114 POSTER

Ursodeoxycholic acid decreases proliferation of normal intestinal epithelial cells in vivo and in vitro

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Introduction: Ursodeoxycholic acid (UDCA) prevents chemical carcinogenesis and aberrant crypt proliferation in a murine model of colon cancer. It also prevents colitis-related colon cancer, where carcinogenesis potentially can be attributed to enhanced proliferation during tissue regeneration. We investigated therefore the effects of UDCA on growth of normal rodent intestinal epithelial cells and on the expression of proliferation-relevant genes.
Materials and Methods: Two groups of six C57BL/6J mice were fed with standard diet containing either no or 0.4% UDCA. After three weeks the mice were killed and the colon was dissected. Formalin-fixed, paraffin-embedded tissue sections were stained immunohistochemically for proliferation marker Ki-67 (clone TEC-3) and the percentage of Ki-67 expressing cells was quantified. In parallel, colonic epithelial cells were isolated and total RNA was analysed using three Affymetrix 430A 2.0 chips per group to identify proliferation-relevant genes affected by UDCA. The gene expression result was validated by quantitative PCR.
For checking UDCA effects in vitro, rat intestinal epithelial cell line IEC-6 was used. MTT test was performed on cells treated with increasing concentration of UDCA (0 to 800 μM) for three days. UDCA treatment-related expression changes of genes identified by the arrays were validated by using RNA of UDCA-treated IEC-6 cells.
Results: UDCA reduced the number of Ki-67 expressing cells to 60% of the value in the non-treated group. There was no change in total number of cells per crypt. The microarray results showed more than 2.5 fold suppression of more than 10 proliferation-regulating genes by UDCA, including TCF4. All alterations were confirmed by PCR. IEC-6 cells showed in MTT test 40% and 50% decrease in proliferation at 600 μM and 800 μM UDCA, respectively. Real-time PCR of the RNA isolated from the cells showed 2 to 25-fold suppression of the proliferation-regulating genes identified in mice, thus confirming the microarray data.
Conclusions: Our results indicate that UDCA decreases the proliferation of normal colonic epithelial cells both in vivo and in vitro. This decrease is concomittant with the suppression of several genes which positively regulate proliferation. This model is suitable for the investigation of target genes of UDCA and of the mechanism of its chemopreventive action.

115 POSTER

Effects of MMR status on colon carcinoma cell survival after 5-FU treatment in vitro and in vivo

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Introduction: Whether mutations in the hMLH1 gene, which is a component of the mismatch repair (MMR) system, increase or decrease the sensitivity of colon carcinomas to 5-fluorouracil (5-FU) is a matter of controversy. We reinvestigated the effects of hMLH1 on the response of colon carcinoma cells to 5-FU in vitro and in a mouse model in vivo by using the established semiisogenic cell system HCT116/HCT116Chr3.
Materials and Methods: HCT116 (MMR−) or HCT116Chr3 (MMR+) cells were treated with 30 μM 5-FU for 48 h and clonogenic survival, long term growth and the activation of the signalling pathways were followed by Western blots and by FACS. For the in vivo experiments cells were injected into nude mice, the resulting tumours were treated with 50 mg/kg 5-FU for 5 consecutive days and the tumour growth was followed.
Results: The clonogenic survival, determined in relation to the nontreated controls, was higher in the MMR− than in the MMR+ cells. The long term total cell survival, however, determined in relation to the total number of surviving cells in the nontreated controls, was lower in the MMR− cells. The lesser total survival of MMR− cells was associated with a higher overall cell death, detectable by trypan-blue staining and a higher necrosis, determined by LDH release. The number of double strand breaks, measured through the extent of histone phosphorylation, and the apoptosis, determined as the extent of PARP cleavage, were higher in the MMR+ cells. The tumours originating from the MMR+cells were less responsive to treatment with 5-FU than the MMR− cell line-derived tumours. Thus the clonogenic survival result reflected the differences in the extent of apoptosis between the two cell lines while the result in vivo corresponded to the overall cell death in vitro.
Conclusions: The comparison of the in vivo and in vitro results of 5-FU treatment indicate that the long term total cell survival is a better predictor of the therapeutic effect of 5-FU than the clonogenic assay. The data in vivo support the clinical reports showing that MMR− tumours respond better to 5-FU therapy than the MMR+ tumours.

116 POSTER

Effective combination of green tea EGCG and EGFR-TKI erlotinib for chemoprevention and therapy in head and neck cancer

