ties with IC_{50} values in low nanomolar range as evaluated by MTT assays in various cancer cells such as breast cancer, prostate cancer, pancreatic cancer and non-small-cell lung cancer cells. The *in vivo* anti-tumor effects of this group of compounds are being investigated in animal models.

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Activation of c-Jun-N-terminal-kinase by R- and S-flurbiprofen results in cell cycle arrest in human colon carcinoma cells

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The unspecific cyclooxygenase inhibitor S-flurbiprofen and its "inactive" enantiomer R-flurbiprofen have been previously found to inhibit tumor development and growth in APC min mice, TRAMP mice as well as in rats. The mechanisms underlying the antiproliferative effects of R- and S-flurbiprofen are unknown. In the present study we show that both R- and S-flurbiprofen inhibit survival of three colon cancer cell lines which differ in the expression of COX-2 (HCT-15: no COX-2, Caco-2: inducible COX-2 and HT-29: constitutive COX-2). The IC_{50}s for S- and R-flurbiprofen ranged from 250-450μM. Both flurbiprofen enantiomers induced apoptosis in all three cell lines as indicated by DNA- and PARP-cleavage. In addition, R- and S-flurbiprofen treatment resulted in a G1-cell cycle block. These effects were associated with an activation of c-Jun N-terminal kinase (JNK), an increase of the DNA binding activity of the transcription factor AP-1 and downregulation of cyclin D1 expression. Supershift experiments indicated that R- and S-flurbiprofen-induced AP-1 activation was associated with a shift in its Jun-protein composition from c-Jun towards JunB. The latter is known to repress cyclin D1 expression. Inhibition of JNK activity prevented the R- and S-flurbiprofen-induced AP-1 DNA binding activity, the repression of cyclin D1 expression and the G1-cell cycle block. However, JNK inhibition had no effect on flurbiprofen-induced apoptosis. These data suggest that the cell cycle inhibitory effects of R- and S-flurbiprofen are mediated at least in part through activation of JNK and subsequent down-regulation of cyclin D1 whereas R- and S-flurbiprofen-induced apoptosis is largely independent of JNK activation. Although *in vitro* effects of R- and S-flurbiprofen were indistinguishable, only R-flurbiprofen inhibited HCT-15 tumor growth in nude mice, suggesting that additional anti-tumoral effects which are specific for R-flurbiprofen only become operative *in vivo*.

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Cks1, a subunit of cyclin-dependent kinases, as a novel target for the treatment of colon cancer

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The level of p27Kip1 protein, a cyclin-dependent kinase inhibitor, is indicative for tumor progression and tightly regulated by several mechansims during the cell cycle-one of them is degradation mediated by the ubiquitin conjugating enzyme complex SCFSKP2 during G1-phase of the cell cycle. Recently, it was demonstrated that Skp2 is overexpressed in various transformed cell lines and in human cancer and that Skp2 overexpression correlates directly with grade of malignancy and inversely with p27 levels in human lymphomas. Here, we report that Cks1, a subunit of cyclin-dependent kinases, is directly affecting p27Kip1 protein levels in human tumor cells while protein levels of cyclin B1, which needs to be degradated by the proteasome after ubiquitination in order to allow the cells to exit mitosis, remain unaltered. Microarray analyses revealed that Cks1 mRNA is more abundant in human colon cancer tissue samples than in normal tissue samples. Further we demonstrated that depletion of Cks1 does affect cell proliferation as well as anchorage independent growth in tumor cell lines indicating that Cks1 in involved in maintaining a transformed cell phenotype. These data provide some evidence that Cks1 could be a novel target for treatment of colon cancer and suggest regulation of the tumorsuppressor p27Kip1 as a possible mechanism.

Signal transduction modulators

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Pharmacogenomic expression profiling of renal cell carcinoma in a phase II trial of CCI-779: identification of surrogate markers of disease and predictors of outcome in the compartment of peripheral blood

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While it is evident that the transcriptomes of primary malignancies differ considerably from corresponding normal tissue, it is currently unknown whether in the context of active solid tumor burden there exist correspondingly distinct markers of gene expression in the peripheral blood of affected individuals. One of the main objectives of clinical pharmacogenomic studies is to determine whether easily obtained (often non-target) tissues such as peripheral blood can be used to identify surrogate markers of disease and predictors of outcome *in vivo*. In the present study we identified pharmacogenomic markers of advanced renal cell carcinoma (RCC) in peripheral blood mononuclear cells (PBMCs) from patients during a phase II trial of the investigational drug CCI-779 which targets the mTOR pathway. RNA samples of PBMCs from RCC patients at baseline (n=45) and normal volunteers (n=20) were hybridized to oligonucleotide arrays containing 12,626 unique transcripts and statistical analyses identified a subset of disease genes significantly changed between the groups. A supervised learning approach identified minimal sets of genes expressed in PBMCs capable of accurately predicting RCC versus normal state. To explore the molecular basis for this differentially expressed gene set, RCC PBMC expression profiles were compared with RCC tumor profiles in silico, with PHA-stimulated PBMC expression profiles ex vivo, and with PBMC expression profiles from end-stage renal failure patients. At the conclusion of the clinical trial, the original baseline expression data in RCC PBMCs were reassessed to determine whether biomarkers eventually predictive of outcome were present in blood samples prior to initiation of CCI-779 therapy. Both unsupervised and supervised approaches identified gene sets in RCC PBMCs that resulted in stratification of responders and non-responders into groups with significant differences in time to disease progression. The present disease gene set lays the foundation for biomarkers that will be explored in larger phase III clinical trials and may eventually aid in the diagnosis and treatment of renal cell carcinoma. Of equal importance, the pattern of gene expression within the easily obtained compartment of peripheral blood correlated with longer time to disease progression could eventually assist in the stratification of patients with higher likelihood for positive responses to CCI-779 therapy in this disease setting.

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Cellular and molecular markers of metastatic potential as targets for micrometastasis detection in gastrointestinal cancer

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Background: Tumor-cell dissemination and metastasis are complex processes whose outcome depend upon cancer cells properties and host-tumor interactions. Metastatic phenotype included activation of growth factors signalling pathways (G), differentiation (D), deregulated adhesion (DAd), epithelial-mesenchymal transition (EMT), invasiveness (Inv) and angiogenesis (A). In order to target micrometastasis (MM) in patients with gastrointestinal cancer (GC) we have developed a model system based on multi parametric immunocytochemistry (IC) and molecular analysis of metastasis-related (MR) markers.
Methods: As surrogate model of GC the following human tumor cell-lines (CL) were used: colorectal Gp5d, LoVo, DLD1, LS513, HT29; gastroesophageal OE19 and pancreatic MBQ-OJC1. In addition hematopoietic (H) CL were analysed. Monoclonal antibodies (mAb) against the following MR antigens (Ag) were selected for IC: EGFR, cytokeratins types I-II (CK), Ep-CAM and N-cadherin. Ag-mAb reaction was developed with avidin-biotin-complex and alkaline phosphatase. Molecular analysis were performed us-