(27/44) according to allelotyping). In case of BC RASSF1A promoter region methylation was observed with the same frequency as allele alterations (57%, 12/21) according to LOH, but the sum of all aberrations in NL3-001 locus determined by TaqMan real-time PCR was even higher, constituted up to 81% (17/21). Althow duplication and multiplication (25% for BC and even more for RCC) contributed in these values considerably. Among inactivating events homozygous deletions (HD) also contributed a large portion, equal to 19%(4/21) for BC and 20%(4/20) for RCC. Due to the highest frequency, methylation of RASSF1A CpG promoter region can be considered as an event of earliest carcinogenesis. This TSG appeared to be useful for oncology patient treatment using genotherapy approach.

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Implications of N-terminal truncated p73 for cancer

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Most human cancers harbour aberrations of cell cycle control in the RB/p16-pathway resulting in deregulated E2F activity, cell cycle progression or apoptosis. Apoptosis by E2F1 has recently been linked to activation of p73 which displays significant structural and functional homology to the tumor suppressor p53. However, instead of mutational inactivation, overexpression of wild-type p73 has been reported in various tumor types compared to normal tissues arguing against a classical tumor suppressor function. Recently, N-terminally truncated, transactivation-deficient p73-isoforms (deltaTA-p73) have been identified as a second class of p73 proteins. Since overexpression of p73 in tumors includes deltaTA-p73, we further characterized these novel p73-isoforms. We show that deltaTA-p73 retains DNA-binding competence but lacks transactivation functions resulting in an inability to induce growth arrest and apoptosis. Importantly, deltaTA-p73 acts as a dominant-negative inhibitor of p53 and full-length p73 (TA-p73). Inhibition of p53 involves competition for DNA-binding, whereas TA-p73 can be inhibited by direct protein-protein interaction. Moreover, we show that deltaTA-p73 overexpression results in malignant transformation of NIH3T3 fibroblasts and tumor growth in nude mice, thereby providing evidence for an oncogenic function of deltaTA-p73. Thus, in analogy to cancer associated conversion of the cellular tumor suppressor gene p53 into oncogenic p53 mutants, increased expression of N-terminally truncated p73 isoforms apparently conveys the TP73 gene with oncogenic activity that appears to be actively selected for during tumor development. Thus, the newly identified p73-Isoforms represent attractive targets for molecular anticancer therapy. This work was supported by the Deutsche Krebshilfe.

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Hypoxia hypersensitizes the slow-growing cells of solid tumors to glycolytic inhibitors

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Since current cancer chemotherapy selectively kills rapidly dividing vs slow or non-dividing cells, the slow-growing cells of solid tumors represent a particularly difficult form of multidrug resistance to overcome. However, due to the hypoxic conditions which many of these slow growing populations of tumor cells are under, a window of selectivity opens for treatment with glycolytic inhibitors. We demonstrate in three distinct *in vitro* models of "hypoxia" (A, B and C) that tumor cells are hypersensitive to inhibitors of different steps of the glycolytic pathway i.e. 2-deoxy-D-glucose and oxamate. Model A are osteosarcoma cells (143B) treated with agents which interfere with mitochondrial oxidative phosphorylation; Model B are Rho 0 cells, a variant derived from 143B cells, which due to their deficiency in mitochondrial DNA cannot perform oxidative phosphorylation and Model C are 143B cells grown under varying levels of reduced external oxygen i.e. 10, 5, 1, 0.5, 0.1 & 0%. In all three models increased levels of lactic acid correlates with hypersensitivity to inhibitors of glycolysis. Overall, the data indicate that as a consequence of "hypoxia", cells switch from aerobic to anaerobic metabolism and by relying on glycolysis as a sole source of ATP synthesis, become hypersensitive to glycolytic inhibitors. Thus, the *in vitro* data suggest that addition of inhibitors of glycolysis to conventional chemotherapeutic protocols should increase treatment efficacy by targeting the slow-growing cells found in most, if not all, solid tumors.

