protein gene BS69. In conclusion, adenoviral vectors efficiently transduce ACC cells in vitro and in vivo. Combined adenoviral vector-mediated delivery of suicide and cytokine genes eradicates tumors in mice. Adenoviral vector infection of ACC cells modulates gene expression profile, upregulating genes involved in cortisol production and stress response.

537. Transcriptionally Targeting the Survivin Promoter to Human Gliomas

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Survivin, a novel member of the IAP (inhibitor of apoptosis) protein family, is associated with malignant transformation and is overexpressed in human brain cancers. On this basis, we hypothesized that the human survivin promoter may be an excellent candidate for glioma gene therapy by transcriptional targeting. To that end, we compared three recombinant adenoviral vectors (reAds), reAdGL3BCox-2, reAdGL3BMidkine, and reAdGL3BSurvivin, in which the reporter gene luciferase is driven by the Cox-2, Midkine, or surivin promoter, respectively, in glioma cell lines and primary glioma cells. The data showed the survivin promoter exhibited the highest activity among the three reAd vectors in both established and primary glioma cells. We also compared the survivin promoter activity between in D65 glioma tumor cells and in normal non-transformed astrocytes. The results indicate there is 6-12 fold higher activity in tumor cells than in the astrocytes. Also, the survivin promoter is down regulated in human brain tissue compared to the cytomegalovirus promoter. Next, the conditionally replicating adenoviral vector (CRAd), CRAd-Survivin-RGD, in which the Ad E1 gene is driven by the survivin promoter, and the Ad infectivity is enhanced by capsid modification with RGD, was seen to efficiently kill the multiple established glioma tumor cells (oncolysis). These CRAd agents driven by the survivin promoter were further shown to efficiently replicate in both the glioma cells (D65) and in U118MG xenografts both *in vitro* and *in vivo*. Also we observed the anti-tumor effects of the CRAd agents in an animal model. The tumor growth was inhibited more than 60% by i.t. injection of CRAd-Survivin agents. In conclusion, the survivin promoter is a promising tumor-specific promoter for transcriptional targeting based on its activation in glioma cells, efficient reporter gene expression, adenoviral replication in gliomas and repression in normal cells. This promoter has a "tumor on" and "normal tissue off" profile, an important parameter for cancer gene therapy. To that end, therapeutic vectors based on this approach may translate into treatment of human gliomas in the clinic.

538. Fiber Chimeric Oncolytic Adenoviral Vectors Mediate Efficient Gene Transfer and Oncolysis of Head and Neck Cancer and Melanoma Cells In Vitro and In Vivo

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Current treatment modalities for melanoma and head and neck cancers (HNC) are not completely effective and there is an urgent need for development of novel therapeutic strategies. Oncolytic adenoviruses (OAVs) designed to selectively replicate and kill cancer cells are rapidly emerging as promising agents for oncolytic virotherapy. However, low expression levels of the adenovirus receptors, CAR and integrins in melanoma and HNC cells have imposed significant hurdles to the use of adenovirus type 5 (Ad5)-based vectors. To improve gene transfer and mediate efficient oncolysis of these cells, fiber chimeric vectors in which the fiber knob was replaced with the fiber knob domain of Ad35 were generated. Marker gene expression mediated by the fiber chimeric vectors in melanoma and HNC cell lines was approximately 10-fold higher than that obtained with parental Ad5 virus. The fiber chimeric structure was then built into an oncolytic adenovirus, in which the expression of the E1a gene is placed under the control of E2F-1 promoter. The cytotoxicity mediated by fiber chimeric vectors in melanoma and HNC cells was 100-fold better than that mediated by the parental Ad5 vector. In addition, the fiber chimeric structures improved the virus yields as much as 100-fold in several melanoma and HNC cells. These results correlated with low levels of CAR and high levels of CD46 on the surface of the cancer cell lines. The results of in vitro transduction, cytotoxicity and virus yields assays correlated with the results of in vivo anti-tumor efficacy studies in FaDu xenograft tumor model in nude mice. These findings have important implications for the potential treatment of melanoma and HNC using fiber chimeric oncolytic adenovirus.

539. Antitumor Activity of an hTERT-Specific, GFP Expressing Oncolytic Adenovirus

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Oncolytic viruses are designed to selectively replicate and lyse tumor cells by using mutations that restrict the lytic viral cycle to tumorigenic tissue or by using tumor-specific promoters to control essential early viral genes. However, various preclinical and clinical studies with oncolytics suggest that, though tumor specificity and replication are achieved, viral replication alone may not be sufficient to inhibit tumor growth. Also, several reports have indicated that oncolytics expressing additional therapeutic genes may also improve anti-tumor effects. Here we describe the construction and functional characterization of a green fluorescence protein (GFP)-expressing oncolytic adenovirus designated Ad/hTC-GFP-E1, whose transgene, GFP, and viral E1A gene are both under the control of a synthetic promoter (hTC) consisting of fusion sequences from the human telomorase reverse transcriptase (hTERT) promoter and minimal cytomegalovirus early promoter (CMV). hTC is used twice to express viral genes and transgenes separately, at high levels and in a tumor-specific manner, providing tumor restricted viral replication and additional effects mediated by the transgene of choice. Our virus' tumor specificity has been demonstrated by Western blot, fluorescence-activated cell sorting analysis, crystal violet staining, and cell viability assays. In addition, our virus has been shown to