both c-myc and cyclin D1. Together, these data show that prostate cancers consistently have elevated free eIF-4E – a consequence of elevated eIF-4E expression and/ or reduced 4EBP1 expression/ function. As such, eIF-4E represents a novel target for prostate cancer therapy. Indeed, treatment of prostate cancer cells with siRNAs or ASOs specific for eIF-4E dramatically reduces eIF-4E expression and suppresses proliferation in concert with markedly diminished expression of cyclin D1 protein. Moreover, systemic treatment of CaP xenografts with eIF4E-specific ASOs or siRNAs suppresses xenograft tumor growth. These data firmly implicate eIF-4E in the development and progression of both human and experimental prostatic adenocarcinomas and indicate the therapeutic utility of targeting eIF4E.

46 POSTER

Biological testing of the tyrphostin tyrosine kinase inhibitor Adaphostin (NSC 680410) as a vascular endothelial growth factor secretion inhibitor in human and murine tumor cells and tumor-activated bone marrow stromal cells

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Background: Vascular endothelial growth factor (VEGF) is a proinflammatory and proangiogenic factor whose production by host and tumor cells contributes to cancer progression and metastasis. Drugs decreasing endogenous VEGF secretion may contribute to cancer prevention and inhibition. However, production of VEGF by host and tumor cells has redundant and heterogeneous mechanisms that hamper discovery of the most efficient inhibitors and their targeted mechanisms. Preliminary studies on the tyrphostin tyrosine kinase inhibitor adaphostin (NSC 680410) suggest its potential ability to inhibit VEGF secretion in human T-lymphoblastic leukemia cell lines. Herein, the inhibitory activity of Adaphostin on VEGF secretion was tested in different solid tumor cell lines that secrete VEGF under basal and cytokine-induced conditions and in bone marrow stromal cells (BMSCs) activated by tumor-derived soluble factors.
Materials and Methods: Human (MDA-MB-435 breast carcinoma, PC3 prostate carcinoma, HT-29 colon carcinoma, SK-HEP-1 hepatoma) and a murine tumor cell line (B16 melanoma) characterized by the ability to secrete VEGF were used. Primary cultured BMSCs were obtained from human and murine organ samples. Adaphostin was added to cultured tumor and host cells at a 0.1-to-10 μM concentration range for 24 and 48 hours. Measurement of human and murine VEGF secretion in control and Adaphostin-treated cells was performed by ELISA. Cytotoxic and proapoptotic effects of Adaphostin were assessed in cultured cells by the MTT assay and apoptotic cell markers.
Results: Following 12 hour-culture under basal conditions with 1 μM Adaphostin, the secretion of VEGF from human cancer cells decreased by 40-to-50% in the absence of any evidence of toxicity or proapoptotic signs. Not statistically significant alterations of VEGF secretion were observed in normal human BMSCs cultured under basal conditions with 1 and 5 μM adaphostin for up to 48 hours. Addition of conditioned medium (CM) from MDA-MB-435 breast carcinoma cells, PC3 prostate carcinoma cells, and HT-29 colon carcinoma cells to human BMSCs for 1 hour increased VEGF secretion by 5-folds. Addition of Adaphostin to tumor-activated BMSCs decreased tumor-induced VEGF production by 50-to-100%. Two BMSC-derived cytokines (IL-18 and TNFalpha), given as recombinant murine proteins at 10 ng/ml for 4 hours, also significantly increased (by 50%) VEGF production from cultured B16 melanoma cells. Addition of 1 μM Adaphostin to B16 melanoma cells 30 min prior to cytokine stimulation completely abrogated cytokine-dependent VEGF secretion. Moreover, the VEGF concentration was also significantly increased by 3-fold in the 6 hour-supernatant of primary cultured murine BMSCs given B16 melanoma-CM for 1 hour. Tumor-induced VEGF was abolished by Adaphostin when added to BMSCs 30 min prior to tumor-CM.
Conclusions: Current results demonstrate the ability of Adaphostin to inhibit VEGF secretion by solid tumor cells and tumor-activated BMSCs. Combination of this tyrosine kinase inhibitor with other antiangiogenic drugs are under study to test possible synergistic drug regimens. This work was supported in part by NCI Contract NO1-CO-12400.

