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.

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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.

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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.

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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.

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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