SMA and TGF-ß1 proteins. Twenty eight days after BDL, rats pretreated with rAAV/eGFP or PBS had significant cholestatic liver injury; whereas, rAAV/Cygb modulated HSC differentiation preserving liver architecture and reducing the rise in collagen synthesis, as assessed by hydroxyproline content of liver tissue. To explore the role of Cygb during progressive liver fibrosis, we administered rAAV-2 by pv injection to SD rats at week 8 during a 12 week course of CCL4 injections or day 12 after BDL. In both models, rAAV/eGFP did not prevent progression of liver fibrosis, whereas, rAAV/Cygb administration reduced expression of markers of HSC activation and improved fibrosis.

We demonstrate that Cygb, delivered to liver by rAAV-2 vector, reduces activation of HSC resulting in less extracellular matrix deposition in both toxic and cholestatic models of liver injury, even when given after the development of liver fibrosis. Manipulating Cygb expression is a potential novel therapeutic strategy for reducing hepatic fibrosis.

447. AP20187-Inducible Insulin-Like Effects in Diabetic Muscle and Liver Transduced with AAV

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Diabetes Mellitus, characterized by insulin deficiency (type I) or resistance (type II), derives from insulin action impairments in hormone target tissues: muscle, liver and adipocytes. Insulin regulates metabolism and glucose homeostasis through binding to a specific membrane receptor (IR) with tyrosine kinase activity. Induction of the insulin receptor signaling in hormone target cells may represent a tool to rescue glucose homeostasis in both insulin and insulin receptor deficiencies. Recently we have described that homodimerization of the chimeric insulin receptor LFv2IRE induced by the small dimerizer drug AP20187 results in insulin like actions in hepatocytes trasduced with adeno-associated viral vectors (AAV).

Here we show that AAV-mediated LFv2IRE expression in murine muscle and liver followed by systemic AP20187 administration results in reversible homodimerization and tyrosine-phosphorylation of the chimeric receptor which peaks 6 hours after drug administration. More importantly AAV vectors expressing LFv2IRE were administered to muscle and liver of the Non-Obese Diabetic (NOD) murine model of type I Diabetes. Significant increases in hepatic glycogen content were observed following AP20187 systemic administration to AAV-treated NOD mice. The analysis of glucose uptake from diabetic muscle transduced with AAV following AP20187 administration is in progress. The ability of the LFv2IRE-AP20187 system to induce insulin-like actions in hormone target tissues in diabetic mice can be further exploited to obtain glucose homeostasis thus representing a potential novel therapeutic strategy for pathological conditions due to either insulin deficiency or resistance.

448. Suicide Gene-Mediated Conditional Ablation of Tumors Derived from Transplanted Embryonic Stem Cells

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Based on their capacity for self-renewal and pluripotency, embryonic stem cells (ESC) are being developed as regenerative medicines, but transplantation protocols using differentiated ESC have been hindered by the fact that even small numbers of undifferentiated ESC within a transplant can lead to the development of teratomas. Within the limitations of current technology, it has not been possible to ensure that a population of differentiated ESC is entirely free of undifferentiated ESC. In this context, the safety profile of ESC transplants would be enhanced if uncontrolled cell growth could be suppressed using external stimuli. To address this issue, we hypothesized that gene transfer to the ESC of conditional elements that could kill the transduced cells if activated, would permit control of inappropriate outgrowth of ESC *in vivo*. To assess this strategy, we developed a replication-defective recombinant lentiviral gene transfer vector containing the herpes simplex virus thymidine kinase (HSVtk) gene (a suicide gene able to convert the nontoxic prodrug nucleotide analog ganciclovir (GCV) to a toxic form), an internal ribosomal entry site (IRES) and green fluorescent protein (GFP) driven by the cytomegalovirus immediate early promoter, and used this vector to genetically modify murine ESC (CGR8 strain). Fluorescence microscopy and FACS analysis demonstrated that 100 % of these cells were killed in the presence of GCV (50 μM) *in vitro*. To assess the ability of the HSVtk suicide strategy to eliminate ESC outgrowth, cloned undifferentiated ESC (10^5 cells) carrying the HSVtk-IRES-GFP transgene were administered subcutaneously into SCID mice to induce teratomas. Teratoma formation was verified by the histological identification of primitive ectoderm, endoderm and mesoderm within the tumor mass. On day 14, when the tumor was 261 ±52 mm^3 in size, intraperitoneal GCV administration was initiated and continued for 4 wk (30 mg/kg, twice per day), while a control group received no GCV. Reduction of tumor mass in the group receiving GCV was evident by 2 wk and the tumors were completely eliminated by 4 wk (10/10 treated, 0/3 untreated). Residual fibrous, fat and cartilage tissue were observed at the site of the tumor in the GCV group, but histologic analysis showed no live tumor cells. Mice followed after cessation of GCV treatment did not show any recurrence of tumor growth, suggesting that the tumor-forming cells were killed and not simply arrested. The HSVtk gene is small (1.3 kb) and the use of an IRES avoids the need for a separate promoter, leaving sufficient space for most transgenes. These data demonstrate that this strategy can be used to deliver a therapeutic gene with additional conditional genetic elements that can be activated to control undifferentiated ESC outgrowth and transduced ESC that have escaped growth control due to insertional mutagenesis associated with the use of integrating vectors.