extremely low concentrations, while displaying an outstanding selectivity over normal cells. Our results suggest that our potent Smac mimetics warrant extensive evaluation as a new class of anticancer agents for the treatment of human cancer by overcoming apoptosis resistance of cancer cells.

385 POSTER

Targeting p53-independent apoptosis in refractory breast cancers

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"Triple-negative" or "basal-like" breast cancers represent a tumor subtype that express neither estrogen/progesterone receptors nor Her2 protein and have a relatively poor prognosis despite conventional therapies. To date little progress has been made in identifying specific molecular pathways associated with these refractory cancers that may be effectively targeted for therapeutic intervention. Our previous work demonstrated that p63, a member of p53 gene family, is upregulated in certain epithelial tumors and is required to promote tumor cell survival through its ability to bind and inhibit the pro-apoptotic activity of the related family member p73. The importance of p63 in normal mammary development implies that dysregulation of p63 might also contribute to breast cancer pathogenesis. Here, we demonstrate that p63 and p73 are exclusively expressed in a subset of primary triple-negative breast carcinomas that exhibit frequent mutational inactivation of p53. Consistent with these findings, we find that p63 and p73 mRNA and protein are also co-expressed in a subset of triple-negative breast cancer cell lines including MDA-MB-468 and HCC1937. To determine the functional role of p63 in this subtype of breast cancer, we tested the effect of endogenous p63 knockdown by lentiviral small hairpin RNA (shRNA) expression in HCC1937 and MDA-MB-468 cells. Inhibition of endogenous p63 in these cells induced the pro-apoptotic bcl-2 family members Puma and Noxa, followed by apoptosis. In contrast, no effect was observed using control shRNA constructs in these cells, nor was any effect observed following expression of p63-directed shRNAs in MCF-7 and Saos2 cells that do not express p63. The induction of Puma, Noxa and apoptosis in triple-negative breast cancer cells is highly dependent on p73 function, as inhibition of p73 by lentiviral shRNA in HCC1937 cells completely abrogated apoptosis following knockdown of p63. These results suggest that p63 promotes survival of breast cancer cells by inhibiting the pro-apoptotic activity of p73. Consistent with these findings, we demonstrate that p63 directly interacts with endogenous p73 and that expression of p63 blocks p73-dependent transcription in a dose dependent manner. Together these findings demonstrate p63 and p73 mediate an essential and tumor-specific survival pathway in triple-negative breast cancers. Therefore, targeting p63 and/or p73 may represent an attractive therapeutic strategy against these refractory tumors.

386 POSTER

Knockdown of PRL levels by siRNA influences response to etoposide in pancreatic cancer cells

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Background: We previously identified several genes involved in stress response as differentially regulated in pancreatic cancer cells following PRL-1 or PRL-2 silencing with siRNA (Proc Am Assoc Cancer Res 2005; 46: 5508). We sought to further investigate the role that PRL phosphatases might play in regulating stress response by treating cells in combination with PRL targeting siRNAs and chemotherapeutic agents as inducers of stress.

Material and Methods: PRL or non-targeting siRNA treated MIA PaCa-2 and PANC-1 cells were treated with the chemotherapeutic agents etoposide and bortezomib. Cell cycle profiles as well as Caspase-3 activity were then evaluated to determine the effect of PRL knockdown.

Results: Etoposide treated MIA PaCa-2 (and PANC1 to a lesser degree) cells with PRL-1 and/or PRL-2 knockdown were morphologically distinct from their non-targeting siRNA control counterparts. Cell cycle analysis confirmed that cell cycle distribution was significantly altered in the etoposide treated cells with PRL knockdown compared to the control siRNA treated cells. However, when cells were treated with the proteasomal inhibitor bortezomib, significant differences in morphology or cell cycle distribution were not observed.

