1.7+0.8g; 20 mg/kg Phor14-beta3, 1.6+0.9g]. Tumor weights in an additional group treated with a mixture of unconjugated Phor14 plus beta 3 did not differ from saline treated controls [2.9+1.4g]. Although the tumor weights did not show differences among CO or n-3FA diet, histological evaluation of tumors showed significant differences: CPE (cytopathological evaluation) values of 5.5+0.5 in saline controls under CO and n-3 FAs diets, 1.3+0.8 in CO vs 0.4+0.3 in n-3 FAs at 20 mg/kg Phor14-beta3 ($p<0.03$) and 1+0.5 in CO vs 0.5+0.2 (10 mg/kg Phor14-beta3) ($p<0.04$) in n-3 FAs fed mice. We conclude that n-3 FAs diet improves the treatment efficacy of Phor14-beta3 by lowering the effective dose and reducing tumor associated cachexia thus improving the overall appearance of the animals.

355

Probing the role of JNK in transformed cell proliferation and survival

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The c-Jun N-terminal kinase (JNK) family of mitogen activated protein kinases (MAPKs) is implicated primarily in stress and immune response pathways, and in some cells contributes to programmed cell death (apoptosis). The role of JNKs in transformed cells is complex. The best-characterized target of JNK is the transcription factor and proto-oncogene c-Jun. Aberrant expression of c-Jun contributes to proliferative and morphologic transformation in model cell systems. There is evidence that c-Jun and JNKs contribute to tumorigenesis *in vivo*. For example, ablation of JNK2 renders experimental animals resistant to skin carcinogenesis1. We have utilized small molecule and gene-based approaches to clarify the role of JNKs in the genesis and potential treatment of cancer. We have developed several chemically diverse classes of potent and selective inhibitors of the JNK enzymes 2. These compounds inhibit the proliferation of a wide range of transformed cells (IC_{50} range 0.3 -5μM) along with a decrease in phospho-c-Jun protein levels as measured by immunoblot analysis. Cell cycle analysis of treated cells reveals a block at the G2/M phase, followed by apoptotic cell death. Gene chip analysis of compound-treated cells demonstrates the involvement of several cyclin genes in JNK-mediated cell cycle progression, as well as other genes that may be involved in the transformed phenotype. The compounds also block the migration and proliferation of human microvascular endothelial cells, a phenotype associated with angiogenesis. Experiments with genetic reagents specifically blocking JNK activity confirm the role of JNK in the phenotypes observed using small molecule JNK inhibitors. Solid tumor cells treated with camptothecin or paclitaxel display a robust induction of JNK activity. Surprisingly, simultaneous inhibition of JNK by either chemical or genetic approaches strongly enhances the ability of diverse classes of chemotherapeutic agents to kill tumor cells. This suggests that JNK plays a protective role in tumor cells treated with traditional chemotherapeutic drugs. In mouse tumor experiments, combining JNK inhibitors with cyclophosphamide has a synergistic effect in blocking tumor growth. Taken together, these results suggest that JNK inhibitors have promise as stand-alone therapy as well as in combination with well-established chemotherapeutic regimens for a variety of cancers.

356

Targeting a protein tyrosine phosphatase, PRL-1, for the treatment of pancreatic cancer

