of information that can be obtained from a single assay, microarrays are an extremely useful tool for candidate biomarker discovery. In addition to gene expression profiling, microarrays can also provide a powerful platform for high-throughput custom sequencing analysis. We designed an Affymetrix CustomSeq® microarray that reads over 300,000 base pairs spanning the entire coding regions of 81 genes. The genes selected for this CustomSeq® microarray regulate important tumor progression processes such as cell-cycle, apoptosis (intrinsic & extrinsic), drug-resistance, pro-survival, proliferation, metastasis, and angiogenesis. Also included in the microarray design are many of the molecular targets for the promising new anti-apoptotic therapies. To validate this new drug development microarray, we PCR amplified EGFR and members of the PI3K/AKT pathway from whole blood and HT29 colorectal tumor cells. After the PCR products were pooled and quantified, the samples were chemically fragmented, hybridized, washed, stained and scanned for fluorescent hybridization signatures indicating the sequence of the amplified genes. We demonstrated the ability of this array to perform high-throughput mutation and SNP screening. Information from this new molecular tool integrates well with gene expression profiling data and will help to implicate important genes, molecular pathways and host-drug interactions that influence cancer growth, development, disease susceptibility, drug resistance and drug response.

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473 POSTER

p53-dependent repression of CHEK1 contributes to apoptosis in colorectal cancer cells: an in vitro and in vivo study

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Background: There are only a few reports on the role of p53-dependent gene repression in apoptotic cell death. Here, we used the plant drug thymoquinone (TQ), to identify potential targets of p53.
Material and Methods: Human colon cancer cells HCT116 p53 +/+ and p53 −/− were treated with 60 μM TQ, and apoptosis-associated genes were analyzed by a cDNA microarray. Drug-induced DNA-damage was characterized by H2A.X foci and H_2O_2 formation. Apoptosis induction was analyzed by flow cytometry, caspase 3- and DASH assay. mRNA and protein expression were determined by *real-time* RT-PCR and Western Blotting, respectively. Using chromatin immunoprecipitation (ChIP), we studied p53 binding at the CHEK1 promoter. HCT116 p53 −/− cells were transfected with a wt-p53 vector in order to restore the p53 function and CHEK1 binding. Furthermore, we investigated the p53, CHEK1 and apoptosis status in a panel of human colon cancer tissues with known p53 mutation status.
Results: Only 17% of genes were dysregulated and might contribute to a significant portion of the TQ response. Strikingly, CHEK1 mRNA and protein were significantly induced in TQ-treated HCT-116 p53 (−/−) cells. Using ChIP, we verified a transcriptional repression of p53 at the CHEK1 promoter. Apoptosis was induced in response to TQ treatment in HCT-116 p53 (+/+) cells but to a much lower extent in HCT-116 p53 (−/−) cells, which fits with the drug-induced, significantly higher DNA damage signal in p53 +/+ cells. Transfection of p53 (−/−) cells with a p53-wt vector decreased the CHEK1 mRNA and protein levels and restored the apoptosis to the level of the p53 (+/+) cells. P53 (−/−) cells transplanted to nude mice intraperitoneally treated with 20 μM TQ also highly up-regulated CHEK1 expression and did not undergo apoptosis in contrast to p53 (+/+) cells. Colon carcinomas with p53 deletions resulting in a truncated non-functional p53 protein had significantly higher CHEK1 mRNA and protein expression levels which were accompanied by poor apoptosis compared to p53 wt-expressing tumors.
Conclusions: CHEK1 repression by p53 in HCT-116 p53 (+/+) cells could be responsible for drug-induced apoptosis, supporting recent findings that transcriptional repression by p53 rather than activation and selective blockade of p53-dependent gene repression accounts for DNA damage-induced apoptosis. In cancer therapy of colorectal cancer, the inactivation of CHEK1 might contribute to the anti-tumor activity of specific DNA-damage-inducing drugs.