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Protein phosphatase 2A, a novel and unexplored anticancer target

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Protein phosphorylation is a major control mechanism intricately coordinated by kinases and phosphatases. While various anticancer drugs target kinases the potential of targeting protein phosphatases (PP) has largely been ignored. Nonetheless, the serine/threonine protein phosphatases 1 and 2A (PP1 & PP2A) play key roles in the cell cycle and apoptosis, and are a valid anticancer target. PP1 and PP2A are known as negative regulators of the cell cycle as they inactivate cdk's and stimulate pRb binding to E2F. Non replicating cells such as neuronal tissue have high PP activity. However, PP's are also crucial for successful mitosis via MAP kinase, histone, condensin, lamin, and vimentin dephosphorylation. PP2A also controls bcl-2 phosphorylation. Inhibition of PP's is counter-intuitive for the treatment of cancer, however, PP inhibition is lethal via aberrant cell cycle movement and mitotic failure. We propose that rapidly dividing cancer cells will be more susceptible to PP inhibition than non-dividing cells. Our lead compound in the development of PP inhibitors is cantharidin (2,3-dimethyl-7-oxobicyclo[2.2.1]heptane-2,3-dicarboxylic-acid-anhydride), a toxin found in blister beetles. It is a potent inhibitor of PP1 (IC_{50}=0.56μM) and PP2A (IC_{50}=0.51μM), small molecule, membrane permeable, not a substrate for p-glycoprotein, stimulates haemopoiesis, and amenable to analogue development. We have shown cantharidin to be cytotoxic in cancer cell lines (HT29, G401, H460, WiDr, A2780, SW480, HCT116, L1210) producing GI50 values of 3.6-16μM; to induce a transient acceleration of cells within in 4h from G1 into S-phase involving a 4-fold increase in 3H-thymidine uptake culminating in G2+M cell cycle arrest within 24h and subsequent apoptotic cell death; and to induce synergistic cytotoxic interactions with the thymidylate synthase inhibitor, Thymitaq. Recently we have shown that PP2A is a better target for drug development than PP1, as the cytotoxicity of cantharidin in cell lines correlated (r=0.87, P<0.01) with PP2A content but not PP1. We have also synthesised two cantharidin analogues that selectively target PP1 (IC_{50}=12.5, 50μM) versus PP2A (IC_{50}=426, >2000μM) which induce no cytotoxicity. Binding and docking studies have led us to synthesise cantharimides which show equipotent PP inhibition and cytotoxicity to cantharidin but which bind to unique grooves in the active site providing the foundation for the development of more selective PP2A inhibitors.

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An integrated approach to the pharmacogenomics and pharmacoproteomics of cancer

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Application of mRNA and protein expression profiling to cancer drug discovery has not proved as straightforward as many expected, in part because clinical tumors are heterogeneous, in part because cancer patients have complex, fragmentary treatment histories. In contrast, the 60 cell lines (the NCI-60) [1-3] used in the NCI drug discovery program have been treated with >70,000 compounds one at a time and independently over a 12-year period. Cell lines in culture do not fully reflect cells *in vivo*, but, historically, most of our knowledge of molecular pharmacology and targets has come from cultured cells, not clinical material. We and our colleagues assessed expression patterns in the NCI-60 using 2-D protein gel electrophoresis [3], high density "reverse-phase" protein arrays, cDNA microarrays [4,5], and oligonucleotide chips [6]. To find patterns in the data, we then developed new data visualizations, including the familiar Clustered Image Map [7], and a tool (MedMiner) that streamlines literature searches on genes and drugs [8]. We and our collaborators next characterized the cells at the DNA level by comparative genomic hybridization (CGH), spectral karyotyping, array-CGH, and SNP chip and then developed algorithms and a program package called LeadScope/LeadMiner [9]. This package makes it possible to predict which molecular substructures will be found in drugs that are active against cells expressing large amounts of a selected gene – and vice versa. Finally, we have also developed the program package GEEVS (GEnome Exploration and Visualization System) to integrate all of the disparate types of data at the DNA, RNA, protein, functional, and pharmacological levels.

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.