270 Overcoming chemoresistance in pancreatic and colorectal cell lines

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Pancreatic carcinoma is a very agrresive and difficult to treat type of tumour, with a high mortality rate. Colorectal carcinoma is a very common cause of cancer-related death.
We have used a panel of pancreatic cancer cells and colorectal cancer cells to determine the effects of the Hsp90 inhibitor 17-AAG, the MEK inhibitor AZD6244 and the PI3K/mTOR inhibitor BEZ235, in cell proliferation assays and in cell cycle distribution of DNA content analyses, measured by flow cytometry. We have ranked the cell lines according to the sensitivity/resistance to the differente inhibitors and found differences among them. The pancreatic cell lines RWP1 and Hs766T were very sensitive to 17-AAG in cell proliferation assays, followed by BxPC3, IMIM-PC-2 and IMIM-PC-1 cells, CFPAC-1 and PANC-1 cells being more resistant to 17-AAG. Interestingly enough, PANC-1 cells did not accumulate in G1, G2/M, or subG1 phases of the cell cycle, whereas MIMI-PC-1 cells and especially IMIM-PC-2 cells underwent apoptosis. The colorectal carcinoma cell line HT-29 was sensitive to 17-AAG and accumulated in G2/M followed by an increase in subG1, whereas the CaCo2 cells were resistant to 17-AAG, accumulate in G2/M but did not undergo apoptosis. Experiments are underway to explain the mechanism of resistance to the Hsp90 inhibitor in these cellular models. The colorectal carcinoma cell lines HGUE-1, CaCo2 and HCT-15 were resistant to AZD6244, whereas the colorectal cacrcinoma cell lines SW-620, SW-480 and HT-29 were sensitive and accumulate in G1. The pancreatic cell lines that were sensitive to AZD6244 (IMIM-PC-2, IMIM-PC-1, Hs766T) accumulated in G1 as well, whereas the most resistant cell lines (BxPC3 and PANC-1) did not. Pancreatic carcinoma and colorectal carcinoma cell lines responded to BEZ235 in a similar fashion in cell proliferation assays and in cell cycle experiments. In general, there was an accumulation in the G1 phase of the cell cycle after BEZ235 treatment. We are characterizing these cell lines in terms of relevant molecules involved in the EGFR/ras/MEK and PI3K/Akt/mTOR pathways to determine the effectiveness of the chemotherapuetic drugs used in our study.

271 Irinotecan leads to mTOR activation through IGF1R/IRS-1-activation in colon cancer

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Numerous studies have established a causal link between aberrant insulin-like growth factors (IGF-1) activation and colon tumourigenesis, indicating that inhibiting the signal transduction networks that they regulate may have therapeutic potential. In this study, we show that irinotecan treatment activate the IGF1R-mTOR pathway in colon cancer, and establishes the inhibition of this pathway as a new approach to potentiate irinotecan therapy in colon cancer treatment. The cellular effects of irinotecan, IRS-1 knockdown and rapamycin were characterized for HT-29 and Caco-2 colon cancer cell lines in vitro. The phosphorylation of IRS-1, Akt, mTOR and p70S6K proteins was analysed by Western blot and the effects on the viability of the cells were detected by the MTT assay and flow cytometry. We further xenografted HT-29 in SCID mice and treated with irinotecan, IRS-1 antisense oligonucleotide (ASO), rapamycin, or the combination of the treatments. Our in vitro results showed that SN38, an active metabolic of irinotecan, increases the IGF1R tyrosine kinase activity and the phosphorylation of IRS-1, Akt, mTOR and p70S6K in both cell lines, and the combined knockdown of IRS-1 not only abrogated this effect but also significantly decreased the viability of the cells compared to the drugs alone. Moreover, in contrast to single-agent therapy, xenografted tumours treated with combination of irinotecan and IRS-1 ASO showed potent inhibition of IRS-1, Akt, and mTOR, which was accompanied by a dramatic reduction in tumour volume. We also tested if blocking mTOR would increase the effect of SN38 in HT29 cell line. Rapamycin treatment reverted the mTOR increased phosphorylation caused by SN38, and decreased the viability of the cells exposed to SN38 alone. Again, the combination of irinotecan and rapamycin also decreased the tumour volume of xenografted mouse. Taken together, our findings identify IGF1R-mTOR pathway activation as a consequence of irinotecan treatment and underscore the potential of a combined therapeutic approach with irinotecan and IRS-1 or mTOR inhibitors for the treatment of colon cancer.
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272 ABCC1 polymorphisms in antracycline induced cardiotoxicity in childhood acute lymphoblastic leukemia

