KP1019 accumulation in P-gp positive cells which could be partly reversed by co-administration of P-gp modulators. KP1019 inhibited P-gp ATPase activity with an K_i of approximately 31 μM. Selection of KB-3−1 cells against increasing KP1019 concentrations for more than year led to only an around 2-fold resistance (KB-1019 cells), and unexpectedly no P-gp expression. Accordingly KB-1019 cells displayed no drug accumulation defect and a unique cross-resistance pattern, indicating an ABC-transporter-independent acquired resistance phenotype.
Conclusion: In summary P-gp has to be considered as significant but weak intrinsic resistance mechanism against KP1019. Acquisition of resistance against KP1019 during chemotherapy seems to be relatively unlikely and acquired resistance based on ABC-transporter overexpression has not to be expected.

631 POSTER

Tumor associated fibroblasts have a profound impact on drug sensitivity of gastric cancer cells

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Background: In most tumor types, including gastric cancer, one of the main obstacles in anti-cancer therapy is the development of drug resistance. Some of the molecular mechanisms involved in acquired drug resistance, such as MDR, are well-characterized. In contrast, cellular mechanisms, i.e. cell-to-cell interactions between the cancer cells and the surrounding stromal cells, are poorly understood. Therefore, the purpose of the present study was to investigate the impact of benign and tumor associated fibroblasts on drug sensitivity of gastric cancer cells. Material and Methods: Fibroblast cultures were originated from benign gastric mucosa and the corresponding primary gastric carcinomas obtained from eight gastric cancer patients. Characterization of the fibroblasts using a panel of cell type specific antibodies confirmed the connective tissue origin. Two gastric cancer cell lines, namely MKN-28 representing the intestinal type and Hs746T indicating the diffuse type of gastric cancer, were used for co-culture experiments using the multicellular spheroid model. Homotypic spheroids consisting of either cancer cells or fibroblasts and heterotypic spheroids consisting of both cell types were established and treated with a variety of clinically relevant drugs. Treatment effects were measured using apoptotic (TUNEL, nucleosome ELISA) and metabolic (MTS) assays. Changes in the protein profiling were identified using 2D-gel electrophoresis followed by MALDI-TOF analysis. Results: Homotypic and heterotypic multicellular spheroids imitated a number of features observed in gastric carcinomas, such as the original differentiation phenotype and a slow proliferation activity. In contrast to homotypic spheroids and heterotypic spheroids containing benign fibroblasts, heterotypic spheroids with tumor associated fibroblasts were less sensitive to most of the drugs tested. Two-dimensional gel electrophoresis revealed that the decreased drug sensitivity of the heterotypic spheroids was associated with changes in the protein expression profile detected in both the gastric cancer cells and the tumor related fibroblasts. Most often, quantitative changes of the proteins were found. In addition, de novo expression of distinct proteins also could be identified. Conclusion: Tumor related fibroblasts, but not their benign counterparts, modulate drug sensitivity of gastric cancer cells. This is associated with profound changes in the protein profile.

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Down-regulation of mitochondrial F1F0-ATP synthase in human colon cancer cells with induced 5-fluorouracil resistance

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5-Fluorouracil (5-FU) is widely used for treatment of advanced colorectal cancer. Unfortunately it is common for such patients to ultimately develop resistance to 5-FU, creating a major problem for chemotherapy. The mechanisms underlying this resistance are largely unknown. To screen for proteins possibly responsible for 5-FU resistance, cells resistant to 5-FU were derived from human colon cancer cell lines, and two-dimensional gel electrophoresis (2-DE)-based comparative proteomics was performed. 2-DE data showed there was lower expression of the alpha subunit of mitochondrial F_1F_0-ATP synthase (ATP synthase) in 5-FU-resistant cells compared to parent cells. Western blotting showed expression of other ATP synthase complex subunits was also lower in 5-FU-resistant cell lines, and that these resistant cells also showed decreased ATP synthase activity and reduced intracellular ATP content. The ATP synthase inhibitor, oligomycin A, strongly antagonized 5-FU-induced suppression of cell proliferation. W hen 5-FU sensitivity was compared to ATP synthase activity in six different human colon cancer cell lines, the positive correlation has been found. Bioenergetic dysfunction of mitochondria has been reported as a hallmark of many types of cancers, i.e., down-regulation of ATP synthase β-subunit expression in liver, kidney, colon, squamous oesophageal and lung carcinomas, as well as in breast and gastric adenocarcinomas. Our findings demonstrate that ATP synthase down-regulation may not only be a bioenergetic signature of colorectal carcinomas, but may also lead to cellular events responsible for 5-FU resistance.

