One clinically interesting pharmacogenomic [10] outcome of this work: L-asparaginase may prove useful for therapy of ovarian cancers that express only low levels of asparagine synthetase. http://discover.nci.nih.gov.

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Inhibition of Akt signaling in tumor cells leads to induction of apoptosis: studies using adenovirus-mediated delivery of an Akt dominant negative mutant

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Akt/PKB is a serine/threonine kinase that plays a critical role in cell survival signaling and its activation has been linked to tumorigenesis in several human cancers. Up-regulation of Akt as well as its upstream regulator PI 3-kinase has been found in many tumors. In addition, the negative regulator of this pathway PTEN/MMAC is a tumor suppressor gene. We have investigated the effects of inhibiting Akt signaling in tumor cells by expression of an Akt kinase dead (KD) mutant in which the two regulatory phosphorylation sites were mutated to alanines. Akt KD, which functions in a dominant negative manner, was introduced into tumor cells using a replication defective adenovirus expression system. As controls we used adenoviruses expressing Akt wild type (WT), p53, MMAC/PTEN, and β-gal. We show that *in vitro* proliferation of human and mouse tumor cells expressing high levels of activated/phosphorylated Akt was inhibited by both Akt KD and p53, in comparison to control viruses expressing either Akt WT or β-gal. Akt KD expression led to an induction of apoptosis similar to p53 expression in tumor cells expressing high levels of activated Akt, whereas control and Akt WT viruses had minimal effect. Expression of MMAC/PTEN induced an apoptotic response selectively in tumor cells in which MMAC/PTEN is deleted or mutated. On the other hand, Akt KD expression had minimal effect in normal cells and tumor cells expressing low levels of activated Akt. In addition, the tumorigenicity of tumor cells transduced with the Akt KD mutant was also significantly reduced compared to control adenovirus-infected cells. These studies validate the usefulness of targeting Akt for new drug discovery efforts and suggest that inhibition of Akt may have a selective antitumor effect.

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The follicular thyroid carcinoma associated PAX8/PPAR-gamma-1 fusion gene permits anchorage independent growth in a follicular thyroid cell line

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A translocation of chromosomes 3p25 and 2q13, seen commonly in follicular thyroid carcinoma (FTC), causes the expression of a fusion protein, which includes the first 9 exons of the thyroid specific transcription factor PAX-8, fused to a full length peroxisome proliferator activated receptor gamma (PPAR-gamma). Expression of the fusion protein (designated PPFP) is restricted to FTC. We have previously demonstrated the impact of this putative oncogene on cell growth characteristics, following transient transfection, where it impairs apoptosis and increases cell numbers *in vitro*. We now report studies designed to assess its oncogenic action, by determining its impact on anchorage independent growth. We generated stable transfectants of an immortalized thyroid cell line (NT cells), following lipofection, using either a PPFP-containing or a control vector (pCDNA3.1). Stable transfectants were selected by growth in geneticin-enriched culture medium. Five thousand cells from a single geneticin-resistant clone were transferred onto soft-agar plates for the control cell line and for each of 5 PPFP cell lines. Each experiment was performed in triplicate. Only a single soft-agar colony was formed from the control cell-line (mean 0.25 ± 0.3 colonies/plate), compared to 8.6 ± 3.3 colonies/plate for the 5 PPFP cell lines ($p<0.03$). These data confirm a true oncogenic role for the FTC-associated PAX8/PPAR-gamma fusion gene, which induces anchorage independent growth in this follicular cell line. These data strongly support our hypothesis that PPFP represents an important initiating oncogene in FTC. Furthermore, the presence of a full-length PPAR-gamma-1 receptor, as part of this oncoprotein, raises the possibility that PPAR-gamma agonists, including the thiazolidenedione group of drugs, may modulate its oncogenic actions.

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A pharmacogenomics strategy for validation of cancer therapeutics using murine xenograph models

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Recent advances in genomics have made possible the identification of thousands of candidate therapeutic targets from diseased tissues using gene expression profiling, proteomics, and genetic technologies. The prioritization and validation of targets arising from genomics approaches has become one of the rate limiting steps in the identification of therapeutic targets. Here we report a comprehensive strategy to facilitate the validation of candidate molecules identified in genome wide transcription profiling experiments. A crucial step in the process is the validation of expression of candidate molecules using in situ hybridization on tissue microarray arrays (TMAs) in conjunction with secondary validation of expression using real time PCR. For each prioritized target a xenograph model is selected based on the expression of the candidate gene against a panel of greater than 42 cancer related cell lines cultured on plastic as well as harvested xenographs from the cancer cell lines grown in nude mice. The xenograph model system is subsequently developed for efficacy studies during the development of therapeutic targets against the candidate molecule. Efficacy is monitored in pharmacology studies as well as the monitoring of surrogate marker genes using real time PCR and ISH on xenograph and TMAs respectively. We have utilized this strategy for the identification of several therapeutic targets arising from large scale expression profiling experiments in several oncology disease indications. The process and characterization of these molecules will be discussed.

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Tumor-selective toxicity of histone deacetylase inhibitors is due to their targeting cell cycle checkpoint points

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Histone deacetylase inhibitors have been demonstrated to be selectively toxic in a wide range of immortalised and tumor cell lines, but normal cells are resistant to killing by these drugs. Animal studies have demonstrated that these drugs have little unwanted toxicity but are effective in killing xenografted tumors, and a number of these drugs are now undergoing Stage I/II clinical trials. The basis of the tumor selective action of histone deacetylases appears to be related to the functional status of a histone deacetylase inhibitor-sensitive G2 checkpoint in the treated cells. This checkpoint imposes a G2 phase cell cycle arrest in drug resistant cells but is defective in drug sensitive cell lines, with these cells dying at some point after transit through mitosis. Reintroduction of a cell cycle arrest provides protection against the toxic effects of the histone deacetylase inhibitors. We have investigated the molecular basis of the toxicity of these drugs. Sensitive cells treated with these drugs during S phase enter mitosis normally but undergo an aberrant mitosis, with chromosomes failing to properly align at metaphase. These cells exit mitosis with very similar kinetics to untreated cells but a high proportion die soon after mitotic exit, suggesting that the mitotic spindle checkpoint which normally detects spindle defects and arrests cells in mitosis until these defects are repaired, is not functioning in drug treated cells. We demonstrate that histone deacetylase inhibitors block normal functioning of this checkpoint, and that cell death is a consequence of cell exiting mitosis without overcoming their spindle defects. Thus the selective toxicity of this class of drugs is based on their targeting two cell cycle checkpoints, a G2 checkpoint which provides the specificity of action and the mitotic checkpoint which results in the toxicity. Histone deacetylase inhibitors are an elegant example of how drugs that target cell cycle checkpoints that are defective in tumour cells can provide the selective toxicity desired in chemotherapeutic agents.