474 POSTER

Inhibition of PDGFR-beta increases sarcoma cell sensitivity to tumor necrosis factor related apoptosis inducing ligand, TRAIL and promotes inhibition of tumor growth in dual therapy using imantanib (Gleevac) and TRAIL

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Background: The activation of the intrinsic and extrinsic apoptotic pathways have been found to sensitize cells to TRAIL. However, the interaction between antiangiogenic agents and TRAIL induced apoptosis has not been well studied. We hypothesize that inhibition of PDGFR-beta will sensitize sarcoma cells to TRAIL and therefore promote synergistic cytotoxicity, and inhibition of tumor growth using antiangiogenic and apoptotic therapy.
Methods: si RNA technology was used to 'knock down' PDGFR-beta in human Ewing's sarcoma cells, TC-71. More than 90% inhibition was achieved in the TC-71si cell line. The expression of death receptors-4 and 5 in the TC-71 versus TC-71si cell lines was then compared. TRAIL induced cytotoxicity in the 'knockdown' versus the wild type cell line was evaluated. The TC-71w cells were then injected into an orthotopic xenograft model of Ewing's sarcoma. An Imantanib (Gleevac) and TRAIL combination was then used to treat mice with either locally advanced chest wall Ewing's sarcoma, or spontaneous pulmonary metastasis secondary to Ewing's sarcoma. Combination treatment was compared to single therapy.
Results: The TC-71si cell line showed increased expression of DR-5 receptors (78% vs 48%) and DR-4 receptor expression (58% vs 35%) compared to the TC-71 cell line. Also, dose dependent TRAIL cytotoxicity was significantly more profound in the TC-71si cells (0% viability) compared to the TC-71 (35% viability) cells at 1500 ng/ml of TRAIL. In our locally advanced chest wall Ewing's model, maximal growth inhibition was seen using the Gleevac and IP TRAIL combination. Ewing's sarcoma chest wall tumors treated with Gleevac plus IP trail grew to an average of 100 mm^3 compared to an average of 1300 mm^3 in the control group without treatment. However, there was no significant difference in the inhibition of tumor growth seen in the the Gleevac and IP versus Gleevac and IN TRAIL. When using the Gleevac and IN TRAIL combination in mice with pulmonary metastasis, only 1 of 6 (16%) mice developed gross pulmonary metastasis versus 4 of 5 (80%) in the control group compared to Gleevac alone (3/6, 50%) versus IN TRAIL (2/6, 33%) or IP TRAIL alone 3/6 (50%).
Conclusion: A combination of TRAIL and Gleevac causes significant inhibition of pulmonary metastasis and primary tumor growth in Ewing's sarcoma. This is the first known report of synergy between apoptosis and antiangiogeneic therapy in a preclinical model.

475 POSTER

Involvement of the multi-ligand cell surface receptor RAGE in Tumor Necrosis Factor-induced cell death

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Tumor Necrosis Factor (TNF) is a potent anti-tumor agent and therefore an ideal tool to identify the signaling pathways that are required to kill a cancer cell efficiently. Our work is focused on a caspase-independent cell death pathway that is characterized by a necrosis phenotype and that requires the increased production of reactive oxygen species (ROS) in the mitochondria.
We have recently shown that the TNF induces increased concentrations of methylglyoxal, a cytotoxic metabolite derived from glycolysis that been considered for a long time as a natural anti-cancer agent. This, together with the TNF-induced phosphorylation of glyoxalase I, leads to the formation of specific methylglyoxal-derived Advanced Glycation End products (AGEs). The effect of AGEs are mediated via cell surface receptors of which the Receptor for AGEs (RAGE) is the best known. RAGE is a multi-ligand receptor involved in tumor growth, invasion and metastasis. Here we report that TNF-induced cell death is mediated via RAGE. Induced overexpression of the ligand-binding domain of RAGE [soluble (s)RAGE] strongly inhibits TNF-induced cell death, as did overexpression of WT RAGE but to a lesser extent than sRAGE. However, overexpression of a mutant of RAGE that lacks the intracellular domain has no significant effect on TNF-induced cell death. We found that TNF induces rapidly nucleocytoplasmic translocations of endogeneous full-length RAGE, which is followed by a considerable reduction in the amount of FL-RAGE and its higher molecular weight complexes. These TNF-induced nucleocytoplasmic translocations of endogeneous FL-RAGE are disrupted by overexpression of sRAGE. This implies that sRAGE may sequester a ligand that is required for TNF-induced nucleocytoplasmic translocations of RAGE and cell death. We further demonstrate that inhibition of a secretory pathway by Brefeldin A completely inhibits TNF-induced cell death as well as the nucleocytoplasmic translocation of FL-RAGE and the subsequent

reduction of FL-RAGE. However, brefeldin A does not affect the TNF-induced NF-κB activation in these cells, indicating that the drug did not interfere with TNF-signaling in general.
In summary, TNF-induced cell death requires a nucleocytoplasmic translocation of the cell surface receptor RAGE. Furthermore, the cell surface RAGE may be a promising target for the induction of cell death in tumor cells.

