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.

90 POSTER

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

cycles (median 2, range 1–8+) of therapy. Three of 9 patients at Sarasar 75 mg BID/ C 75 mg/m2/G 1000 mg/m2 experienced DLT (1 pt: gr 3 N/V (Sarasar and C both given day 1, 2 pts: gr 4 ANC). Other at least possibly drug-related toxicities have included gr 3–4 thrombocytopenia, gr 2–3 nausea and vomiting, gr 2 fatigue/asthenia, gr 2 diarrhea, gr 1 transaminitis, and gr 1 tinnitis. The extent of myelosuppression appears to be enhanced in patients with extensive prior therapy, whereas N/V has been ameliorated with oral antiemetics administered for several days following therapy with C. Two previously treated patients with breast cancer have demonstrated confirmed clinical responses, one CR (chest wall disease) lasting for 7 cycles and one PR (soft-tissue) that is ongoing at 8+ cycles of therapy. These patients received their first cycles at doses of Sarasar/C/G of 75/75/1000 (d1, 8, 15) q 4 weeks, respectively, while all subsequent cycles have been at Sarasar/C/G doses of 75/75/750, respectively. Based upon tolerability over several cycles, accrual is ongoing at the Sarasar 75 mg/m2 BID/ C 75 mg/m2/G 750 mg/m2 (d1, 8) dose level on the every three-week schedule. These results suggest that this novel combination might be active in the treatment of metastatic breast cancer, a tumor type that has demonstrated single-agent responses (albeit modest) to farnesyl transferase inhibitors. Pharmacokinetic and biologic correlative analyses, including farnesyl transferase functional inhibition and surrogate assays, will be presented.

93 POSTER

Genetic validation of activated polyamine catabolism as a novel therapeutic strategy targeting prostate cancer

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Background: Depletion of intracellular polyamine pools invariably inhibits cell growth and thus, represents a viable therapeutic/prevention strategy. Although this is usually accomplished by inhibiting biosynthetic enzymes, we propose that it might be more effectively achieved by activating polyamine catabolism at the level of spermidine/spermine N^1-acetyltransferase (SSAT), an enzyme known to be inducible by various drugs and compounds. Our previous studies have confirmed this strategy in MCF-7 breast carcinoma cells (Vujcic et al., J. Biol. Chem. 275:38309, 2000) . On the basis of unique polyamine homeostatic responses in the prostate gland, we have reason to believe that tumor cells derived from it may be particularly sensitive to this approach.
Methods and Results: SSAT was conditionally over-expressed in LNCaP prostate carcinoma cells via a tetracycline-repressible system. Tetracycline removal resulted in a ~20-fold increase in SSAT mRNA and enzyme activity and a massive accumulation of SSAT acetylated polyamines. This, in turn, led to sustained growth inhibition that unexpectedly, was not associated with spermidine and spermine depletion. Rather, polyamine pools were maintained by a compensatory increase in biosynthetic enzyme activities that gave rise to heightened metabolic flux through polyamine biosynthetic and catabolic pathways. Treatment with the biosynthetic inhibitor α-difluoromethylornithine during SSAT induction interrupted flux and prevented growth inhibition, thus, demonstrating a cause-and-effect relationship. Of the various underlying mechanisms investigated, flux-induced growth inhibition correlated closely with a 50% depletion in the SSAT cofactor, acetylCoA as measured by capillary electrophoresis. Having demonstrate the antiproliferative potential of this approach, we next examined the *in vivo* consequences of SSAT overexpression in mice genetically predisposed to develop prostate cancer. TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) female mice were cross-bred with male transgenic mice that systemically over-express SSAT. At 30 wk of age, the average genitourinary tract weight of TRAMP/SSAT mice was 75% smaller than that of TRAMP mice and by 36 wk, it was ~92% smaller. SV 40 large T-antigen expression in the prostate epithelium were similar in TRAMP and TRAMP/SSAT mice. Consistent with an 18-fold increase in SSAT activity in the TRAMP/SSAT bigenics, prostatic putrescine and acetylated spermidine pools increased remarkably relative to the TRAMP mice while spermidine and spermine pools were minimally affected due to a compensatory increase in biosynthetic activity similar to that seen in LNCaP cells. This heightened metabolic flux resulted in >70% reduction in acetyl-CoA in TRAMP/SSAT prostate tumors while having only a minor effect on acetylCoA levels in the liver. A role for SSAT in fat metabolism is indicated by markedly reduced levels of abdominal and subdermal fat in SSAT transgenic and bigenic mice. Taken together, the antitumor activity deriving from activated polyamine catabolism appears to be related to downstream effects on acetylCoA and fat metabolism.
Conclusions: In addition to elucidating the overall antitumor effects of SSAT overexpression in prostate cancer and defining previously unrealized metabolic consequences, the present findings provide *in vitro* and *in vivo* genetic support for the discovery and development of specific small molecule inducers of SSAT as a novel therapeutic strategy targeting prostate cancer. Given the known high responsiveness of this enzyme system to various anticancer drugs, polyamine analogs, and other agents, such a molecule should not be difficult to identify.

