Telo TAGGG Telomerase PCR ELISA Plus assay.
Cytotoxicity tests showed that both ligands inhibit cell viability with their IC50 values for ligand 1 and ligand 2 respectively, at 72 h incubation time in HL60 cells: 0.15 and 0.19 μM; in HL60AR cells: 46.21 and 16.48 μM; in MCF-7 cells: 1.16 and 0.42 μM; in MDA-MB-231 cells: 16.55 and 5.1 μM. Moreover, it was also reported that ligand 1, showing fluorescence at 365/397 (exc./emis.), binds the growing cells permanently, persisting even through a few cell passages what was observed in a fluorescence microscopy.
Telomerase activity assay showed that both ligands significantly inhibit telomerase activity at the concentration of 0.1 μM. However, the action of both ligands resulted also in Polymerase activity inhibition, which might suggest interactions specific not only to quadruplexes but also to DNA helix or maybe even enzyme structure.
It is suggested that both studied ligands could be strong and selective cancer cells growth inhibitors that results from their telomerase inhibition specific action. It is also possible that the studied compounds could be new promising fluorescent probes for DNA detection and labeling, however further studies concerning their specificity and sensitivity are required.

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Non-clinical pharmacokinetics, distribution and excretion of SNS-314, a novel, selective aurora kinase inhibitor

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Background: The Aurora kinase family is comprised of three proteins, Auroras A, B and C that function as key regulators of cell progression through mitosis and cytokinesis and may be important targets in anti-cancer therapy. SNS-314 is a novel small molecule that potently inhibits all three Aurora proteins in the low-nanomolar range. SNS-314 has robust anti-tumor activity in a wide range of human xenograft tumor models in mice using an intermittent dosing schedule. SNS-314 currently is being investigated in a Phase 1 trial to evaluate its safety and pharmacokinetic properties in humans.
Methods: Pharmacokinetic studies were conducted in mice, rats and dogs dosed with SNS-314 or [14C]SNS-314. Blood, tissue, bile, and urine were collected between 0−48 hours and analyzed via LC/MS/MS. Pharmacokinetic parameters were estimated using WinNonLin. Quantitative whole body autoradiography was used to measure tissue distribution in rats.
Results: Pharmacokinetic studies were conducted in mice, rats and dogs after single and repeated administration. In rising dose pharmacokinetic studies, SNS-314 displays non-linear systemic exposure; the area under the concentration curve increases more than dose linearly. This is most pronounced in rats and mice and occurs to a lesser extent in dogs. Sex-related differences in pharmacokinetic parameters are observed in rodents and to a much lesser extent in dogs. Female rats had 1.3 to 2 fold greater plasma AUC than male rats. SNS-314 is rapidly and extensively distributed in both mice and rats when dosed IV, IP, or PO. Administration at 170 mg/kg to tumor bearing mice shows drug levels persisting in the tumor for more than 96 hours post-dose (T1/2 = 7.5 hr), even though plasma levels were not measurable beyond 40 hours post-dose (T1/2 = 4.7 hr). Whole-body autoradiography indicates [14C]SNS-314 related radioactivity is widely distributed in tissues after an IV bolus dose with maximum concentrations observed 1 hour post dose. Approximately 70% of SNS-314 is eliminated through biliary excretion 48 hours post dose.
Conclusion: The favorable pharmacokinetic properties of SNS-314 including elevated tumor over plasma drug levels support clinical investigation of this oncology agent.

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Relationship between expression of CXCR4 and histological type in adenoid cystic carcinoma of the head and neck

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Background: Adenoid cystic carcinoma (ACC) is one of the most common malignant tumors of the salivary glands characterized by multiple recurrences and distant metastasis resulting in significantly worsening prognosis. CXCR4/CXCL12, a representative chemokine receptor and its ligand, has been reported to be involved in cancer metastasis, especially in breast cancer metastasis. In order to investigate the high invasive and metastatic potentials of ACC, CXCR4 expression in ACC was examined, and analyzed the relation to clinicopathological features and histological type.
Methods: We analyzed immunohistochemical expression of CXCR4 surgical specimen of ACC. We also used two established human tumor lines, ACCY and ACCI, in nude mice derived from ACC of the oral floor. The expression levels of protein and mRNA of CXCR4 in these tumor lines were examined by western blot and RT-PCR.
Results: Patients expressed CXCR4 at high levels showed lung metastasis, regional lymph nodes metastases, and poor prognosis. The solid type and cribriform type with distant metastases showed intense CXCR4 staining, while tubular type and cribriform type with no metastasis were weakly positive. In vivo model, ACCY tumor showed an increased growth rate as the passage levels proceeded, and the histological feature has been changed from a cribriform pattern to a solid one. CXCR4 was highly expressed in 15th passage level than in initial level of ACCY. ACCI tumor in nude mice developed spontaneous metastasis to the neck, and the histological feature changed from a cribriform pattern of ACC to undifferentiated carcinoma. This metastatic tumor (ACCIM) caused spontaneous metastasis to the lung at high incidence when transplanted subcutaneously in nude mice. Expressions of CXCR4 in ACCIM were higher than ACCI, and lung metastatic area was strongly positive immunohistochemically. Both ACCI and ACCIM had high levels of mRNA for human CXCR4 by RT-PCR.
Conclusions: Our results indicate that there is a close relationship between CXCR4 and histological type of ACC, and CXCR4 may play important roles in the process of metastasis and biological behavior of ACC.

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Association of miR-21, miR-31, miR-143, miR-145 and let-7a-1 levels with histopathologic features of colorectal cancer

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Background: MicroRNAs (miRNAs) are endogenously expressed short non-coding RNAs, that repress protein translation through binding to target mRNAs. Although the number of verified human miRNA is still expanding, only few have been functionally described. However, emerging evidences suggest the involvement of altered regulation of miRNA in pathogenesis of cancers and these genes are thought to function as both tumours suppressor and oncogenes. Previous studies, mainly based on microarrays technology applied on colorectal cancer cell lines, showed altered expression levels of several miRNAs in colorectal cancer (CRC).
Materials and Methods: In our study, we examined by Real-Time PCR expression levels of miR-21, miR-31, miR-143, miR-145 and let-7a-1 in bioptic samples of 29 colorectal cancer patients including 3 cases of IUCC Stage I, 11 of Stage II, 6 of Stage III, 9 of Stage IV. For 6 cases of CRC samples also adjacent non-tumor tissue was analyzed. MiRNAs expression levels were correlated with tumor stage, grade, size, anatomical localization, serum CEA levels and p53 protein expression in tumors. For data normalization we tried different approaches (18S rRNA, GAPDH, let-7a-1). Finally, variability of let-7a-1 expression was shown to be the lowest. P values were calculated using Mann-Whitney U test.
Results: Expression levels of all analyzed miRNAs significantly differ in tumor and normal mucosa, miR-21 ($p = 0.0001$) and miR-31 ($p = 0.0006$) were up-regulated and miR-143 ($p = 0.013$) and miR-145 ($p = 0.018$) were down-regulated in tumors. MiR-21 was also correlated with CRC stage. Although the highest levels of miR-143 and miR-145 were in normal mucosa, we identified positive correlation of tumor stage and their expression suggesting altered tumor suppressor function of these miRNAs in early events of colorectal carcinogenesis. Distal CRC showed significant