Materials and Methods: The analysis was performed with a T7-phage-based peptide phage display library that was specifically constructed to detect posttranslational modifications of peptides. For this study we focused on the identification of peptides recognized by proteolytic enzyme activities. 10^9 different substrate peptides were screened against a complex proteome present in cellular lysates derived from untreated and irradiated cells (SW480, 10Gy). In a subtractive screening procedure a differential activity pattern could be monitored and substrates for treatment-specific activities could be distinguished from substrates for background activities. The method was developed and applied in a methodological and discovery-oriented approach.
Results: Radiation-specific substrate peptides were isolated in a clinically relevant radioresistant cell system (SW480). Multiple peptide sequences were selected that are specifically recognized and cleaved by treatment-dependent enzyme activities. A specific recognition sequence was identified to be part of the human nucleoporin protein (Nup50) relevant for controlled nuclear protein shuttling. Nup50 was further investigated in vivo in response to ionizing radiation.
Conclusions: We present a novel technique for the identification of treatment-induced posttranslational peptide modifications in tumor or normal tissue cells. This procedure represents a complementary tool for genome-wide screening approaches. The identified peptides, their specificity and biological counterparts suggest that intact nuclear shuttling and compartimentalization processes co-determine the cellular radiosensitivity.

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Gene expression profiling of cancer associated fibroblasts

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Fibroblasts located in the vicinity of tumor cells, often termed cancer associated fibroblasts (CAFs), contribute to the microenvironment that is important for cancer cell development, growth, invasion and metastatic progression. Hitherto, the defining features of CAFs and their specific differences to their normal counterparts are only incompletely understood. Therefore, based on a laser capture microdissection and microarray-based approach, the aim of this study was to comprehensively characterize CAFs in their *in vivo* environment to identify novel potential targets for anti-cancer therapy. Fresh frozen samples of basal cell carcinoma and normal skin from three different patients were used for laser microdissection of CAFs and normal fibroblasts (NFs). The RNA was extracted, amplified (~200.000 fold) and labeled with Cy3 or Cy5 modified nucleotides. Labeled RNA from CAFs and NFs (500–4000 cells) from each patient was competitively hybridized on cDNA microarrays in replicates. Results were confirmed using quantitative real time PCR. The analysis yielded three gene lists with up-regulated (patient 1: 608, patient 2: 49, patient 3: 228 genes; >1.7, t-test $p<0.05$) and down-regulated genes (patient 1: 552, patient 2: 84, patient 3: 75 genes; <0.57, t-test $p<0.05$). In the group of down-regulated genes only few genes coincide (patient 1 and 2: 14, patient 1 and 3: 5; patient 2 and 3: 3 genes). In contrast, among the up-regulated genes a significant number of genes were overlapping between the different patients (patient 1 and 2: 24, patient 1 and 3: 56; patient 2 and 3: 12 genes). Most of these genes were involved in matrix remodeling and cell-cell or cell-matrix interaction (i.e. SPARC, galectin-2, galgranulin-A, laminin alpha 2) but also in growth regulation (i.e. Grap-2, VAV3) and angiogenesis (i.e. angiopoietin-like-2). The gene lists provide a valuable tool to select genes for further studies of their functional relevance. This approach will be expanded to other solid tumors to identify additional novel CAF-specific genes, crucial for stroma-tumor interaction, which will be exploited for use in novel anti-tumor strategies.

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AG879, a tyrphostin c-erbB-2 inhibitor, leads to transcriptional repression of cyclin D1 in pancreas cancer cells through a p27Kip1 independent pathway

