451 POSTER

Irradiation-driven tumor selectivity of liposome-based gene therapy targeting Akt activation

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The vascular network is a highly accessible target for tumor therapy. However, as for any cancer treatment, the primary goal is to deliver sufficient amounts of drug to the targeted tissue while minimizing damage to healthy organs. Cationic liposomes have been identified as delivery systems significantly more effective at targeting tumor *versus* normal vascular networks. Though, the liposome uptake by the liver restricts their potential as shuttles to selectively target the tumor endothelium. Here, we reasoned that additional selectivity could be found in the nature of the delivered gene and by combining another anti-tumor therapy. Accordingly, the pro-survival PI3K /Akt pathway is thought to be activated by ionizing radiations and a dominant-negative Akt would therefore mostly target tumors (versus non-irradiated organs). Furthermore, we have recently documented that irradiation led to NO-mediated tumor vessel dilation which could thereby enhance the access of liposomes to the tumor. In this study, we have therefore examined whether radiotherapy and the use of dominant-negative Akt plasmid delivered by cationic liposomes could mutually improve their efficacies.
We first used cultured endothelial cells and isolated tumor microvessels to demonstrate that low dose irradiation led to the stimulation of both Ser^{473} Akt and Ser^{1177} eNOS phosphorylations (e.g., activation); the use of a PI3K inhibitor further indicated that the former largely accounted for the increased NO/cGMP production. Using a reporter-encoding plasmid, we then showed that irradiation dramatically enhanced the *in vivo* expression of the tagged protein in the tumor endothelium. Also, using $eNOS^{-/-}$ mice, we documented the key role of NO in mediating the adjuvant effects of X-Ray on plasmid delivery, likely through an increase in tumor blood flow. We then combined local irradiation to the liposome-dominant negative Akt DNA complex administration. In two different protocols associating gene therapy with either a single 6 Gy dose or a 5×2 Gy fractionated scheme, we consistently observed synergistic effects of the combinatory treatment. In fact, the transgene when administered alone, did not reveal any tumor response and the tumor growth delay after irradiation represented less than 50% of the gain obtained when combining both approaches (n=8; $P<0.01$); these findings were obtained in two mouse tumor models.
In conclusion, the combination of low dose radiotherapy and liposome-cargoed dominant-negative Akt gene therapy appears particularly well suited to selectively target tumor vasculature. Besides the intrinsic tumor specificity of local X-Ray administration and the propensity of cationic liposomes to bind tumor vessels, we have further identified a "treatment-driven" selectivity, e.g. the ability of radiotherapy to induce Akt activation in tumor vasculature and to increase the liposome access to the tumor.

452 POSTER

The oncolytic reovirus, Reolysin, augments the anticancer effects of cytotoxic agents in vitro against the ras-mutated human colon cancer cell line HCT116

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The type 3 reovirus, Reolysin, is an oncolytic virus with selectivity for ras-activated cells both in vitro and in vivo. In order to better define the activity of Reolysin in vitro and specifically to study interactions between Reolysin and cytotoxic agents, cell proliferation studies were undertaken in HCT116 cells. Reolysin was highly cytopathic against HCT116 with 60% reduction in cell number at doses as low as 1×10^{-5} pfu and nearly 100% at doses of 0.1 pfu at 7 days exposure. With as little as 2 days of exposure to virus, there was 50% cytotoxicity with doses of 1 pfu. Of interest, Reolysin was equally active against cells that were 80% confluent or at confluence as low as 50%. Despite enhanced cytotoxic activity in ras-mutated cells, previous studies have also demonstrated enhancement of activity in cells with a disrupted ras pathway. In order to test this hypothesis, we studied HCT116 variants in which ras was disrupted by homologous recombination. Of interest, these cells demonstrated equivalent sensitivity to Reolysin on days 2–7 and at doses of $1x\ 10^{-4}$ to 10 pfu. To determine whether Reolysin augmented the effects of various cytotoxic agents, cells were exposed to cytotoxic agents for 3 days with or without Reolysin, 0.1 pfu. The reovirus enhanced the cytopathic effects of fluorouracil at every concentration tested. Similar effects were observed with gemcitabine. Enhancement of the cytotoxicity of doxorubicin and cisplatin was observed, but only at concentrations approaching the IC50 for these agents. No synergy was observed with paclitaxel. Reolysin has in vitro activity against ras-mutated HCT116 and its ras-disrupted variant, and enhanced the cytotoxicity of fluorouracil, gemcitabine, doxorubicin and cisplatin against HCT116, making it a promising agent for clinical trials.