94 POSTER

The cytotoxic effects of 17-AAG, an inhibitor of Hsp90 are enhanced by combination with the PI-3-kinase inhibitor LY294002 in non-small cell lung cancer (NSCLC) cell lines

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NSCLC cells rely on multiple genetic abnormalities that result in several aberrant signaling pathways that in turn mediate cancer maintenance and progression.
Because of cellular signaling redundancy it is anticipated that interruption of a single signaling network or transforming molecule will not significantly affect tumor growth.
As an alternative approach, we have examined the effects of 17AAG a derivative of geldanamycin, a drug that alters the function of heat shock protein 90 (Hsp90), a ubiquitously expressed molecular chaperone that appears to play an essential role in malignant transformation by regulating the stability and activity of multiple oncogenic growth factor receptors and proteins important in promoting tumor proliferation and survival such as EGFR and p-Akt.
A549 (PTEN wild-type) and H157 (PTEN mutant) cell lines with low and high constitutive activated Akt expression respectively were used for these experiments. Exposure of A549 and H157 cells to 17AAG resulted in inhibition of cell growth as measured by MTT assay with IC_{50} concentrations of 5 μM and 500 nM respectively at 72 hours. Flow cytometry at 24 and 48 hours revealed G1/S and G2/M arrest respectively.
We reasoned that using 17AAG to destabilize Hsp90 proteins, while simultaneously targeting directly one of the most dominant signaling pathways, the PI-3 kinase pathway might result in improved tumor cytotoxicity. LY294002, a PI-3 kinase inhibitor inhibited growth at IC_{50} concentrations of 30–40 nM for both cell lines.
Indeed simultaneous exposure for 72 hours to equitoxic concentrations (ratios of IC_{50}) led to supra-additive cytotoxic effects for the A549 and synergistic effects for the H157 cells with inhibition achieved at suboptimal concentrations of the individual drugs.
The effects of the combination on apoptosis and cell cycle were also evaluated and will be presented.
Depletion of target client proteins was examined by immunoblot analysis. Dramatic decreases in p-Akt, p-GSK3β, pERK1/2, c-Raf and cyclin D1 were observed in H157 and to a lesser degree in A549 cells, while induction of apoptosis as evidenced by PARP cleavage, and detection of caspases, 3, 9 and 8 was also seen.
Studies are underway to clarify further the molecular determinants of interaction between Hsp90 and PI-3 kinase inhibitors, aiming at identifying the mechanisms of differential sensitivity and predictors of response. The information obtained from the present study could have direct clinical applications in the treatment of NSCLC (supported by the ASCO CDA and P50CA91007–02).

95 POSTER

Expression of genes relevant for tumour aggressiveness in endometrial cancer

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Objective: The aim of this study was, to analyse the expression of identified genes in endometrial cancer tissue, witch might be responsible for tumour aggressiveness.
Methods: After identification and analysis of differential displayed cDNA in matched paired patients with endometrial cancer with and without metastatic recurrence, three cDNA samples (named edi1, edi-2 and edi-3) were reamplified and sequenced. An NCBI-database request on homologies on these three sequences was done. To measure and to analyse the expression of these three cDNA samples TAQMAN-assay was used on 54 cases (42 without and 12 with recurrent disease). Statistical analysis was done by using Mann-Whitney – U-Test, Cox-regression model and Kaplan-Maier concerning to overall survival and recurrence free interval.
Results: The mean age of patients in this study population was 68 years (range 34–89), the mean weight was 75kg (range 55–132). 71.9% of patients showed no evidence of disease, 17.2% died on disease. In this