phosphatidylinositol 3-kinase (PI3 kinase) activity. DNA from CYR61 transfectants treated with PTX displayed no signs of the classical DNA laddering pattern of apoptotic death. Inhibition of PTX-induced apoptosis in CYR61 transfectants was also demonstrated by TUNEL assay. Moreover, MCF-7/CYR61 cells were unable to induce p53 expression in response to PTX-induced damage. It is tempting to postulate that the angiogenic factor CYR61 -a downstream effector of HRG- might protect breast cancer cells from PTX-induced apoptosis by enhancing alphav beta3-PI3 kinase pro-survival signaling and inhibiting p53 pro-apoptotic functions. We suggest that new anti-HRG, anti-CYR61, and/or anti-integrin alphav beta3 strategies may prevent vessel growth simultaneously rendering tumor cells more sensitive to PTX-based chemotherapy in breast cancer.

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NFkappaB repression by sulfasalazine sensitizes pancreatic carcinoma cells to cytostatic drugs *in vivo*: a new concept of combined chemotherapy

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The pancreatic carcinoma is still characterized by a poor prognosis and ranks 5th among malignancy-associated deaths. Surgical resection is feasible only in a minority of patients and other therapy options based on chemotherapy are only palliative. One reason for this might be the resistance of tumor cells towards cytostatic drugs. To improve the therapy of pancreatic cancer it will be important to understand the mechanisms how tumor cells achieve chemoresistance and to define molecular targets for new therapeutic strategies. Some pancreatic carcinomas are characterized by a constitutively elevated NF-kappaB activity accounting for chemoresistance. In order to elucidate whether blockade of NF-kappaB activity with the anti-inflammatory drug sulfasalazine is suitable for overcoming this chemoresistance *in vivo*, we employed a mouse model with subcutaneously xenotransplanted human Capan-1 pancreatic carcinoma cells. Fourteen days upon tumor inoculation, animals were randomized in six groups, receiving no treatment, treatment with gemcitabine (2.5 mg/kg day, intraperitoneal), treatment with etoposide (6.5 mg/kg day, intraperitoneal), either alone, or in combination with sulfasalazine (70 mg/kg day, oral), or with sulfasalazine alone. Upon treatment with etoposide or gemcitabine alone, tumor sizes were moderately reduced to 50 % and 60-70 %, respectively, as compared to untreated tumors. The corresponding combination groups (etoposide: 20-25 %, gemcitabine: 45-50 %) showed significantly higher reduction in tumor sizes. TUNEL-staining revealed higher numbers of apoptotic cells in tumors from combination groups, and proliferation as indicated by Ki67 staining was strongly reduced. Furthermore, combined treatment of sulfasalazine with the cytostatic drugs led to a decreased blood vessel density. Immunohistochemical staining of the activated p65 subunit revealed that sulfasalazine treatment abolished the basal NF-kappaB activity in tumor cells. These data imply that NF-kappaB inhibition sensitizes pancreatic carcinoma cells to cytostatic drugs *in vivo*. In particular, a combined chemotherapy with the well established anti-inflammatory drug sulfasalazine offers great potential for improved anti-tumor responses in pancreatic carcinomas.

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Validation of reliable assay methods for glutathione quantitation and glutathione s-transferase activity in cancer patients

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Glutathione s-transferases (GST) are members of a superfamily of enzymes that catalyze the reaction of electrophilic compounds with glutathione (GSH) to form inactive conjugates. These enzymes are of great importance in cancer biology since their levels have been correlated with resistance to cytotoxic drugs such as alkylating agents, platinum compounds and anthracyclines. Conversely, recently has been published that a DNA minor groove binder is activated by the GST/GSH systems (Geroni et al, Cancer Res. 2002). GST distribution in cancer patients has been extensively investigated but mixed data have been reported. The objectives of this study were primarily to validate robust and reliable assays for GSH/GST detection, suitable for routine clinical use and to explore the correlation between blood and tissue levels for both. Matched blood and tissue samples (normal and malignant) from 52 cancer patients (NSCLC and SCCHN) were investigated. GSH concentration and GST activity were measured by an enzymatic assay. GST content was also analysed by HPLC. Moreover, since the existence of regions of tissue heterogeneity is well documented within the tumor, multiple samples from seven cancer specimens have been analysed. Data were evaluated for either intra- and inter-patient variability to verify whether GSH/GST exhibit heterogeneity in samples from different areas of the same specimen. Both GST activity and GSH levels were higher in cancer than in normal tissue. The difference was statistically significant in NSCLC ($p=0.0004$ and $p=0.0002$, respectively for GSH and GST) and borderline in SCCHN ($p=0.03$ and $p=0.02$, respectively for GSH and GST). Moreover GSH levels in whole blood showed a highly significant correlation with GST activity in matched cancer samples in both malignancies ($p=0.003$, $r=0.53$ in NSCLC, $p<0.0001$, $r=0.89$ in SCCHN). The strong correlation found between GST activity in cancer tissue and GSH level in whole blood indicates that GSH could have a clinical relevance as a surrogate marker of GST activity in tumor tissue and should be further investigated. However, tissue heterogeneity analysis suggested that GSH and GST levels could be linked to tissue variability in both normal and tumour tissues. Since statistical analysis indicates that heterogeneity is a true biological fact rather than an analytical artefact, it is recommended to have a larger sample of tumor tissue for GSH and GST biochemical analysis to confirm the validity of GSH as biomarker.

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Activation of wt p53 protein in normal and tumor cells by a novel anti-cancer drug CHS 828

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CHS 828, a novel cyanoguanidine, represents a new class of drugs for cancer treatment, with an unknown primary mechanism of action. It is known that anti-cancer drugs induce p53 response thereby triggering the cell cycle arrest or apoptosis We investigated the effect of anti-cancer drug CHS 828 on p53 induction in normal and tumor cells. We observed dose-dependent upregulation of wt p53 by CHS 828 in MCF-7 cells as well as in human and mouse fibroblasts. The drug, however, failed to induce p53 protein in PARP-1 deficient cells even at a 30-fold higher dose and after prolonged treatment. Combined treatment of PARP-1 -/- cells by CHS 828 and MDR modulators did not alter p53 expression. CHS 828 inhibited cell proliferation and DNA replication in tested cells. Interestingly, the DNA synthesis as well as proliferation of PARP-1 -/- cells was inhibited by about three-fold lower drug concentration then their normal counterparts. The treatment of mouse cells by CHS 828 for 48h impaired the integrity of plasma membrane in PARP-1 deficient mouse cells as evidenced by Trypan blue dye exclusion test. The effect of CHS 828 on p53 in normal cells seems to be cell cycle dependent. Treatment of quiescent cells resulted in downregulation of p53 protein. These results show that the drug is able to activate p53 response depending on cell cycle and PARP-1 functional status. The inactivation of PARP-1 sensitizes cells to the novel anti-cancer drug CHS 828.

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PPARgamma ligands modulate PPARgamma and RARbeta expression in human glioblastoma cell lines

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A recent approach to cancer therapy is the control of cell growth and induction of apoptosis via ligands of nuclear hormone receptors (NHR). Among NHR, much attention has been focused on peroxisome proliferator-activated receptor gamma (PPARgamma), that has been involved in the control of growth and/or differentiation of several malignant cell types. Currently, we are evaluating whether PPARgamma may be a molecular target for novel therapies in glioblastoma. We have already reported that PPARgamma is expressed in glioblastoma tumors and cell lines (A172, U87-MG,