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Pancreas cancer is the fourth leading cause of cancer death among adults in the United States and has the worst prognosis of any type of cancer. We used cDNA expression array analysis to identify new targets for pancreatic cancer drug development. Comparison of gene expression profiles from 9 pancreatic cancer cell lines and normal pancreas cells for over 5,000 genes showed frequent (5 out of the 9 cell lines) and significant overexpression (three-fold or higher) in 30 genes. One of these genes encodes the protein tyrosine phosphatase type IVA, member 1 (PRL-1). PRL-1 is an immediate-early gene in regenerating liver and is also expressed in mitogen-stimulated fibroblasts. The expression of PRL-1 is associated with cell proliferation and differentiation due to its ability to regulate the protein tyrosine phosphorylation and dephosphorylation of substrates that remain unknown. RT-PCR and Northern blotting confirmed overexpression of the PRL-1 gene in 9 pancreatic cancer cell lines compared to normal pancreas. To further validate PRL-1 as a molecular target, we used antisense oligonucleotides to inhibit the expression of PRL-1 in pancreatic cancer cells and analyzed the effects on cell proliferation and apoptosis. The human PRL-1 sequence (residues 1-173) was used as a probe to search a non-redundant database of sequences using PSI-BLAST. Top ranked sequences were the known structures of human SHP-2 tyrosine phosphatase (2SHP) and a family of C. elegans phosphatases. Analysis of PRL-1 sequence using 3-D structure prediction programs confirmed the similarity with 2SHP and several other tyrosine phosphatases including the lipid phosphatase domain of PTEN. The sequence identity and similarity between PRL-1 and 2SHP is 23% and 40% and that between the other human tyrosine phosphatases is 40% and 55% respectively. Using this structural information we constructed a homology model with the software INSIGHT II. The PRL-1 model indicated a conserved hydrophobic core, but a changed specificity pocket without any major distortion of the active site. Docking studies were performed utilizing two bis-(paraphosphonophenyl) methane, which occupied the active pocket with a low binding energy. The homology model shows the presence of a unique unoccupied cavity within the PRL-1 binding pocket, which will be explored using 3-D database searches and identified novel inhibitors will be tested for enzyme inhibition.

357

Combined expression of pTa and Notch3 in T cell leukemia identifies the requirement of preTCR for leukemogenesis

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Notch receptors are conserved regulators of cell fate and have been implicated in the regulation of T cell differentiation and lymphomagenesis. However, neither the generality of Notch involvement in leukaemia, nor the molecules with which Notch may interact have been clarified. Recently, we showed that transgenic mice expressing the constitutively active intracellular domain of Notch3 in thymocytes and T cells developed early and aggressive T cell neoplasias. Although primarily splenic, the tumors sustained features of immature thymocytes, including expression of pTalpha, a defining component of the pre T cell receptor, known to be a potent signalling complex provoking thymocyte survival, proliferation and activation. Thus, enforced expression of Notch3, which is ordinarily down-regulated as thymocytes mature, may sustain preTCR expression, causing dysregulated hyperplasia. This has been successfully tested in this paper, by the observation that deletion of pTalpha in Notch3 transgenic mice abrogates tumor development, indicating a crucial role for pTalpha in T cell leukemogenesis. Strikingly, parallel observations were made in humans, in that all T cell acute lymphoblastic leukaemias examined showed expression of Notch3 and of the Notch target gene HES-1, as well as of pTalpha a and b transcripts, whereas the expression of all these genes was dramatically reduced or absent in remission. Together, these results suggest that the combined expression of Notch3 and pTalpha sustains T cell leukemogenesis and may represent novel pathognomonic molecular features of human T-ALL.

358

Quadruplex formation in the c-MYC promoter inhibits protein binding and correlates with *in vivo* promoter activity

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Previously, we have determined that a G-quadruplex interactive compound, the cationic porphyrin TMPyP4, can cause down-regulation of the c-MYC proto-oncogene in tumor cell lines. Subsequently, we have found that a region of the c-MYC promoter, termed the NHE III1, is able to form two different intramolecular G-quadruplex structures *in vitro*; a chair- and basket-type. Through site-directed mutagenesis of the c-MYC promoter, we have provided evidence that the chair-type quadruplex is a biologically relevant structure *in vivo*. Here, we show that a specific protein, identity to be determined, is able to bind to a 27-base long oligomer corresponding to the NHE III1 only if the oligomer cannot form a G-quadruplex; when the oligomer is mutated such that the chair-type quadruplex can no longer form, the ability of this protein to bind is increased several fold. However, mutations to the basket-type quadruplex, or of bases not involved in formation of either quadruplex, have no effect on binding. This exactly correlates with c-MYC

promoter activity from constructs bearing these same mutations. We hypothesize that the ability of TMPyP4 to stabilize the chair-type quadruplex in the NHE III1 prohibits normal binding of this protein, and results in the decrease in c-MYC expression we have seen previously. A model is proposed, which explains how quadruplex formation occurs normally in the c-MYC promoter and regulates expression through the relative ability/inability of this protein to bind to the DNA.

