S17

ONCOLYTIC MEASLES VIROTHERAPY FOR OVARIAN CANCER

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Live attenuated measles virus (MV-Edm) has potent and selective oncolytic activity against ovarian cancer. A single MV-Edm infected tumor cell fuses with neighboring cells to form a multinucleated syncytium but the virus caused minimal cytopathic damage on non-transformed cells. To tailor measles virus for cancer therapy, the virus was genetically modified to express the soluble extracellular domain of human carcinoembryonic antigen (MV-CEA). Virus replication and viral gene expression in infected cells resulted in production and release of CEA into the extracellular space/circulation. This MV-CEA virus is being translated into a phase I clinical trial for ip delivery into patients with recurrent ovarian cancer. We also determined the biodistribution of viral replication and gene expression in MV-susceptible transgenic mice. Viral genome was most abundant in the spleen and no viral RNA was detected in brain, lungs and heart. Mice were also injected with MV-eGFP and we found very few infected mesothelial cells but a strong green fluorescence was apparent in the spleen and the greater omentum. Immunohistochemical analysis indicated that the majority of the infected cells were macrophages. We are currently in the process of modifying the tropism of MV-CEA to infect only ovarian cancer cells and not non-target cells.

S18

"COMBINATION THERAPY USING ADGFPFASL$_{TET}$ AND CERAMIDASE INHIBITORS OVERCOMES RESISTANCE TO APOPTOSIS IN DU145 PROSTATE CANCER CELLS"

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The application of gene therapy to cancer has a difficulty not in the choice of therapeutic genes *per se* but delivery of a corrective signal to every cell in the cancer. Thus, studies on the delivery of therapeutic genes or how to amplify the response to gene therapy are urgently required. In the DU145 model of prostate cancer, the resistance to induction of apoptosis through the Fas receptor signalling pathway is due to overexpression of apoptotic resistance genes including cFLIPs [Hyer et al., Can Biol. and Ther. 1(4): 405-410, 2002]. We have also determined that expression of a FasL-GFP fusion gene overcomes resistance in infected cells [Hyer et al., Mol. Ther. 2(4):348-358, 2000]. To overcome the delivery issue (<30%), we have determined that overexpression of the GFPFasL fusion protein overcomes resistance, kills the cell apoptotically, and produces apoptotic vesicles that will signal Fas to induce apoptosis (i.e. bystander activity) [Hyer et al., Can. Gene Ther. 10(4):330-339, 2003]. However, because of expression of apoptotic resistance genes some cancers, including the DU145 model, are resistant to the apoptotic vesicles and are thus, relatively insensitive to vesicle-mediated bystander activity. To overcome this, we have examined the role of ceramide in this process by using small molecule ceramidase inhibitors. We now demonstrate that the acid ceramidase inhibitor, LCL204, which acts by increasing intracellular ceramide levels, is highly efficient at activating cell death in DU145 cells at nontoxic doses when combined with AdGFPFasL virus at MOIs achievable *in vivo. In vivo* experiments support this therapeutic approach. We will present selected data at the meeting to shed light on the mechanisms of this combined therapy. This work is supported by DOD N6311601MD10004 and NIH R24 CA82933.

S19

REPLICATIVE VIRAL VECTORS FOR CANCER: *QUO VADIS?*

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The inability of standard replication-defective vectors to achieve effective transduction of tumors *in vivo* has been a major obstacle to gene therapy for cancer. Use of replication-competent virus vectors would be more efficient, as each tumor cell that is successfully transduced would itself become a virus-producing cell, thus initiating further transduction events *in situ*. Indeed, clinical trials of oncolytic virotherapy have already been initiated for a number of different replicating virus agents; however, while promising results have been observed, the limited persistence of most cytolytic viruses *in vivo* and the robust immune responses resulting in their clearance still make it difficult to address the most prevalent problem in cancer, i.e., the re-growth and metastasis of malignant cells after treatment.

To address this issue, we have been developing replication-competent retrovirus (RCR) vectors, which are highly selective for rapidly dividing cancer cells due to the intrinsic inability of retroviruses to infect quiescent normal cells. Moreover, although retroviruses are not intrinsically cytolytic, their ability to integrate permanently into the target cell genome and achieve persistent infection of cancer cells allows efficient, widespread, and stable seeding of suicide genes throughout an entire tumor mass as they replicate, thereby allowing synchronized cell killing triggered by pro-drug administration. Residual cancer cells serve as a reservoir for persistent viral infection that spreads to metastatic sites, enabling multiple rounds of pro-drug administration to achieve further prolongation of survival.

Further testing of RCR vectors in different tumor models, comparison of different RCR vector designs, and development of GMP-grade manufacturing processes for clinical trials now under discussion with the FDA, is currently being pursued through an international consortium, including researchers at UCLA, USC, Memorial Sloan Kettering Cancer Center, and Mayo Clinic in the United States, Hopital Pitie-Salpetriere in France, Aarhus University in Denmark, and the University of Veterinary Sciences in Austria. With the use of replicating vectors, the original promise of retrovirus-mediated gene therapy for cancer may finally be fulfilled.

S20

LENTIVIRAL VECTORS BASED ON HIV-1, HIV-2 AND SIV

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Retroviral vectors have been used for many years for gene delivery both *in vitro* and *in vivo*. Lentiviruses are a class of retroviruses characterised by their ability to integrate their genomes into cells which are non dividing. As such they have made a significant impact in gene delivery, successfully targeting cells which are not able to be transduced by conventional murine or avian oncoretroviral based vectors. Gene transfer with lentiviral vectors is extremely efficient and high level gene expression can be achieved in a large variety of cell types. Despite the specific characteristic of entry into non dividing cells, lentiviral vectors are also extremely efficient at entering into cells which are dividing. Thus, *in vivo*, they have the advantage of facilitating entry to all cells within a particular tissue rather than those at any particular stage of the cell cycle. Since our identification of the HIV-1 packaging signal, we have developed HIV-1 and latterly HIV-2 and SIV based vectors for gene delivery. We have shown high level gene expression in a number of target tissues *in vitro* and *in vivo*. Recently, we have used these vectors to target tissues involved in transplantation such as the heart and have demonstrated successful gene transduction in the transplanted heart. Using genes which can suppress the immune response, we have demonstrated evidence of prolongation of cardiac survival in the transplant scenario.