47 POSTER

CRx-026: discovery and clinical development of a syncretic anti-mitotic agent with significant anti-cancer activity

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Background: A 'syncretic' drug comprises multiple biologically active compounds that interact synergistically to provide an optimal therapeutic effect with minimal side effects. CRx-026, is a novel syncretic anti-cancer agent, comprising chlorpromazine and pentamidine, that achieves its synergistic effects through a dual action in mitosis, selectively inhibiting hsEg5/KSP, a mitotic kinesin essential for centrosome separation, and PRL phosphatases, which play an important role in regulating mitotic progression and proper chromosome separation. By modulating the activity of these two targets in a concerted fashion, CRx-026 achieves a synergistic effect greater than either component agent achieves on its own.
Methods &Results: *In vitro*, CRx-026 exerts anti-proliferative and cytotoxic effects across a spectrum of human cancer cell lines. Additionally, we have tested CRx-026 in pair-wise combination with 48 anti-cancer agents representing all major mechanistic classes. CRx-026 synergized with several chemotherapeutic classes – especially with taxanes and vinca alkaloids. *In vivo*, CRx-026 elicits substantial anti-tumor activity in the xenograft tumors, A549, HCT116 and HT29. Results demonstrate that CRx-026, but not its components, had significant anti-tumor activity which was comparable or superior to paclitaxel or 5-FU. Moreover, CRx-026 has a more favorable side effect profile than paclitaxel and 5-FU, with improved survival and overall lower toxicity effects. Finally, we have confirmed several *in vitro* synergies in xenografts where CRx-026 was synergistic both with paclitaxel and with vinorelbine, enhancing anti-tumor activity without increasing toxicity in xenografts.
Conclusions: CRx-026 is a novel anti-cancer agent with obvious anti-cancer activity in both *in vitro* and *in vivo*. The two components of CRx-026 are now being integrated into a novel pharmaceutical composition, and we are currently advancing this new anti-cancer drug through clinical development. Phase I studies are currently underway in a standard dose escalation schema for patients with solid tumors to determine safety and pharmacokinetics. Multiple treatment regimens are currently being explored to help optimize future clinical development. Data are expected in late 2004.

48 POSTER

In vivo selection of vascular extravasating phage: novel means to isolate prostate tumor-homing peptides

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Background: New tumor targeting agents are required to advance cancer diagnosis and therapy. Bacteriophage (phage) peptide display technology is an emerging approach to identify and improve peptides as tumor-targeting agents. However, only a few of the peptides identified by *in vitro* phage selections have been clinically useful. This failure may be attributed to poor peptide solubility, affinity, specificity, and stability *in vivo*. It is our hypothesis that the *in vivo* application of phage display would have the advantage over *in vitro* selection strategies in that one can "select" in the environment of the whole animal, peptides that can extravastate tumor vasculature and selectively and stably bind to a given tumor. To this end we developed *in vivo* schemes to select for phage that target prostate tumors in mice.
Material and Methods: First, the biodistribution and pharmacokinetics of a fUSE 5 phage library (15 amino acid peptides displayed on cpIII) in scid mice was determined. Next, the library was cleared through non-tumor bearing mice in an effort to enrich for extravasating phage. The pre-cleared library was injected into human PC-3 prostate carcinoma-bearing scid mice. After one hour, tumors were removed, phage were eluted with acidic or neutral buffers (with 0.5% CHAPs detergent) and amplified. After four rounds of selection phage clones were analyzed. Peptides displayed on phage with the highest titer for the tumors or PC-3 cultured cells were chemically synthesized and their binding properties analyzed.
Results: Biodistribution studies demonstrated that fUSE5 phage half-life in the blood was ~18 min with optimal tumor/tissue localization of ~1 h. Only those phage extracted from the prostate tumors with detergent exhibited specific binding to PC-3 carcinoma cells. The phage and synthesized peptides bound PC-3 cells and PC-3 xenografted tumor tissue but did not bind appreciably to human PC-3M (metastatic form of PC-3), OVCAR3 ovarian, MDA-435 breast, or LS174T colon carcinoma cell lines. The peptides exhibited a K_d of 100pM for binding to live PC-3 cells. While the peptide target(s) remain to be identified, they may represent new classes of prostate tumor biomarkers. Future work will employ the peptides as an affinity ligand for target identification and, once radiolabeled, as potential cancer imaging or therapeutic agents.