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Background: We have previously shown that green tea polyphenols particularly epigallocatechin gallate (EGCG), had a synergistic anti-tumor activity with a epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) Erlotinib, in cell culture and nude mouse xenograft model of squamous cell carcinoma of the head and neck (SCCHN) (Int. J. Cancer, in press, 2008). However, the mechanism of their anti-tumor synergism is not fully understood.
Materials and Methods: To this end, annexin V-PE staining was performed to study apoptosis, and western blotting, for the expression pattern for the cell cycle and apoptosis regulatory proteins. Lenti-virus based expression system using short hairpin RNA (shRNA) was employed to ablate the expression of specific proteins in this experiment.
Results: The treatment of head and neck cancer cell lines with Erlotinib showed time-dependently increased (up to 15 folds) expression of cell cycle regulatory proteins, both p21 and p27 and an apoptosis regulatory protein Bim, and also induced Puma in some cell lines. Cells underwent G1 arrest with very little apoptosis (up to 10%). Furthermore, ECGC alone (30 μM) had very little or no effect on the expressions of these proteins varying among the cell lines and induced minimal apoptosis (8−10%). However, simultaneous treatment of EGCG and Erlotinib synergistically increased apoptosis (~40−45%) and strongly inhibited (up to 80% reduction) the expression of p21 and p27 without affecting the induction of Puma and Bim, while Erlotinib induced both Foxo1a and p53 transcription factors in some cell lines. EGCG also induced these proteins. Ablation of p53 by shRNA suggested that p53 is dispensable for the expression of Bim, p21 or p27 and for the synergistic apoptotic effect. These results indicate that Erlotinib induced both checkpoint proteins p21 and p27 and apoptosis regulatory proteins Bim and Puma probably by activating Foxo1a transcription factor. EGCG synergistically increased the anti-tumor activity of Erlotinib by inhibiting the checkpoint proteins p21 and p27 without affecting the expression of Bim and Puma.
Conclusions: Therefore, the combination of green tea EGCG and Erlotinib is highly promising for the further development of cancer prevention and therapy particularly for head and neck cancer.
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Drug delivery

117 POSTER

ANG1005, Paclitaxel conjugated to the angiopep brain transport vector for the treatment of brain cancer: preclinical studies

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Background: The blood–brain barrier (BBB) is mainly formed by brain capillary endothelial cells which are closely sealed by tight junctions and express high levels of active efflux transport proteins, including P-glycoprotein (Pgp). As a result, the overwhelming majority of small molecules, proteins and peptides do not cross the BBB. In the present study, we investigated the utilization of a new peptide based drug delivery technology that provides a non-invasive and flexible platform for transporting drugs into the central nervous system.
Material and Methods: In situ brain perfusion were used to assess the brain uptake of our conjugates. Analysis of tissues was done after extraction by LC-MS-MS or HPLC. Xenograft models of glioblastoma (U87) were established by intracranial stereotaxic injections of U87 cells in mice and rats.
Results: Angiopep2-Cy5.5 is very rapidly transported in the brain parenchyma as visualized in the brain after intravenous and in-situ brain perfusion. Higher fluorescence was also detected in the brain tumor compared to the normal brain. Based on these properties, we have created several new drug entities, the most advanced of which is ANG1005 formed by chemical conjugation of our peptide vector (angiopep-2) to three

molecules of paclitaxel. In contrast to free paclitaxel, which is normally prevented from reaching the brain by the Pgp efflux pump, ANG1005 is efficiently transported across the BBB, with approx. 100 fold higher transport rate compared to free paclitaxel and 10 fold higher transport rate than Temozolomide measured using in-situ brain perfusion in rats. In addition, ANG1005 is homogenously distributed in rat brains. ANG1005 was detected by LC-MS-MS in both normal brain and brain tumors in mice 30 minutes after i.v. injection; detected brain levels of 2.1 μM are above the therapeutic concentrations of paclitaxel. The effect of ANG1005 was evaluated on glioblastoma (U87) xenograft tumor growth in immune deficient mice and resulted in a significant increase of survival of mice treated with ANG1005 of 27%. In a rat glioblastoma (U87) brain orthotopic model, administration of ANG1005 resulted in a shrinking of IC tumors measured by MRI.
Conclusion: The AngioPep peptide vector can be used to transport small drugs to the brain parenchyma for the treatment of brain cancers. ANG1005 is currently under evaluation in two phase 1 clinical trials for the treatment of primary and secondary brain tumors in humans.

118 POSTER

Comparative pharmacokinetic study of abiraterone acetate in a capsule and tablet formulation

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Abiraterone acetate is a novel 17-hydroxylase/c17−20 lyase inhibitor, blocking the conversion of pregnenolone to dehydroepiandrosterone (DHEA) and progesterone to androstenedione, thus inhibiting the production of testosterone and oestrogen precursors. Consistent with the continued androgen signalling in a substantial proportion of castration resistant or hormone refractory prostate cancer patients, this compound has shown impressive decrease in PSA levels associated with significant antitumour activity in Phase I and II studies. The present study was carried out to assess and compare the pharmacokinetic profile of abiraterone following administration of capsules or tablets of abiraterone acetate, under fed (after an experimental high fat-high caloric meal) and fasted conditions, in patients with progressive prostate cancer despite GnRH analogue treatment. The study was two armed (fed or fasted) with patients enrolled in either group 1 (Study Day [SD] 1 capsules, SD 2 tablets) or group 2 (SD 1 tablets, SD 2 capsules) at a dose of 1000 mg abiraterone acetate. Blood samples collected pre-dose and 1, 2, 4, 6, 8, 24, 48, 72 hours post dose were analysed by LCMSMS and pharmacokinetic parameters derived. A 2×2 crossover model was preformed with fixed effects for sequence, formulation and period, and random effect for subject nested within sequence, on AUC(0-t), AUC(0-∞) and Cmax, using capsule formulation as the reference in 27 evaluable patients. The confidence interval for fixed effect was 95%. Our study shows that the 90% confidence intervals for AUC and Cmax are between the 119–293% in fasted state, and in the fed state, 65–141%. The only significant difference in the two formulations was in the Tmax in the fasted state suggesting that the absorption is faster in the capsules compared with the tablets. As previously determined, high fat-high caloric meal significantly increased the exposure to abiraterone.