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Anthracyclines, such as doxorubicin and daunorubicin are used as chemotherapeutic agents in wide spectrum of cancers, also in childhood acute lymphoblastic leukemia. The usage of anthracyclines is limited by their cardiotoxic side effect. ABC-transporter genes play an important role in the protection of the heart against xenobioticums, but genetic polymorphism can alter gene expression or transporter function.
Our objective was to find associations between the development of cardiotoxicity and single nucleotide polymorphisms (SNP) in genes coding ABC-transporters, genes regulating the expression of ABC-transporters and other genes involved in the distribution and metabolism of xenobiotics.
We performed our analyses on data from 235 paediatric acute lymphoblastic leukemia patients treated with the ALL-BFM 90 or ALL-BFM 95 protocols. Clinical data of the cardiac function was collected from the patients medical records. Genotypes were determined either by PCR-RFLP, Real Time PCR TaqMan probe or 48-plex PCR followed by single base extension (using the GenomeLab SNPstream genotyping platform). We examined altogether 54 single nucleotide polymorphisms in the following genes: *ABCB1, ABCC1, ABCC2, ABCC3, ABCC10, ABCG2, CYP3A4, CYP3A5, CAR, SXR, MTHFR, GGH, RFC1, MVP* and *STMN1*. Presently we have been performing statistical analysis using multivariate and Bayesian network analysis.
Two SNPs, the ABCC1 rs3743527 and ABCC1 rs246221 were found to be associated with significantly altered fractional shortening. Left ventricular fractional shortening was decreased in the patients with the ABCC1 rs3743527 TT genotype at the end of the treatment echocardiography compared to patients with CT or CC genotypes ($p = 0.001$). Regarding the ABCC1 rs246221 SNP, the CT and TT genotype patients had reduced fractional shortening at the time of the latest echocardiography compared to the CC patients ($p = 0.027$).
Our results suggest that genetic variants in the ABCC1 gene are associated with the grade of anthracycline induced left ventricular dysfunction. These polymorphisms should be further examined to individualize chemotherapy in the future.

273 Effect of food on the pharmacokinetics (PK) of axitinib in healthy volunteers (HVs)

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Background: Axitinib is a potent, selective inhibitor of VEGFR 1, 2, and 3 with significant antitumour activity in various tumour types. A definitive food-effect study conducted using a previous crystal polymorph (Form IV) of axitinib indicated ~23% lower plasma exposures obtained with food compared to overnight fasting. This open-label, phase I randomized, single-dose, 3-period, 6-sequence, crossover definitive food-effect study was conducted using polymorph Form XLI commercial image tablets of axitinib.
Methods: A single 5 mg oral dose of axitinib (Form XLI) was administered to 30 HVs under 3 treatment conditions: overnight fasting (10 hours before and 4 hours after dose administration), with a high-fat, high-calorie meal, or with a moderate-fat, standard-calorie meal. The moderate-fat meal assessment was included since it simulated conditions under which oncology patients would take study medication. Subjects (5 per group) were randomized to 6 different sequences of fasted and fed conditions. In each of the 3 periods per sequence, subjects received a single dose of axitinib followed by a washout period of $\geqslant$7 days. Serial blood sampling was performed predose and at intervals postdose to measure axitinib plasma concentrations. Plasma concentrations of axitinib were measured using a validated LC/MS (liquid chromatography/mass spectrometry) method. Axitinib plasma pharmacokinetic parameters were estimated using standard noncompartmental methods.
Results: Compared with overnight fasting (AUC_{inf} 145 ng·hr/mL), a high-fat, high-calorie meal produced a mean 19% increase in axitinib plasma exposure (162 ng hr/mL) while a moderate-fat, standard-calorie meal produced a mean 10% decrease (125 ng hr/mL). Compared with fasting, C_{max} was 11% higher with a high-fat, high-calorie meal and 16% lower with a moderate-fat, standard-calorie meal (25.9, 28.8, and 21.8 ng/mL, respectively). Intersubject variability was >50% for both AUC_{inf} and C_{max} across all 3 treatment arms. There were no significant differences in adverse effects between the 3 treatment arms, and all were mild to moderate in severity.
Conclusions: The effect of high-fat and moderate-fat meals on axitinib exposure did not demonstrate a consistent trend. In addition, the presence of food or type of meal had a minimal effect on axitinib PK. These results indicate that food does not substantially affect axitinib PK in HVs. Single oral doses of axitinib were well tolerated in HVs on all 3 treatment regimens.