Conclusions: We employed *in vivo* phage display in human prostate tumor-bearing mice to identify peptides that extravasate the vasculature and specifically target the tumor cells, not just the surrounding vasculature. This finding is significant because previous *in vivo* selections preferentially retrieved phage that bound vascular components (Arap, Nat Med 8:121). Thus, our studies may facilitate the development of a range of cancer cell-surface or internalizing molecules not previously realized.

49 POSTER

DNA aptamers that recognize the MUC1 tumour marker

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Mucins are high molecular weight glycoproteins that provide a protective layer on epithelial surfaces and are involved in cell-cell interactions, signalling, and metastasis. The membrane-bound MUC1 mucin is expressed in normal mucosas and the aberrant expression of its under-glycosylated forms has been reported in various carcinomas of the epithelium. MUC1 is a human tumour antigen expressed in breast, pancreatic and ovarian cancers. Agents able to bind tightly and specifically to the surface of malignant cells would greatly benefit cancer diagnosis and treatment, whereas the targeting of cell surface receptors would have significant implications on inflammation and immunity. While antibodies have the ability to specifically recognise some tumour cell markers, their large size and own immunogenicity markedly limit their pharmacological value. The development of nuclease resistant DNA molecules, termed *aptamers*, has provided a new alternative to antibodies. Using the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) methodology, one can generate vast libraries of oligonucleotide ligands (DNA, RNA, or unnatural products), screened rapidly for specific sequences that have appropriate binding affinities and specificities to the clinically relevant marker.
We have identified synthetic DNA oligonucleotide aptamers that bind to the MUC1 tumour marker with low nanomolar affinity via the 20 tandem repeat sequence of the MUC1. These specific aptamers were selected from an initial library that contained a degenerate region of 25 bases to result in 4^{25} random-sequence DNA molecules. Ten rounds of in vitro selection and amplification were performed, to confer affinity maturation of aptamers for MUC1. Selected aptamers were cloned, sequenced and found to be sharing some unique consensus sequences. The affinity of each aptamer for MUC1 was studied by qualitative and quantitated methods such as ELISA, BIAcore, and EMSA. Affinities on the nanomolar range have been identified and confirmed.
Efforts in our laboratory are now focusing on optimization of their delivery, functionality and structural properties. Fluorescent labelled aptamers have been successfully used in the identification of MUC1 expressing tumour cell lines such as the MCF7 breast tumour cell line and can be used in future diagnostic assays, whereas radiolabelled aptamers can be used clinically to enable imaging and therapy of the tumour marker bearing cancer cells.

50 POSTER

Inhibition of histone deacetylase 2 increases apoptosis and P21 expression

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Background: Histone deacetylases (HDACs)1 and 2 share a high degree of homology and are known to co-exist within the same protein complexes containing other transcriptional co-repressors. Despite their close association, studies have shown that each possesses unique functions, which cannot be compensated by the other. This study describes the regulation of HDACs1 and 2 in colorectal and cervical cancers and the function of HDAC2 in apoptosis.
Materials and Methods: A combination of quantitative RT-PCR and tissue array was used to determine the expression of HDAC1 and HDAC2 in colorectal cancer and matched normal mucosal samples. Immunohistochemistry was used to determine the expression of HDACs1 and 2 in cervical cancers and dysplasias. HDAC2 expression in HeLa cells were knocked-down by specific siRNAs designed against HDAC2 (Dharmacon Inc). The efficacy of knock-down was confirmed by Western blot analysis. Apoptosis was assayed by annexin V-FLUOS staining and flow cytometry. P21 expression was determined by Western blot analysis using a specific antibody (Snata Cruz Biotechnologies).
Results: Both HDACs1 and 2 are upregulated at the mRNA (n=16) and protein (n=45) levels in colorectal cancer. The upregulation of HDAC2 was more robust and occurred more frequently in the samples. It also occurred early in the carcinogenic process, with 4 of the 5 polyps showing upregulation of HDAC2 compared to normal mucosa. In cervical carcinoma (n=9), the expression of both HDACs1 and 2 was correlated with the severity of cervical dysplasias and invasive carcinomas of the cervix. However, HDAC2 expression showed a clear demarcation of higher intensity staining at the transition region of dysplasia. Further, more cells were stained for HDAC2 than HDAC1 in cervical dysplasia. The functional significance of HDAC2 upregulation was determined by knocking down the expression of HDAC2 with HDAC2-specific siRNA. Cells displayed an increased number of cellular extensions reminiscent of cell differentiation after HDAC2 knockdown. There was also an increase in apoptosis, associated with an increase in P21 expression.
Conclusion: The results suggest that histone deacetylases, especially HDAC2, are important enzymes involved in the early events of carcinogenesis, making them candidate markers for tumor progression and targets for cancer therapy.

