(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.