zyme activity. Structure-based design methods have been used to significantly enhance selectivity and potency for both, quadruplex specificity and telomerase inhibition, by addition of a third substituent at the 9-position of the acridine ring. We report here on biological studies of trisubstituted compounds, including two with extended substituents at the 9-position (BRACO19, JCACI9). Enhanced quadruplex affinity shown by molecular dynamics based on the G-quadruplex crystal structure was paralleled by an increased differential between concentrations needed to inhibit telomerase activity in the TRAP reaction versus those which result in overt cytotoxicity in a 4 d SRB assay. JCACI9, the compound with the more extended 9-amino substituent, inhibited the TRAP reaction of MCF-7 breast cancer cell lysates at 2 μM, but had an IC_{50} for growth inhibition in MCF-7 cells of 45 μM. BRACO19 had an IC_{50} of 6 μM and produced telomerase inhibition at 1 μM. SBACI11 however, an agent with a short 9-substituent, lacked differential of growth and enzyme inhibition in this cell line. Specific telomerase inhibitory effects were accompanied by induction of cellular senescence as determined by β-galactosidase staining in long term MCF-7 cultures treated with BRACO19 and JCACI9 concentrations below acute cytotoxicity. A 2- to 5-fold increase in β-gal positive cells was found starting at 7 days of continuous treatment (6 population doublings, PD). In contrast, telomere shortening in the order of ~ 1 kb measured by Southern blotting was seen after 33 PDs. We suggest therefore that these two events are not necessarily related, and that this behavior might be indicative of G-quadruplex inhibitors producing senescence by direct interference with telomere maintenance/capping rather than successive shortening. Thus, G-quadruplex interactive agents seem not to require the extended time lag characteristic of pure inhibitors of the catalytic activity of telomerase.

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The unfolded protein response pathway plays a key role in anti-cancer drug sensitivity

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All cells possess signaling pathways that allow them to detect environmental changes that adversely affect cellular processes and activate protective responses. An important stress protection pathway is the unfolded protein response (UPR), which is activated by conditions that interfere with protein folding in the endoplasmic reticulum (ER). Protein folding in the ER can be affected by environmental stresses, such as glucose deprivation or hypoxia, and pharmacological agents, such as tunicamycin and thapsigargin. UPR activation triggers signaling cascades that alter transcription, cell cycle progression, protein synthesis and stability, and cell survival. It has also been shown that agents that induce the UPR lead to dramatic changes in sensitivity to several classes of anti-cancer agents, including topoisomerase II (topo II) inhibitors, and DNA alkylating agents. For example, agents such as tunicamycin lead to a rapid depletion of topo II and resistance to etoposide. Conversely, the induction of the UPR increases sensitivity to cisplatin by an unknown mechanism. A key regulator of the UPR is BiP (GRP78), a resident ER chaperone. BiP binds to several effectors of the UPR and blocks their activation. When unfolded proteins accumulate in the ER, BiP releases UPR effectors to bind the unfolded proteins, thus allowing activation of the pathway. Over-expression of BiP prevents UPR induction, providing a tool for directly assessing how this pathway affects anti-cancer drug targets. We used CHO cells that over-express BiP to assess the contribution of the UPR in altered sensitivity of cells to topo II targeting drugs and alkylating agents. In the absence of stress, cells over-expressing BiP have the same levels of topo II and sensitivity to etoposide as the parental cells. However, unlike the parental cells, in the presence of UPR inducing agents, the level of topo II in BiP over-expressing cells remained unchanged and resistance to etoposide was not seen. This result demonstrates that induction of the UPR pathway is directly responsible for resistance to topo II targeting agents. Interestingly, CHO cells over-expressing BiP are highly resistant to cisplatin, even in the absence of UPR inducing conditions. This result indicates that BiP regulates proteins that play an essential role in determining cellular sensitivity to cisplatin. Experiments are underway to identify proteins regulated by BiP that are important for sensitivity to cisplatin.

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Identification of a new drug target using RNAi-based functional genomics

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A Genome-wide RNAi screen was performed to obtain functional information on genes involved in cell division in C. elegans and furthermore to identify similar gene functions in other organisms. We inhibited the expression of 91% of the 19,500 predicted open reading frames of the entire genome using RNA-mediated interference (RNAi). By using an *in vivo* time-lapse differential interference contrast microscopy assay we so far identified more than 750 genes required for the first two cell divisions in C. elegans embryos. One of these genes is H38K22.2, which plays an important role in these early divisions. Inhibition of the gene product of H38K22.2 by RNAi results in a severe cell division phenotype, the characteristics of which are formation of multiple female pronuclei, wrong spindle positioning and delayed P1 division, resulting in an early death of the embryo. We characterized the human homologue RP42, which shows 36% identities and 56% positives to the C. elegans gene. The RP42 protein shows a specific localization on the centrosomes during the cell cycle in human cells. Expression of the RP42 transcript in human tissues is generally very low, with the highest levels found in proliferating tissues. Importantly, expression is also up regulated in various tumours, namely lung, colon and pancreas. Expression of RP42 can be inhibited to 90% in human cells using an RNAi based assay. Functional results will be presented.

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PDGF receptor inhibition in tumor stroma, with STI571 or PDGF B-chain aptamers, enhances the effects of chemotherapy in experimental solid tumors by increasing tumor drug uptake

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Most solid tumors display interstitial hypertension. The increased interstitial fluid pressure (IFP) in tumors has been proposed to act as a barrier for tumor drug uptake. Many solid tumors also display PDGF receptor expression in tumor stroma cells. Furthermore, PDGF receptor activation in loose normal connective tissue increases IFP. Based on these observations we investigated the effects of PDGF receptor inhibition on tumor IFP, tumor drug uptake, and on the anti-tumor effects of chemotherapy. Studies were performed on subcutaneous PROb colon adenocarcinomas and KAT-4 thyroid carcinomas grown in syngeneic rats and SCID mice, respectively. Both tumor models display extensive tumor stroma formation and PDGF receptor expression restricted to stroma cells. PDGF receptor inhibition was achieved by systemic treatment with STI571 or PDGF B-chain aptamers. Inhibition of PDGF receptor signaling reduced tumor IFP in both models by approximately 30%. PDGF receptor inhibition increased tumor transvascular transport in the PROb tumor model and increased the uptake of Taxol in the KAT-4 tumor model. Similarly, an increased uptake of EPO906 (epothiloneB, a novel non-taxoid microtubuli stabilizing agent) occurred in KAT-4 tumors, but not in liver and kidney. Most interestingly, combination treatment of PROb tumors with STI571 and 5-FU, or of KAT-4 tumors with STI571 and Taxol or EPO906, dramatically enhanced the anti-tumor effects of the cytotoxic drugs. Treatment with only STI571 did not produce anti-tumor effects. The synergistic interactions were not observed *in vitro*, and co-treatment with STI571 did not lead to anti-angiogenic effects. Different STI571 treatment schedules, together with weekly EPO906B administration, confirmed the association between the beneficial therapeutic effects of co-treatment, and the reduction of IFP and enhanced tumor drug uptake induced by STI571 pre-treatment. In conclusion, our study demonstrates that PDGF receptor inhibition in tumor stroma cells reduces tumor IFP, increases the tumor uptake of chemotherapy drugs, and enhances their therapeutic effects. The study thus identifies inhibition of PDGF receptor signaling in tumor stroma as a novel, possibly general, combination strategy for enhancement of the therapeutic effects of standard chemotherapeutics. (Parts of the study were performed with financial support from Novartis, Basel, Switzerland)