imaging was highly sensitive to detect only a non-palpable cluster of cancer cells in vivo.

447 POSTER

Retargeting adenoviral gene therapy vectors to bladder tumours over-expressing EGFR and other cell surface markers

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The efficacy of adenovirus-mediated cancer gene therapy is potentially compromised by relatively low levels of expression of the human coxsackie-adenovirus receptor (hCAR) in a variety of tumour biopsy panels and cell lines. Several reports have described methods to by-pass the natural tropism of the adenovirus for hCAR, including genetic modification of adenoviral fibre-knob domains, adenobodies, diabodies and bi-specific fusion proteins (Dmitriev *et al*, 2000; *J. Virol.* **15**, 6875–84). Relative over-expression of epidermal growth factor receptor (EGFR) in human bladder tumour specimens compared with normal bladder urothelium correlates with poor prognosis. Retargeting of adenoviral cell-surface binding from hCAR to EGFR therefore represents an attractive means of achieving tumour-selective entry of adenoviral gene therapy vectors. In this report we describe the production of a fusion protein composed of the extracellular domain of hCAR fused with a 53 amino acid peptide encoding the mature form of human epidermal growth factor (EGF) (sCAR-EGF_{53}). Using a *lacZ*-expressing replication-defective adenovirus, we show that this protein preferentially targets tumour cell lines expressing low levels of hCAR and high levels of EGFR. Pre-incubation of the fusion protein with the adenovirus improves the infectivity of low-hCAR/high-EGFR cells from 2–5% to 95–100%. Reversal by anti-EGFR neutralising antibody confirms the specificity of the retargeting. Of equal significance, our fusion protein also hinders entry into high hCAR-expressing cells. We show that the sCAR-EGF_{53} protein increases marker gene expression in a variety of human bladder tumour cell lines, and that the improvement correlates well with CAR/EGFR expression status of the cells as determined by Western blotting. The design of our fusion protein construct permits substitution of EGF_{53} by other ligands, allowing the straightforward production of a number of fusion proteins designed to target cell surface molecules that are over-expressed in tumours. We demonstrate selective targeting of bladder tumour cell lines based upon knowledge of their expression profiles, using a panel of bi-specific fusion proteins.

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Targeting protein kinase CK2 for induction of apoptosis as an approach to cancer therapy

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Protein kinase CK2 (formerly casein kinase 2 or II) has been found to be dysregulated in cancers. Its role in cell growth and proliferation has long been known. However, besides its function in cell proliferation, CK2 can also exert a potent suppression of apoptosis mediated by agents such as etoposide, heat shock, and removal of survival factors (e.g., Ahmed et al., Trends in Cell Biology, 12: 226–230, 2002). We have now found that CK2 can block death receptor-mediated apoptosis induced by factors such as TNF-α, TRAIL, and Fas-ligand in responsive cells. Because of the extensive role of CK2 in cell growth and suppression of apoptosis, we examined the effects mediated by its downregulation. Various prostate cancer cells and squamous cell carcinoma of the head neck were treated with antisense CK2α. This resulted in extensive induction of apoptosis in a dose and time-dependent manner. Extension of these studies to xenograft models showed that prostate cancer tumors were eradicated when treated with antisense CK2α in vivo. Under similar experimental conditions the normal cells and tissue were relatively resistant to the effect of the antisense. We are also developing an approach to targeted delivery of the antisense to the tumors in vivo by encapsulating the antisense in sub-50 nm Tenascin nanocapsules which are taken up by the tumor cells via the caveolar route. Preliminary results suggest the feasibility of this approach for eradication of the xenograft tumor. These observations provide an initial "proof of the principle" for the potential feasibility of targeting CK2 for cancer therapy. [Supported by N.C.I. and V.A. Medical Research funds].