51 POSTER

HSV-tk gene transduction enhances proliferation rate and COX-2 expression in rat gliosarcoma cells

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Background: Transduction of tumor cells with the Herpes Simplex Virus thymidine kinase (HSV-tk) gene and consequent treatment with ganciclovir is widely used for suicide gene therapy of brain tumors. Recently we observed that HSV-tk gene transduction of rat gliosarcoma (9L) cells enhances the expression of cyclooxygenase-2 (COX-2) and the release of prostaglandin E_2 (PGE_2). It is well established that COX-2 overexpression with increased production of COX-2-derived prostaglandins are associated with multiple aspects of carcinogenesis, including the control of cell proliferation and increased resistance to apoptosis and chemotherapy.
Aims: In the current work we aimed to: a) determine the effect of HSV-tk gene transduction on proliferation rate of 9L cells; b) evaluate the effect of nimesulide (NIM), a selective COX-2 inhibitor, on proliferation rate of wild-type (9L) and HSV-tk transduced (9L/HSV-tk) 9L cells.
Results: Western blot analysis of COX-2 protein expression in 9L and 9L/HSV-tk cells showed that COX-2 is overexpressed in HSV-tk transduced cells, while wild-type cells did not express COX-2 at detectable levels. COX-2 overexpression in HSV-tk transduced cells was accompanied by increased release of PGE_2, assessed by radioummunoassay, into the culture medium ($2.5\pm$ 0.2 *vs* 102.3 ± 9.4 ng/10^6 cells for 9L and 9L/HSV-tk cells, respectively). In order to determine the effect of HSV-tk gene transduction on cell proliferation rate, 9L and 9L/HSV-tk cells were incubated for 120 hrs in 24-well culture plates and the number of attached cells was counted every 24 hrs. We found that proliferation rate of 9L/HSV-tk cells was 2 to 3-fold higher, compared to that of wild-type 9L cells. To evaluate whether increased release of PGE_2 accounts for the observed enhancement in proliferation rate in the 9L/HSV-tk cells, we investigated the effect of NIM on proliferation rate of both wild-type and HSV-tk transduced cells. Incubation of 9L and 9L/HSV-tk cells with NIM at a concentration which inhibits COX-2 activity, completely abolished PGE_2 release in both wild-type and HSV-tk transduced cells for as long as 96 hrs. However, at this concentration, NIM failed to affect proliferation rate of 9L and 9L/HSV-tk cells.
Conclusions: Taken together, we demonstrate herein that HSV-tk gene transduction enhances proliferation rate and COX-2 protein expression and activity in rat gliosarcoma cells. Additionally, the enhanced proliferation rate of HSV-tk transduced cells appears to be independent on prostaglandins overproduction since treatment with the selective COX-2 inhibitor failed to abolish the enhancement of the proliferation rate.

52 POSTER

Attenuated immunogenicity and toxicity of PEGylated recombinant methioninase (PEG-rMETase) in primates

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Background: Methionine depletion by recombinant methioninase (rMETase) has been demonstrated to be an effective antitumor regimen in tumor-bearing mouse models. However, the therapeutic potential of rMETase has been limited by its short plasma half-life and immunological effects, including high antibody production in mice and monkeys and anaphylactic reactions in monkeys.
Materials and Methods: In order to improve the therapeutic potential of rMETase, a PEG-rMETase conjugate has been developed by coupling the enzyme to methoxypolyethylene glycol succinimidyl glutarate (MEGC-PEG-5000). In this study, we evaluated the pharmacokinetics, antigenicity and