Conclusions: Our results indicate that PRL-1 and PRL-2 knockdown might affect how pancreatic cancer cells respond to certain chemotherapeutic agents. We are currently evaluating cell cycle distribution and induction of apoptosis in these cells lines using other chemotherapeutic agents with known activity against pancreatic cancer (gemcitabine, erlotinib and 5-fluorouracil). This should aid in identifying chemotherapeutic agents, which might successfully be used in combination with PRL inhibitors.

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387 POSTER

A phase Ib trial of ARQ 501, a checkpoint pathway activator, in combination with docetaxel in patients with advanced solid tumors

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ARQ 501 is a DNA damage checkpoint pathway activator whose effect is to induce selective cell death in cancer cells through E2F1 pathway, independent of cell's p53 status. In preclinical models, ARQ 501 demonstrates potent anticancer activity with a high therapeutic index, an effect greatly enhanced by the addition of a variety of cytotoxic agents, including taxanes. Therefore, a phase Ib dose escalation study began in patients with advanced solid tumors to determine the maximum tolerated dose (MTD), pharmacokinetics, and preliminary antitumor activity of ARQ 501 in combination with docetaxel. In all arms of the study, docetaxel (50, 75 or 100 mg/m^2) was given every 21 days. Within this 21 day cycle, several schedules of ARQ 501 administration were investigated. These included: (1) ARQ 501 given days 1–5 and docetaxel administration on day 3 of each cycle; (2) ARQ 501 infused over one or three hours followed by an infusion of docetaxel on day 1 of each cycle. In this schedule, additional weekly infusions of ARQ 501 were added, as tolerated, to achieve a final schedule of weekly ARQ 501. With the first schedule, MTD was reached at a dose of 120 mg/m^2/day (600 mg/m^2 per 21 day cycle). With weekly ARQ 501 administration, maximum doses administered to date are 390 mg/m^2 for the one hour infusion and 550 mg/m^2 for the three hour infusion times. In all cases, anemia has been the major adverse event, which has limited dose escalation. Evidence to date suggests that this is due to hemolysis of circulating mature red cells as a result of oxidative stress (unrelated to the checkpoint activation). Other adverse events have been generally mild and include neutropenia, hyperglycemia, hypomagnesemia, fatigue, pyrexia, naseau, and hyperbilirubinaemia.

As of May 30, 2006 38 patients have been enrolled with monitored data available for 31 patients. Nine of 11 patients enrolled in schedule 1 were evaluable for efficacy. Of these, 5 patients achieved a best response of stable disease (SD) or better (9.4 to 23.6 weeks). Of note, 2/4 ovarian cancer patients, who had failed prior therapies with platinum and taxanes, achieved a partial response (PR) per decrease in CA 125 levels. One also showed a 42% reduction per RECIST, but no confirmatory scan was performed. To date, 27 patients have been treated on schedule 2. Twelve patients remain on study, and 18 patients are evaluable for efficacy. Fourteen patients achieved a best response of SD or better (6 to 32 weeks). Three patients achieved a PR (unconfirmed), including a 47.6% regression at week 12 in a patient with head and neck cancer, a 51% regression at week 6 in a patient with pancreatic adenocarcinoma, and a 33.9% regression in a patient with ovarian cancer.

These data suggest that the combination of the checkpoint pathway activator ARQ 501 with a taxane is well tolerated and has encouraging signs of anti-tumor activity, particularly in ovarian cancer. Phase II investigation in this condition is warranted.

388 POSTER

Expression profile of histone deacetylases and histone H4 acetylation in selected B- and T-cell lymphomas

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Background: Histone deacetylase inhibitors (HDACi) are in clinical trials for a variety of malignant diseases. Interestingly, in hematological malignancies the HDACi SAHA and depsipeptide have shown remarkable efficacy in cutaneous T-cell lymphoma (CTCL) with relatively fewer responses in diffuse large B-cell lymphoma (DLBCL). The reason for this suggested class effect of HDACi in B- and T-cell malignancies is unknown. In a study on breast cancer, low levels of histone H4 acetylation prior to treatment with an HDACi were predictive of response. In the present investigation, we have examined the expression of selected HDACs and the acetylation of histone H4 in CTCL and DLBCL.