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The poor prognosis of pancreatic cancer with the current traditional modalities makes molecular targeted therapies a high priority. c-erbB-2 provides cell survival and proliferative signals through Cyclin D1. Our hypothesis is that inhibition of cyclin D1 expression will induce cell cycle arrest and apoptosis of pancreatic carcinoma. We investigated in a human pancreatic cell line L3.5sL (SL) that has a constitutively active c-erbB-2, the effects of c-erbB-2 inhibition through two different interventions: 1) degradation of its mRNA (siRNA) to prevent protein expression and 2) blockade of its catalytic activity in its tyrosine kinase domain (AG879, tyrphostin small molecule). AG879 treatment induced G1 cell cycle arrest and apoptosis. Treatment with siRNA also inhibited proliferation. The effect of these two approaches on $p27^{Kip1}$ expression was distinctly different. siRNA treatment decreased expression of c-erbB-2 and increased $p27^{Kip1}$. However, AG879 at the IC50 dose did not alter expression of c-erbB-2 or $p27^{Kip1}$ but repressed transcription of cyclin D1 mRNA 50% as measured by RT-PCR assay and cyclin D1 promoter activity fourfold as measured by luciferase promoter assay. Furthermore, AG879 had no effect on proteasome activity, excluding degradation as a plausible pathway. Since the cyclin D1 promoter has STAT3 and STAT5 binding sites, we assessed whether these STATs were involved in regulating Cyclin D1. STAT5 was not detectable in SL cells but STAT3 was. STAT3 function requires phosphorylation on Y^{705} and S^{727}. Y^{705} pSTAT3 was decreased 30% after AG879 treatment but S^{727} pSTAT3 was completely inhibited at 3 hr after AG8790 treatment. Mobility shift assays confirmed that STAT3 binding to the cyclin D1 promoter was decreased beginning 3 hr after AG879 treatment. We propose that AG879 at doses that do not decrease c-erbB-2 protein expression but induce G1 cell cycle arrest and apoptosis inhibits S^{727} STAT3 phosphorylation and that this is a critical step in repressing cyclin D1 transcription. In contrast, lowering expression of c-erbB-2 protein causes cell cycle arrest through upregulation of $p27^{Kip1}$ Further elucidation of the mechanism involved in regulating S727 STAT3 phosphorylation may provide insights into novel therapies for pancreas cancer.

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17-allylamino-17-demethoxygeldanamycin overcomes trail resistance in colon cancer cell lines

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Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is a promising candidate for treatment of cancer, but its cytotoxicity is limited in some cell lines. The mechanisms of this resistance have not been fully elucidated, but both AKT and NF-κB pathways have been shown to modulate cytotoxic responses. We studied the effect of combination of TRAIL and the hsp90 inhibitor 17-AAG, which we have shown to enhance the cytotoxicity of oxaliplatin in colon cancer cell lines through inhibition of NF-κB. In a series of 9 colon cancer cell lines IC50 values for a 72-hour exposure to TRAIL ranged from 30 to 3000 ng/ml. Cytotoxicity assays demonstrated additivity or synergism of the TRAIL/17-AAG combination in all cell lines, with combination indices at IC50 ranging from 0.65 to 1. The sensitizing effect of 17-AAG was greater in the TRAIL-resistant cell lines. 17-AAG enhanced TRAIL-induced activation of caspase 3 in all cell lines tested. In TRAIL-resistant cell lines, the combination of 17-AAG and TRAIL resulted in activation of either extrinsic or intrinsic apoptotic pathways in a cell line-specific manner. In the RKO cell line AKT inhibition was associated with activation of the mitochondrial apoptotic pathway, while in HT29 cells inhibition of NF-κB was permissive of caspase-8 dependent apoptosis. In both cell lines the combination resulted in down-regulation of X-linked inhibitor of apoptosis protein (XIAP), which may facilitate the activation of effector caspases. The ability of 17-AAG to target multiple putative determinants of TRAIL sensitivity warrants their further investigation in combination.

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A phase I, pharmacological and biological study of Sarasar® (lonafarnib, SCH 66336), cisplatin and gemcitabine in patients with advanced solid tumors

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Sarasar (lonafarnib, SCH 66336) is a novel, oral tricyclic farnesyl transferase inhibitor (FTI) with broad anti-tumor activity in human xenograft models. Preclinical studies have shown that Sarasar is synergistic with cisplatin (C) and additive with gemcitabine (G). The purpose of this study is to determine the dose-limiting toxicity (DLT), maximum tolerated dose (MTD), and biologic and pharmacokinetic behavior of the combination of Sarasar (75–125 mg BID daily), C (75–100 mg/m2 IV day 1), and G (750–1000 mg/m2 IV weekly d1, 8 or d1, 8, 15) given every 3–4 weeks. The study was amended to investigate an every three-week schedule and to delay institution of Sarasar until day 8 of course 1, in order to better differentiate the emetogenic effects of Sarasar and C. To date, 21 pts (median age 53, range 37–72; median PS 1) have received a total of 40