119 POSTER

The effects of treatment sequencing on the antitumor activity of vandetanib and paclitaxel in a model of ovarian carcinoma xenograft

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Background: We have previously shown (Cesca et al, AACR 2008) that vandetanib (ZACTIMA™), an inhibitor of VEGFR2, EGFR and RET signaling, induced vascular morphological and functional changes in human ovarian carcinoma xenografts grown in nude mice (reduced vessel area, increase in mature vessels and diminished vascular permeability). Furthermore, vandetanib pretreatment reduced tumor uptake of paclitaxel (PTX) at early timepoints after its injection. The purpose of this study was to use this information to guide the schedule of combination treatments.
Materials and Methods: PTX distribution, after continuous or intermittent (five days suspension) vandetanib pre-treatment, was analyzed by HPLC in plasma and tumors at 1 h and 24 h after PTX injection. In parallel tumors, tumor perfusion was assessed by determining Hoechst 33342 content by HPLC. The antitumor activity of combination treatment was examined by giving vandetanib (50 mg/kg/day p.o., five days time course), before or after PTX (20 mg/kg i.v.) in A2780−1A9 tumor bearing mice. Tumor growth was evaluated, and response expressed as the best T/C % and growth delay (T-C), compared with vandetanib alone.
Results: Pretreatment with vandetanib for five days resulted in a 30% decrease in tumor uptake of PTX measured one hour after PTX injection. However, after suspending vandetanib treatment for five days the PTX biodistribution in tumor was similar to that in controls not treated with vandetanib. Diminished H33342 levels in the tumor after vandetanib treatment (20% to 30%) suggested that the decrease in PTX biodistribution was associated with reduced tumor perfusion. The administration of PTX followed by vandetanib resulted in greater antitumor activity (T/C = 18%) compared with the reverse sequence (T/C = 40%). Repeating these treatments for 3 cycles, with five days break between cycles, further increased the growth delay (T-C = 21 days for PTX followed by vandetanib and T-C = 13 days for vandetanib followed by PTX).
Conclusions: These findings indicate that there is an improved antitumor activity of vandetanib plus PTX, compared with either agent alone. The data also suggest that, in this model, the combination of vandetanib and PTX had greater antitumor effects when vandetanib was given after PTX.

120 POSTER

Customized PEG linkers improve tumor delivery of RNA antagonist oligonucleotides

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Background: Locked Nucleic Acid (LNA) antisense oligonucleotides (LNA-ONs) are novel RNA antagonists capable of potently silencing mRNA targets in vitro (at low to sub-nanomolar concentrations) and in vivo. Nevertheless, systemic delivery of LNA-ONs may be further improved if more favorable pharmacokinetic profile, cell penetration, and specific tumor targeting were possible. We describe here the utility of Customized PEG linkers that enhance cellular uptake of LNA-ONs resulting in potent down-modulation of target mRNA in human tumor cells and improved tumor delivery of LNA-ONs in tumor-bearing mice.
Material and Methods: Customized PEG linkers were synthesized by incorporating either cell penetrating peptides (CPP) or folate into the PEG polymers, which were then converted to releasable linkers before being conjugated with anti-survivin or anti-ErbB3 LNA-ONs. Target gene down-modulation by PEG-LNA-ON conjugates was evaluated using Real-Time (RT)-PCR. The PEG conjugates were administered intravenously to tumor-bearing mice to study the biodistribution and target knockdown by hybridization methodology and RT-PCR, respectively.
Results: When CPPs or folate were attached to the PEG-LNA-ON conjugates, marked intracellular delivery of the PEG conjugates was demonstrated by fluorescence microscopy that was comparable to cells transfected with lipofectamine. CPP-PEG-LNA-ON conjugates have shown concentration-dependent and target-specific mRNA down-modulation in a panel of tumor cell lines. In contrast, folate conjugates did not improve down-modulation of the target mRNA in the absence of transfection and are trapped in intracellular vesicles. Both enhanced tumor accumulation of oligonucleotides and improved mRNA down-modulation in human tumor tissue implanted in mice was observed with PEG conjugates compared with naked LNA-ONs.
Conclusions: Customized PEG linkers have improved the in vitro cellular uptake of LNA-ONs. PEG-LNA-ON conjugates with CPP or folate can efficiently enter cancer cell without transfection. However, only CPP-PEG-LNA-ONs have improved knockdown of target mRNA without transfection. Both the enhanced permeation and retention effect and targeted delivery probably promote LNA-ON accumulation and modulation of gene expression in the tumor. Customized PEG linkers may provide a promising approach for more efficient in vivo delivery of oligonucleotides including LNA-ONs and siRNAs.