PACA-2 and S2103 pancreatic cancer cells in colony formation assay (CFA) showed that OAd-IFN combination was more efficacious in inhibiting colony formation than triple combination with OAd-LUC, a counterpart of OAd-IFN expressing luciferase. Triple combination with OAd-IFN was also more efficient than double therapies with 5-FU+radiation, OAd-LUC + radiation, and OAd-LUC+5-FU. As CFA is an assay frequently used to access the proliferating capacity of single cells in vitro, our data suggests that IFN expressed virus decreased the number of TIC/CSC. This is supported by our in vivo studies showing that inclusion of OAd-IFN in combination with radiation or with 5-FU+radiation results in superior tumor shrinkage than all therapies not involving IFN or including OAd-LUC. In addition, tumors treated with OAd-IFN combination have a longer recurrence interval compared with all other treatments suggesting reduced levels of TIC.

Although further studies are necessary to understand the impact of our OAd-IFN virus in pancreatic cancer stem cells, we believe IFN expressed by our virus made quiescent CSC more susceptible to treatments. Because our virus can express high levels of IFN restricted to the tumor site, we believe that a higher percentage of these cells will be affected by IFN delaying clinical recurrence of tumors, and improving therapy effectiveness.

322. Benign Herpes Simplex Virus Vector Design for Efficient Delivery of Large or Multiple Transgenes To a Diversity of Cells

Yoshitaka Miyagawa,[1] Fang Han,[1,2] Gianluca Verlengia,[3] Hiroaki Uchida,[4] Michele Simonato,[3] Justus B. Cohen,[1] Joseph C. Glorioso.[1]

[1]Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA; [2]Tsinghua University, Beijing, China; [3]Department of Medical Sciences, University of Ferrara, Ferrara, Italy; [4]University of Tokyo, Tokyo, Japan.

Viral vectors derived from herpes simplex virus (HSV) have the potential to revolutionize gene therapy due to their ability to accommodate large and multiple therapeutic transgenes. However, current HSV gene therapy vectors express toxic levels of an immediate-early (IE) protein, ICP0, whose function is required for robust and sustained transgene expression. Here we report the development of a new generation of HSV vectors that are IE-gene independent and non-toxic, yet capable of persistent transgene expression in a variety of human primary non-neuronal cell types. We identified a CTCF motif cluster upstream of the latency promoter and a known long-term regulatory region as key elements for the protection of transgene expression cassettes from global silencing of the viral genome in the absence of all viral IE gene products. Using this new HSV vector system, we have observed vigorous expression of full-length dystrophin cDNA (14 kb) for several weeks in a dystrophin-deficient muscle cell line. We further tested our vectors for transgene expression in rodent brain. While we detected variable persistence of gene expression from the latency locus, we were surprised to observe vigorous long-term reporter gene expression from one other locus despite the absence of gene expression from this locus in non-neuronal cells. These findings demonstrate that transgene expression in neurons is operatively different from that in non-neuronal cells and suggest that multiple loci can be used for expression of foreign genes in the nervous system. In addition, our data raise the prospect that our highly defective HSV vector system will be applicable as a safe delivery tool for large and multiple therapeutic genes to a wide range of non-neuronal tissues.

323. Adenoviral Compartmentalized Liver Transduction Improves Safety and Yields Longerm Transgene Expression

Gustavo Cabrera,[1] Blanca Segura,[1] Martin Granados,[1] Ana Luisa Esparza.[1]

[1]Global BioTherapeutics Inc, Mexico City, Mexico.

Still unresolved complications associated with adenoviral mediated hepatic-based gene therapy are a strong immunological response with damage to the organ and short-term gene expression. Our group hypothesized that the two components at the core of these drawbacks are: 1) the astronomically high quantities of vector infused and 2) the viremia associated with the conventional routes of vector administration. To stringently manipulate these two variables and assess their effect on adenoviral mediated liver transduction our group implemented compartmentalized liver transduction. Compartmentalized liver transduction is based on known adenoviral spatio-temporal kinetics and is achieved by the direct intra-parenchymal injection of adenovirus into a blood-flow isolated portion of the liver; blood flow is reestablished after viral endocytosis has been completed. Implementing compartmentalized liver transduction in a rat model, gene expression and transgene presence was confined to the site of injection, no inflammation nor liver damage was observed, reporter protein expression with low quantities of vector (10^2) as well as linear dose response curves were documented and prolonged transgene expression was achieved. Our results conclude that compartmentalized liver transduction is a safe and effective mode of vector delivery with a promising future in the field of gene therapy.

324. Effect of Decorin-Expressing Adenovirus on Pathologic Fibrosis; Decreased Collagen Synthesis and Elevated MMP Expression

Hyo Min Ahn,[1] Youjin Na,[1] Il-Kyu Choi,[1] Won Jai Lee,[2] Yong Oock Kim,[2] Ju Hee Lee,[3] Dae Hyun Lew,[2] Hyun Roh,[2] Chae-Ok Yun.[1]

[1]Department of Bioengineering, College of Engineering, Hanyang University, Seoul, Korea; [2]1Institute for Human Tissue Restoration, Department of Plastic & Reconstructive Surgery, College of Medicine, Yonsei University, Seoul, Korea; [3]3Department of Dermatology and Cutaneous Biology Research Institute, College of Medicine, Yonsei University, Seoul, Korea.

Background: Decorin is a natural transforming growth factor-b1 (TGF-b1) antagonist. Reduced decorin synthesis is associated with dermal scarring and increased decorin expression appears to reduce scar tissue formation. To investigate the therapeutic potential of decorin for keloids, human dermal fibroblasts (HDFs) and keloid-derived fibroblasts (KFs) were transduced with decorin-expressing adenovirus (dE1-RGD/GFP/DCN), and we examined the therapeutic potential of decorin-expressing Ad for treating pathologic skin fibrosis

Method: Decorin expression was examined by immunofluorescence assay on keloid tissues. HDFs and KFs were transduced with dE1-RGD/GFP/DCN or control virus, and protein levels of decorin, epidermal growth factor receptor (EGFR), and secreted TGF-ß1 were assessed by western blotting and ELISA. And collagen type I, III, and MMP-1, 3 mRNA levels were measured by real-time RT-PCR. Additionally, we immunohistochemically investigated expression levels of the major extracellular matrix (ECM) proteins in keloid spheroids transduced with dE1-RGD/GFP/DCN.

Results: Lower decorin expression was observed in the keloid region compared to adjacent normal tissues. After treatment with dE1-RGD/GFP/DCN, secreted TGF-ß1 and EGFR protein expression were decreased in TGF-b1-treated HDFs and KFs. Also, type I, III collagen mRNA levels were decreased and expression of MMP-1,