Material and Methods: CTCL (n = 75) and DLBCL (n = 31) samples were examined for expression of HDAC1, HDAC2, HDAC6, and acetylated H4 by immunohistochemistry. Stained samples were grouped in three expression categories (negative/low, moderate, high) based on the proportion of positive cells and staining intensity in each sample. Comparisons were done using Chi-square tests with exact probabilities.

Results: Both malignancies showed high HDAC1 expression ranging from 38.7% in DLBCL to 59.2% in CTCL (P = 0.10). Almost all samples (97%) showed moderate or high level of HDAC2 expression. High HDAC2 expression ranged from 31.2% in CTCL to 60.6% in DLBCL (P < 0.0001). In total, 21 CTCL showed high HDAC2 expression. These included 12 of 17 cases with aggressive histology or phenotype. HDAC6 showed low to moderate level of expression in both lymphomas. Low level of HDAC6 was observed in 29.4% of DLBCL and 30.7% of CTCL (P = 0.89). High level of acetylated H4 was more common in DLBCL (35.3%) compared to CTCL (14.3%) (P = 0.02). Furthermore, high H4 acetylation seemed to be more common in DLBCL belonging to the activated B-cell like (ABC) category (42.1%, 8 of 19) than in the less aggressive germinal center B-cell (GCB) type (10%, 1 of 10) (P = 0.12).

Conclusion: The prevalence of HDAC2 expression in both malignancies, and particularly in the more aggressive phenotypes of CTCL, suggest a possible involvement of HDAC2 in the development of malignant lymphomas. The low level of H4 acetylation in CTCL might be encouraging as a possible biomarker for HDACi response. These observations should however be validated in prospective studies.

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Hypermethylation induced SPARC, TIMP-3 and PENK down-regulation in endometrial cancer

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Background: Endometrial cancer remains the leading cause of gynecological cancer. This study was to investigate the genes down-regulated in the endometrial cancer.

Methods: Initially, we performed a genome-wide analysis of gene expression in a set of 55 sporadic endometrioid endometrial adenocarcinomas and compare this to 29 normal endometrium controls using microdissection and high density oligonucleotide microarray. To identify signaling pathways that are associated with cervical tumorigenesis, microarray expression data were imported into a PathwayAssist software. We further measured methylation of the three genes using methylation-specific polymerase chain reaction (MSP-PCR) in a total of 76 endometrioid endometrial cancers

Results: We obtained a dominant signaling pathway in which 25 genes were coordinately regulated in endometrial cancer. These genes encode for proteins that are part of a signaling pathway associated with cell cycle progression and invasion. Of the 25 genes, SPARC (5q31.3−q32), TIMP-3 (22q12.1−12.3) and PENK (8q23−q24) were down-regulated in cancer for 2.9-fold, 3.48-fold and 3.12-fold, respectively, when compared to normal. The results showed hypermethylation of SPARC, TIMP-3 and PENK in 99%, 25% and 95% of these cancers, respectively. Furthermore, we found the hypermethylation of TIMP-3 was correlated to clinical stage of the tumors and total survival of the patients.

Conclusions: The results obtained from this study indicate that hypermethylation induced down-regulation of SPARC, TIMP-3 and PENK might be related to the development and progression of endometrioid endometrial cancer. Demethylation and reactivation of the three genes may be as an adjunct therapy for the endometrial cancer.

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390 POSTER

Secreted frizzled-related protein 4 inhibits proliferation and metastatic potential in prostate cancer

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Background: Activation of the Wnt signaling pathway is implicated in aberrant cellular proliferation in a variety of cancers. In prostate cancer, Wnt 3A signaling promotes cell growth through an androgen-dependent interaction with downstream beta-catenin signaling. Secreted Frizzled-Related Protein 4 (sFRP4) inhibits Wnt signaling by binding and sequestering the Wnt ligand. Our group has already demonstrated that increased expression of membranous sFRP4 predicts for a good prognosis in localized prostate cancer. Thus the aim of this project was to investigate the phenotype of sFRP4 overexpression in androgen-dependent and androgen-independent prostate cancer models.