476 POSTER

Inhibition of EMMPRIN (CD147) sensitizes human breast cancer cells to anoikis

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Overexpression of extracellular matrix metalloproteinase inducer (EMMPRIN or CD147), a member of the immunoglobulin family and a glycoprotein enriched on the surface of tumor cells, promotes invasion, metastasis, growth and survival of malignant cells, and confers resistance to some chemotherapeutic drugs. However, the molecular mechanisms underlying the actions of EMMPRIN are not fully understood. In this study we sought to determine whether EMMPRIN contributes to the malignant phenotype of breast cancer by inhibiting anoikis, a form of apoptosis induced by loss or alteration of cell-cell or cell-matrix anchorage, and to explore the signaling pathways involved. We found that human breast carcinoma cells expressing high levels of EMMPRIN formed aggregates with large surface area, had higher viability, and were resistant to apoptosis in the absence of attachment. Knockdown of EMMPRIN expression by RNA interference (siRNA or shRNA) sensitized those cancer cells to anoikis, as demonstrated by activation of caspase-3, increased DNA fragmentation and decreased cellular viability. Furthermore, we observed that the accumulation of Bim, a pro-apoptotic BH3-only protein, was reduced in EMMPRIN-expressing cells, and that silencing of EMMPRIN expression elevated Bim protein levels and enhanced cellular sensitivity to anoikis. Inhibition of Bim expression by siRNA decreased the sensitivity to anoikis in cells with low EMMPRIN. Treatment of cells with a MEK inhibitor (U0126) or proteasome inhibitor (epoxomicin) also upregulated Bim accumulation and rendered cells sensitive to anoikis. These results indicate that expression of EMMPRIN protects cancer cells from anoikis, and this effect is mediated by a MAP kinase-dependent reduction of Bim via proteasomal degradation. Since anoikis deficiency is a key feature of neoplastic transformation and invasive growth of epithelial cancer cells, our study on the role of EMMPRIN in anoikis resistance and the mechanism involved underscores the potential of EMMPRIN expression as a prognostic marker and novel target for cancer therapy.

477 POSTER

Inhibition of mTOR or apopotic pathway induces autophagy and radiosensitizes PTEN null prostate cancer cells

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Background: The PI3K/Akt pathway plays a critical role in oncogenesis, and dysregulation of this pathway through loss of PTEN suppression is a particularly common phenomenon in aggressive prostate cancers. The mammalian target of rapamycin (mTOR) is a downstream signaling kinase in this pathway, exerting prosurvival influence on cells through the activation of factors involved in protein synthesis. The mTOR inhibitor rapamycin and its derivatives are cytotoxic to a number of cell lines; recently, mTOR inhibition has also been shown to radiosensitize endothelial and breast cancer cells in vitro.
Hypothesis: Because radiation is an important modality in the treatment of prostate cancer, we tested the ability of the mTOR inhibitor RAD001 (everolimus) to enhance the cytotoxic effects of radiation on two prostate cancer cell lines, PC-3 and DU145.
Results: We found that both cell lines became more vulnerable to irradiation after treatment with RAD001, with the PTEN deficient PC-3 cell line showing the greater sensitivity. This increased susceptibility to radiation is primarily driven by induction of autophagic cell death. Furthermore, we demonstrate that blocking apoptosis with caspase inhibition and Bax/Bak siRNA in these cell lines enhances radiation-induced mortality in an autophagic dependent process.
Conclusion: Together, these data highlight the emerging importance of mTOR as a molecular target for therapeutic intervention, and lend support to the idea that non-apoptotic modes of cell death may play a crucial role in improving tumor cell kill.