359

Additive/Synergistic interaction between MKP-1 inhibitors and anti-cancer drugs in human non-small-cell lung cancer cell H292

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A group of novel MKP-1 inhibitors developed in MAXIA has been shown to suppress tumor growth both in *in vitro* and *in vivo* by activation of JNK and subsequent induction of apoptosis (see abstract by Allan Kaspar, et. al.). The JNK pathway has been reported to be targeted by several marketed anti-cancer drugs such as cisplatin (CDDP), paclitaxel and adriamycin for their apoptosis induction. Therefore, it is rationale to hypothesize that inhibition of MKP-1, a downregulator of JNK activity, may enhance the chemotherapeutic effects of those antineoplastic agents that depend on the JNK pathway. This study investigated the combination therapy of MAXIA's MKP-1 inhibitor, MX7091, with cisplatin, paclitaxol and adriamycin. Apoptosis induction and general cytotoxic activity were evaluated in the non-small-cell lung cancer (NSCLC) cell line H292 by the cell death ELISA assay and the 3-4,5-dimethylthiazol-2-yl-2,5-diphenyl-tetrazolium bromide (MTT) assay, respectively. Concurrent exposure of H292 cells overnight with MX7091 (1 uM) and cisplatin (10 uM) led to an 11 fold increase of apoptosis whereas MX7091 alone only induced six-fold increase of apoptosis and cisplatin (10 uM) alone did not significantly affect cell viability (1.4 fold). MTT assays revealed the similar observation in cell viability in both H292 cells and BxPC-3 cells (a pancreatic cancer cell line). Western blot analysis showed that JNK and c-Jun phosphorylation was synergistically enhanced by combined use of MX7091 and cisplatin as compared with each agent alone. These results suggest a synergistic interaction between MX7091 and cisplatin and are in agreement with a previous report that overexpression of MKP-1 can block the JNK activation and apoptosis by cisplatin. Paclitaxel (0.5 uM) or adriamycin (0.5 uM) alone induced apoptosis at 4.2 and 8.2 folds, respectively. Combination of MX7091 with paclitaxel or adriamycin resulted in 13.4 and 11.9 fold increase in cell apoptosis, respectively, indicating additive/synergistic interactions between MX7091 and those two drugs. More experiments are being performed to study the combination index (CI) of these drug combinations in different cancer cells. Animal studies are also being conducted to explore the *in vivo* synergism of the MKP-1 inhibitors and cisplatin and other anti-tumor agents.

360

New differentiation-involution inducing agents for the treatment of breast cancer

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Breast cancer displays many properties that are exhibited during the developmental cycle of the mammary gland. The mammary gland comprises stromal and epithelial cells that communicate through an extracellular matrix (ECM) to control the function of the gland. The stromal cells control the ECM composition and its network structure in the mammary gland. Upon hormonal stimulation during pregnancy and lactation, stromal adipocyte dedifferentiate into preadipocyte causing a change in the ECM content and signal the ductal epithelial cells to proliferate. Once lactation seizes, stromal preadipocytes redifferentiate into adipocytes and change the ECM content such that the proliferating epithelial cells to undergo apoptosis and the mammary gland to remodel back to its resting or lead to adult nulliparouse state. This process is called involution. An imbalance or incomplete involution can lead to breast cancer. Existing breast cancer drugs such as anti-hormone, and apoptosis inducing agents, act mainly on epithelial cells. MAXIA developed a new class of anti-breast cancer agents that mimic involution and function through stromal fat cells and the ECM. These compounds induce differentiation of preadipocytes into adipocytes and downregulate integrins, cadherins and Wnts. The changes caused by our compounds induce growth arrest and apoptosis of breast cancer cells and lead to tumor regression and prevention *in vivo*. Western blot analysis revealed that cylin D1, a down stream target of the integrin, cadhedrin and Wnt signaling pathways, is also down regulated. The compounds showed additive/synergistic activity in the *in vivo* model with the anti-estrogen Tamoxifen. Thus, we have discovered a new class of differentiation/involution inducers that promise a new effective treatment for breast cancer when used alone or in combination with anti-hormonal therapies.