Material and Methods: sFRP4-overexpressing androgen-dependent (LNCaP) and androgen-independent (PC3) prostate cancer models were established *in vitro*. Changes in proliferation and metastatic potential were assessed using Cell Titer 96® system, soft agar assays and matrigel invasion chambers. Immunofluorescence and immunohistochemistry were used to identify changes in adhesion molecules *in vitro* and *in vivo* respectively.

Results: sFRP4 overexpression in both cell line models results in a morphologic change to a more epithelioid cell type with increased membranous beta-catenin and cadherins (E-cadherin in LNCaP, N-cadherin in PC3) resulting in more adhesions between cells. Functionally, sFRP4 overexpression is associated with a decreased rate of proliferation (p = 0.0002) and decreased anchorage-independent growth in both systems (p < 0.0001) and decreased invasiveness of PC3 cells (p < 0.0001). Furthermore, in human localized prostate cancer (n = 224) increased membranous sFRP4 expression is associated with increased membranous beta-catenin expression (p = 0.02).

Conclusions: These data suggest that sFRP4 is an inhibitor of prostate cancer growth and metastasis independent of hormonal status with correlative evidence in human disease. Consequently, sFRP4 is a potential new therapeutic target for androgen-independent prostate cancer.

391 POSTER

The significance of Pyk2 in hepatocellular carcinoma invasiveness

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Background and Objective: Our previous study showed that proline rich tyrosine kinase (Pyk2) is over-expressed in tumor tissues as compared to their adjacent non-tumor tissue. Statistical analysis suggests that this over-expression is significantly correlated with tumor growth and venous invasion. Over-expression of Pyk2 is also significantly correlated with shorter overall and disease-free survival. However, the precise mechanism of Pyk2 on tumor invasiveness is still unclear due to the limited reports. In the current study, we aim to investigate the role of Pyk2 on the invasiveness of hepatocellular carcinoma cells by both *in vitro* functional study and *in vivo* animal models.

Materials and Methods: In the *in vitro* study, plasmids containing the full length or dominant negative form of Pyk2 was transfected into HCC cell line (PLC). After selection with antibiotics (G418), stable clones with the expression of full length or dominant negative form of Pyk2 was isolated. The invasiveness was compared according to their ability to adhere to the extracellular matrix, colony formation assay and wound healing assay. The mechanism of the Pyk2 signaling was investigated by western blotting and immunoprecipipation assay.

In vivo tumor models were done in athymic nude mice. Tumors produced from the different transfectants were implantanted into the liver of the mice. After 48 days the mice were sacrificed and tissue samples were collected. Tumor growth pattern including invasiveness was examined by H&E staining. Tumor cell proliferation (Ki67) and apoptosis (TUNEL) were compared among the groups of mice with liver tumor from different transfectants.

Results: The full length Pyk2 transfectant possessed the highest cell motility as compared to the vector control and C-terminal transfectants by wound healing assay. Pyk2 full length transfectant also presented significantly stronger adhesiveness towards collagen I, fibronectin and laminin by adhesion assay. It promoted the anchorage-independent growth as well as the anchorage dependent growth by the soft agar assay and colony formation assay. Western blotting and co-precipipation assays indicated that Pyk2 forms a signaling complex with c-Src. Phosphorylation of c-Src, MEK and ERK 1/2 were up-regulated in full length Pyk2 transfectants as compared to the vector control and C-terminal transfectants.

The tumors from the full length Pyk2 transfectants got the highest incidence of Ki67 positive staining tumor cells and least apoptotic cells as compared to empty vector control and C-terminal transfectants.

Conclusion: Over-expression of Pyk2 may contribute to an invasive phenotype of HCC.

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Peroxiredoxin II protects cancer cells in a way of proteasome inhibition

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Background: Peroxiredoxin II, a cytosolic isotype of human 2-Cys peroxiredoxin(Prx), can behave either as a peroxidase or as a molecular