453 POSTER

Validation of pharmacodynamic assays to determine the clinical effect of an antisense to the XIAP inhibitor of apoptosis

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XIAP is a potent endogenous inhibitor of caspases shown to be overexpressed in human tumours. Recently, a 19-mer second generation antisense oligonucleotide targeting the protein was approved for Phase I evaluation in the UK. Validation strategies and performance data on 4 different assays are presented that will be incorporated as endpoints during this trial. Immunohistochemistry (IHC) of XIAP in tumour biopsies was validated using fixed sections of tissues obtained from XIAP-null mice and matched (wild-type) control animals. These functioned as both positive and negative controls to determine the specificity of the methodology and as replicate quality controls (QCs) in order to assess reproducibility, within-day and between-day precision and stability of the protein. An immunostaining method producing a weighted mean that takes account of both staining intensity and percentage of expressing cells was adopted to score sections. The M30-Apoptosense™ Elisa detects a caspase cleaved fragment of cytokeratin 18 that is released into the circulation after apoptotic cell death and is believed to represent a plasma surrogate marker enabling a quantitative assessment of apoptosis occurring in the patient's tumour. Validation of this technique focussed on generating an independent positive quality control to perform measurements on precision, kit-to-kit QC, and stability studies. Between-day precision data obtained using this control ranged from 3.6–6.7%. Kit to kit variability was less than 10%, while samples stored at −80ºC were demonstrated to be stable for at least four months. To facilitate validation of a Western blotting method for XIAP, we employed replicate cell pellets consisting of a genetically-engineered breast cancer cell line (MDA-MB-231/X-G4) stably expressing an RNAi to silence XIAP as a negative control and 2 positive control cell lines. XIAP was quantitated by densitometry as a ratio to GAPDH and by reference to a 5 point calibration curve (1.8–147 pg/ug) constructed using recombinant GST-XIAP protein added to a surrogate protein matrix. Calibration curves were linear with a mean r^2 value of 0.997. Between day precision was 29% for the low XIAP expressing control and 23% for the high XIAP expressing control. Finally, real time RT-PCR to quantitate XIAP mRNA was validated using two different housekeeping genes (GAPDH and B2M), high and low expressing cell lines as QCs and an 8 point calibration curve constructed from a XIAP cDNA plasmid.

454 POSTER

Highly active, cancer specific promoters for gene therapy of small cell lung cancer

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One of the major problems in conventional, non-invasive cancer treatment is the lack of specific targeting to the cancer cells. For targeted gene therapy, the lack of cancer specific receptors has proven a major obstacle. Less specific receptors can be used for targeting by having the expression of a therapeutic gene regulated by a cancer specific promoter. The identification of novel highly active, cancer specific promoters would improve the potential of cancer gene therapy.
For small cell lung cancer (SCLC) there is currently no satisfactory treatment, wherefore development of novel modalities, such as gene therapy, is highly in demand. Using global gene expression analyses (oligonucleotide arrays), we have identified a number of highly and specifically expressed genes in SCLC (Pedersen N. *et al*., *Cancer Res.* **63**, p1943, 2003). Several of the genes identified by the array analysis are known to be transcriptionally regulated in a developmental manner and several reflect the neuroendocrine origin of SCLC.
The gastrin releasing peptide (GRP) gene is highly expressed in SCLC tumors and cell lines and the GRP promoter has been suggested for gene therapy for SCLC. We found expression levels of a reporter gene from the GRP promoter was as strong as the SV40 promoter in a number of SCLC cell lines. However, as GRP is expressed in some normal adult tissues, this may limit its use for gene therapy.
The insulinoma-associated antigen-1 (INSM1), in contrast, is exclusively expressed during early embryonal development, but has been found re-