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Genetic variation in P-glycoprotein gene (ABCB1) and tipifarnib exposure

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Background: Farnesyl transferase inhibition is a novel approach to cancer chemotherapy in both solid and hematologic tumors. Tipifarnib (Zarnestra™; R115777) is a potent farnesyl transferase inhibitor currently under clinical development as a monotherapy or as a combination therapy with other antitumor agents. P-glycoprotein (P-gp) is an efflux transporter that contributes to transport of drugs from intracellular to extracellular compartments. Hence, interindividual variations in P-gp function may influence drug bioavailability, predisposition to treatment resistance as well as drug-drug interactions among compounds subject to drug efflux mediated by P-gp. The aim of the present study was to evaluate the influence of functional genetic variations in the P-gp gene (*ABCB1*) in relation to clinical pharmacokinetics of tipifarnib.
Material and Methods: 24 healthy volunteers who participated in a food-effect study with a single 100 mg oral dose of tipifarnib were included in the present study. Pharmacokinetic data from the unfed state were utilized for all association analyses. Three synonymous but functional single nucleotide polymorphisms (SNP) in the coding region of the *ABCB1* (C1236T, G2677T, C3435T) were genotyped. Additionally, the key functional C3435T SNP in exon 26 of the *ABCB1* was characterized in a patient sample (N= 29) with advanced solid tumors administered multiple oral doses of tipifarnib (200 mg b.i.d, 4 days).
Results: A high degree of linkage disequilibrium (LD) was observed among the three *ABCB1* SNPs with p-values for all pair-wise LD <0.002. There was no deviation from the Hardy-Weinberg equilibrium in the sample (p values: 0.23–0.69). No significant association was found between haplotypes consisting of any combination of one to three of the *ABCB1* SNPs and tipifarnib C_{max} and AUC_{0-72h} (p values: 0.26–0.99). These observations were consistent with the analysis of the C3435T SNP in relation to tipifarnib C_{max} and AUC_{0-10h} in the patient sample (p values: 0.28–0.57).
Conclusions: Tipifarnib plasma exposure is not appreciably influenced by common genetic variants in *ABCB1*. These preliminary data suggest that P-gp is not involved in tipifarnib absorption in humans.

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E2F-1 induction and MEK inactivation coordinates with p53-generated signals to switch chemotherapy-induced growth arrest to apoptosis in human colorectal HCT116 cancer cells

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Cancer chemotherapeutic agents exert their cytotoxic effect by inducing DNA damage and activating apoptosis. The tumour suppressor protein p53 is an important modulator of apoptosis and whose mutation often affects the sensitivity of cancer cells to chemotherapy. In human colon cancer cell HCT116, anti-metabolite anticancer drug 5-FU triggers a p53-dependent apoptosis, whereas DNA damaging agent adriamycin results in growth arrest albeit both agents are strong p53 activators. To investigate the molecular mechanisms leading to the differential outcomes of DNA damage, we compared the gene expression profiles induced by 5-FU and ADR. We found that 5-FU and ADR induced a similar expression profiles in p53 responsive genes, indicating that differential cellular response to 5-FU and ADR is not due to differential activation of p53 target genes but depends on additional molecular events. Further analysis revealed the activation of E2F-1 pathway in response to 5-FU treatment, which was not observed in ADR-treated cells. Over-expression of E2F-1 in HCT116 cells resulted in apoptosis and partially abrogated the G2/M arrest induced by ADR, which mimics a 5-FU-like phenotype. In addition, signaling pathway analysis indicates that 5-FU treatment results in inactivation of MEK/ERK pathway but ADR did not. Inhibition of this pathway by MEK inhibitor U0126 resulted in a significant apoptosis, suggesting that MEK/ERK pathway is required for the survival of HCT116 cells. Thus, our data suggest that