478 POSTER

Gefitinib reverses TRAIL resistance in human bladder cancer cell lines via inhibition of AKT-mediated XIAP expression

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Background: Inhibitors of the epidermal growth factor receptor (EGFR) display activity in subsets of solid tumors, but identifying responsive tumors prospectively has been elusive, and it is not clear how to best exploit the biological effects of EGFR inhibitors.
Materials and Methods: We measured DNA synthesis by 3H-thymidine incorporation and DNA fragmentation associated with apoptosis by propidium iodide staining and FACS analysis. We quantified the expression of various target proteins by immunoblotting. We knocked down expression of AKT or XIAP by transient transfection with commercially available siRNA constructs. We studied the effects of therapy on the growth of orthotopic 253J B-V xenografts in nude mice.
Results: The EGFR inhibitor gefitinib (ZD1839, Iressa) blocked cell proliferation at relevant concentrations in 7/18 human bladder cancer cell lines. Sensitivity to gefitinib was loosely associated with expression of E-cadherin and lack of expression of vimentin characteristic of tumor cells that have not undergone the epithelial-to-mesenchymal transition (EMT). The drug had modest effects on DNA fragmentation and also failed to promote apoptosis induced by conventional chemotherapeutic agents (gemcitabine and paclitaxel). However, it did interact with recombinant human tumor necrosis factor related apoptosis-inducing ligand (TRAIL) to induce high levels of apoptosis in gefitinib-sensitive but not gefitinib–resistant lines. The molecular mechanisms involved downregulation of active AKT and XIAP expression and were mimicked by chemical inhibitors of the PI3 kinase/AKT but not of the MEK/ERK pathway. Furthermore, direct siRNA-mediated knockdown of AKT resulted in downregulation of XIAP and TRAIL sensitization, and knockdown of XIAP itself was sufficient to reverse TRAIL resistance. The effects of gefitinib plus TRAIL on the growth of TRAIL-resistant orthotopic 253J B-V xenografts will be presented.
Conclusions: Our results demonstrate that EGFR pathway activation limits TRAIL-induced apoptosis via an AKT- and XIAP-dependent mechanism in EGFR-dependent human bladder cancer cells. The data provide the conceptual framework for a further evaluation of the combination in relevant preclinical models and clinical trials in patients.

479 POSTER

ZIO-101: a new organic arsenic in advanced cancers

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Background: Arsenics are potent anti-cancer drugs. Organic arsenics are much less toxic than inorganic arsenics (like arsenic trioxide [As_2O_3]). ZIO-101 (S-dimethylarsino-glutathione; Figure), a new organic arsenic, is active against diverse cancers in experimental models and has a LD50 about 50-fold higher than As_2O_3. ZIO-101 is 5–10-fold more efficient in entering cancer cells than As_2O_3. Finally, ZIO-101 more specifically affects the pro-apoptotic signaling pathway than does As_2O_3. These features result in more damage to mitochondria and more cell-killing with ZIO-101 than with As_2O_3.
Methods: Combined data from 3 phase-1 ongoing studies evaluating safety, activity and pharmacokinetics of ZIO-101 in subjects with advanced cancers failing many prior therapies. Starting dose was 78 mg/me2/d IV for 5 d every mo with 20−40% dose increases.
Results: 49 subjects were treated including 29 with diverse advanced solid cancers and 20 with blood and bone marrow cancers. Detailed data are available on 33; data in 16 more will be presented. Median age is 61 y (43−85 y); 16 were male. The maximum administered dose (MAD) was 595 mg/me2/d, the estimated maximum tolerated dose (MTD), 500 mg/me2/d and the dose limiting toxicity (DLT), transient confusion and ataxia. Clinical benefit was reported in 10 subjects (30%) including acute myelogenous leukemia (AML) and solid cancers (colorectal, kidney, head and neck and pancreas cancers). 3 subjects with AML had substantial deceases in blood leukemia cells, and 1 subject had a reduced RBC transfusion need. 5 subjects with solid cancers had stable disease for 3+ to 7+ mo and 1 subject had a mixed response. Therapy with ZIO-101 at the MTD was safe: fatigue was the only toxicity $\geqslant$ grade-2 occurring in $\geqslant$ 25% of subjects. Clinically-important QTc-prolongation, a limitation of As_2O_3, did not occur. Pharmacokinetic (PK) studies at 214 mg/me2/d: tmax = 1 h (no SD), Cmax = 685 μg/L (SD ±130 μg/mL), t1/2 = 13.9 h (SD ±0.3 h) and AUC0-∞ = 14.9 mg h/L (SD ±2.6 mg h/L).
Conclusions: Clinical and PK data show ZIO-101 is safe at doses resulting in blood levels with substantial anti-cancer activity in experimental models.