449 POSTER

Treatment of pancreatic cancer by TGF-beta2 suppression mediated by the antisense oligonucleotide AP 12009: Preclinical efficacy data

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Background: With a 5-year-survival rate of less than 1% pancreatic cancer is one of the most aggressive human cancers. Overexpression of TGF-beta2 in malignancies such as pancreatic cancer and glioma is associated with advanced tumor stage due to its pivotal role in malignant progression by inducing key mechanisms including immunosuppression, metastasis, angiogenesis and tumor cell proliferation. In pancreatic cancer patients TGF-beta2 levels were more than 3-fold elevated as compared to healthy controls.
Methods: Aiming at a highly specific anti-tumor therapy, AP 12009, a phosphorothioate antisense oligonucleotide specific for the human TGF-beta2 mRNA, has been developed and tested for its anti-tumor activity in a variety of preclinical studies with pancreatic cancer cells.
Results: AP 12009 significantly reduced the TGF-beta2 secretion in several human pancreatic cancer cell lines (Hup-T3, Hup-T4, PA-TU 8902). In functional assays AP 12009 inhibited pancreatic tumor cell proliferation in a dose-dependent manner by up to 76%. Migration of PA-TU 8902 cells was completely blocked as compared to untreated controls in a spheroid migration model in contrast to a TGF-beta2 antibody which had no effect. Additionally, AP 12009 reversed TGF-beta2 mediated immunosuppression in an allogenic system with pancreatic cancer cells targeted by IL-2 activated PBMC (LAK cells) derived from healthy donors. After AP 12009 treatment LAK cell cytotoxicity was increased in a donor-dependent manner up to 401% of the untreated control (effector/target ratio 20:1).
Conclusions: AP 12009 has already shown efficacy in phase I/II clinical studies as therapy for high-grade glioma. Based on this successful application of AP 12009 in clinical studies in glioma patients and the presented preclinical efficacy in tumor proliferation, migration and inhibition of escape from immunosurveillance in pancreatic cancer cells a multi-site dose-escalation trial with AP 12009 in pancreatic carcinoma has been started.

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The TGF-beta1 antisense oligonucleotide AP 11014 for the treatment of non-small cell lung, colorectal and prostate cancer: preclinical studies

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Background: Tumor derived transforming growth factor beta (TGF-beta) is a pivotal factor for malignant progression by inducing metastasis, angiogenesis and tumor cell proliferation and by mediating the tumors' escape from immunosurveillance. In colorectal, non-small cell lung and prostate cancer in particular expression of the TGF-beta1 isoform has been correlated with tumor progression and poor clinical prognosis. Significantly elevated TGF-beta1 plasma levels to more than 3-fold in colon cancer and NSCLC and to more than 4-fold in prostate cancer further support the role of TGF-beta1 as a key tumor promoter.
Methods: The in vitro effects of AP 11014, a TGF-beta1 specific phosphorothioate antisense oligonucleotide, on TGF-beta1 secretion, proliferation, migration of and immunosuppression by various cancer cell lines were determined.
Results: AP 11014 significantly reduced TGF-beta1 secretion by 43–100% in different NSCLC (A549, NCI-H661, SW 900), colon cancer (HCT-116) and prostate cancer (DU-145, PC-3) cell lines. Tumor cell proliferation was inhibited in a dose-dependent manner in all cell lines. In a scratch assay AP 11014 reduced migration of a NSCLC (SW 900) and prostate cancer (PC-3) cell line by max. 65% after 24h. Additionally, AP 11014 reversed immunosuppression mediated by NSCLC (NCI-H661, A-549), colon (HCT-116) and prostate cancer (PC-3) cell derived TGF-beta1 by increasing LAK cell cytotoxicity up to 368% of the untreated control (effector/target ratio 5:1).
Conclusion: These preclinical data clearly indicate antisense mediated suppression of TGF-beta1 by AP 11014 as a highly promising approach for the therapy of non-small cell lung, colorectal and prostate cancer in humans. Based on these data a clinical trial with AP 11014 in TGF-beta1 overexpressing tumors is in preparation.