361

Novel inhibitors of MKP-1 have potent anti-cancer activity *in vivo*

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The mitogen-activated protein kinase phosphatases (MKPs) are a subfamily of dual-specificity phosphatases that are capable of dephosphorylating and inactivating members of the mitogen-activated protein kinase (MAPK) family. Activation of c-Jun N-terminal kinase (JNK), a member of the MAPK family, has been shown to be involved in mediating apoptotic cell death. JNK activity is negatively regulated by MKP-1, which has been shown to dephosphorylate JNK and to protect cells from certain apoptotic stimuli. Additionally, MKP-1 overexpression has been observed in patients with prostate, ovarian, and lung cancers. MAXIA has developed small molecule inhibitors of the phosphatase MKP-1, MX7091 and related analogues, which induces JNK-activation and apoptotic death of tumor cells. *In vitro* phosphatase assays show that MX7091 and related compounds selectively inhibit MKP-1 activity. Treatment of cancer cell lines with nanomolar concentrations of MX7091 results in JNK activation, caspase activation, and apoptotic cell death. Additionally, MX7091 synergizes with the chemotherapeutic cisplatin to induce apoptosis of tumor cells. MX7091 efficacy correlates with overexpression of MKP-1 in tumor cells, consistent with MKP-1's role in maintaining cell survival. MX7091 significantly reduces tumor size and induces tumor remission, while increasing animal survival time in *in vivo* models of pancreatic, colon, and non-small cell lung cancer. These studies demonstrate that MAXIA's MKP-1 inhibitors represent a novel class of anti-tumor agents with potential clinical utility.

362

alpha2-6-sialylated neolacto-series gangliosides serve as receptors for the anticancer drug rViscumin

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rViscumin is a heterodimeric cytotoxic plant protein currently in clinical phase 1 trials. Like ricin and other type II ribosome inactivating proteins (RIP) rViscumin consists of an enzymatically active A-chain being responsible for the toxicity towards tumor cells through inactivation of the translational machinery of the cell and a B-chain with carbohydrate binding activity. The B-chain is responsible for binding to the surface of the target cells, subsequently leading to internalisation. In this study we set out to investigate potential differences in the carbohydrate specificity of rViscumin and ricin which could explain the good tolerability and efficacy of rViscumin during preclinical and clinical development. In recent literature rViscumin as well as ricin are described as galactose-specific carbohydrate binding proteins. Employing solid phase binding assays rViscumin was shown to preferentially bind to terminally alpha2-6-sialylated neolacto-series gangliosides IV6Neu5Ac-nLc4Cer, VI6Neu5Ac-nLc6Cer, and VIII6Neu5Ac-nLc8Cer. Only marginal binding of rViscumin to galactose-terminated neutral glycosphingolipids was determined, whereas reinvestigation of ricin specificity demonstrated this type II RIP as galactose-binding protein. In cytotoxicity assays with human promyelotic HL-60 cells and human bladder carcinoma 5637 cells IC_{50} values of 1.16 pM and of 12.1 pM rViscumin were determined, respectively. CHO-K1 cells were resistant to rViscumin treatment up to a concentration of 5.26 nM tested. A direct correlation of rViscumin cytotoxicity and the expression of the receptor ganglioside IV6Neu5Ac-nLc4Cer was shown by means of a specific anti-Neu5Acalpha2-6Galbeta1-4GlcNAc-R antibody. The data revealed 3.7x106 and 1.5x106 receptor molecules per HL-60 and 5637 cell, respectively. CHO-K1 cells were negative, lacking alpha2-6-sialylated gangliosides. Moreover, CHO-K1 cells were rendered susceptible towards rViscumin cytotoxicity after exogenous application of human granulocyte gangliosides (HGG) which contain predominantly alpha2-6-sialylated gangliosides. From these data we conclude that rViscumin in contrast to ricin has