that other member(s) of programmed cell death participate in the completion of EGFR-TKI induced cell death in NSCLC.

In about half of the patients, the acquired resistance to EGFR-TKI monotherapy upon prolonged treatment is due to T790M secondary mutation in the kinase domain of EGFR. However, a significant portion of patients develop acquired resistance without alterations in the primary sequence of EGFR kinase domain. A few mechanisms, including receptor cross-talks and transphosphorylation by non-ErbB family members, were suggested as possible explanations of acquired EGFR independence, though the physiological relevance of these results obtained from in vitro experiments still remains unclear. As an alternative pre-clinical model system of the acquired resistance to the current reversible TKI therapy, orthotopic tumor xenografts mice were treated with EGFR-TKI for a prolonged period of time. Refractory clones obtained from the lung of such mice display a series of histological and cellular phenotypes distinct from its parental cells. A subgroup of mice developed acquired resistance through T790M mutation. Other mice, however, developed resistance independent of T790M mutations. The molecular mechanisms leading to the acquired resistance are discussed.

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Specific inhibition of hypoxia-induced vascular endothelial growth factor expression by flavonoids in human lung cancer cells

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Flavonoids are a group of polyphenolic secondary metabolites important for plant biology and human nutrition. Epidemiological studies have shown that these compounds may have an important role in explaining the favorable effects of vegetables and fruits against cancer, in special lung cancer. Previous studies have reported that some flavonoids, such as quercetin, luteolin or fisetin, induced apoptosis in several cancer cell types. Another important property related to cancer chemoprevention may be their ability to inhibit tumor angiogenesis. Cells exposed to hypoxia up regulate the expression of several transcription factors, including the hypoxia inducible transcription factor (HIF), which induces the coordinated expression of many genes involved in glucose metabolism, pH regulation and angiogenesis. Among these genes, the vascular endothelium growth factor (VEGF) plays a key role in the stimulation of tumor angio and lymphangiogenesis. We investigated the effects of a group of 40 structurally related flavonoids on the expression of VEGF and HIF-1 alpha in human lung cancer cells. We found that several of these compounds inhibited VEGF production under hypoxic conditions at non cytotoxic concentrations. We also investigated the molecular pathways involved in the inhibition of VEGF expression, including MAP kinase and PI3-kinase-signaling pathways, and the expression and transactivation of HIF-1 factor. This research leads us to analyze the structure-activity relationships for these compounds and the relevance of each pathway in the inhibition of VEGF production. Overall, it is concluded that among the wide range of biological effects that flavonoids may exert, inhibition of angiogenesis may be of great relevance in their anticarcinogenic properties.

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The combined effect of a non selective and a selective cyclooxygenase-2 inhibitor and 5- fluorouracil treatment on HCA-7 human colorectal carcinoma cell line

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Background: 5-FU is included in many major chemotherapeutic regimens which have been statistically judged to be effective adjuvant therapy for patients with colorectal cancer. However, 5-FU itself does not substantially improve survival rates. Several NSAIDs have been tested in combination with a number of cytotoxic drugs. We hypothesized that treatment of cancer cells with 5-FU combined with indomethacin (INDO) or NS-398 might have a synergistic antiproliferative effect.

The aim of the study was to investigate whether INDO a nonselective cyclooxygenase (COX) inhibitor or NS-398, a COX-2-selective inhibitor, influence the cytotoxic effect of 5-fluorouracil on high COX-2 protein expressing HCA-7 colorectal cells. Considerable research effort was directed towards understanding the mechanism how these COX inhibitors modify the cytotoxicity of 5-FU.

Materials and methods: Sulphorhodamine B proliferation assay was used to measure the effect of 48 h 5-FU±INDO or 5-FU±NS-398 treatments on HCA-7 cells. COX-2 protein levels were analysed by Western blot and immunofluorescent method. PGE2 production was measured by ELISA. To investigate the cell cycle and apoptotic cells FACS analysis was used.

In order to understand the relative insensitivity of HCA-7 cells against 5-FU (IC50 value: 1mM) we studied the rate limiting enzyme of 5-FU catabolism dihydropyrimidine dehydrogenase (DPD)

Results: INDO or NS-398 treatment alone did not influence the proliferation of HCA-7 cells. INDO or NS-398 combinated with 5-FU significantly enhanced the proliferation inhibition caused by 5-FU alone. ($p<0.01$). The PGE2 production was reduced by 90% after 48 hours treatment with INDO or NS-398 which was similar range in case of 5-FU combinations as well. COX-2 protein levels were relatively unchanged. FACS analysis showed a delay in S phase progression and a marked decrease of G2/M fraction after treatment with 5-FU + INDO or 5-FU + NS-398.

The combined treatments also caused a significant increase in the number of apoptotic cells compared to 5-FU alone ($p<0.01$). High DPD enzyme activity was demonstrated in HCA-7 cells which was strongly reduced by both INDO and NS-398 as well.

Conclusion: 5-FU cytotoxicity against HCA-7 cells was augmented by combination with COX inhibitors which could be due at least partly to the increase of apoptotic cells and increase of the amount of 5-FU available for the anabolism as a consequence of the reduction of DPD activity.

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NSC-mediated tumor selective therapy - towards glioma clinical trials

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Neural stem cells (NSCs) display inherent tumor-tropic properties that can be exploited for targeted delivery of anti-cancer agents to invasive and metastatic tumors. Malignant gliomas are the most common primary brain tumors and are considered among the deadliest of human cancers. We postulate that NSC-mediated therapy of glioma will increase tumor-selectivity and decrease systemic toxicities, and thus potentially achieve therapeutic indices sufficient to eradicate invasive and residual tumor cells that are otherwise lethal. We generated a v-myc immortalized, clonal human NSC line, HB1.F3, which has been modified to stably express the cytosine deaminase (CD) therapeutic transgene (HB1.F3.CD). CD coverts 5-fluorocytosine (5-FC) prodrug to chemotherapeutic 5-fluorouracil (5-FU). Pre-clinical safety data in mice indicate that the HB1.F3.CD NSC line is non-toxic, non-immunogenic, non-tumorigenic, and chromosomally and functionally stable over at least 15 passages. Identification of a single copy and single insertion site for both v-myc and CD genes was determined by LAM-PCR and confirmed by Q-PCR. We believe that use of this stable, sustainable, and expandable NSC line will circumvent the problems associated with characterization, senescence, and replenishment sources of primary stem cell pools. Our pre-clinical therapeutic efficacy studies using the HB1.F3.CD NSCs in combination with 5-FC prodrug in laboratory animals demonstrated 70-90% of anti-tumor responses, as measured by decreased tumor burden or increased survival time in glioma-bearing mice (and in solid tumor metastases to brain, and medulloblastoma models). Our data also indicate that HB1.F3.CD cells can be labeled with iron oxide superparamagnetic nanoparticles, which allows in vivo MRI monitoring of NSC migration to intracranial glioma in tumor-bearing mice. We now propose the use of HB1.F3.CD NSCs in human patients with recurrent high-grade glioma. The NIH Recombinant Advisory Committee has approved the use HB1.F3.CD cells for recurrent glioma at a public hearing December, 2007. We are developing a pilot study in patients with recurrent high-grade glioma to assess the safety and feasibility of HB1.F3.CD NSCs injected directly into the brain parenchyma at the time of surgical tumor resection, in combination with oral 5-FC. We postulate that HB1.F3.CD

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Development of anti MUC1 DNA aptamers for the imaging of breast cancer

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