



CLINIGENE CURRENT GENE THERAPY WEEKLY

From May 17th to May 24th 2010

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PMID:
20490718

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Inhibition of JNK1 expression decreases migration and invasion of mouse hepatocellular carcinoma cell line in vitro.

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c-Jun N-terminal kinase (JNK) is located in focal adhesion plaque (FAP). JNK is necessary to growth, morphogenesis, and differentiation of cells; especially JNK1 has a close relation with tumors. In this study, we silenced JNK1 by using short hairpin RNA (shRNA) and examined the effect on migration and invasion of mouse hepatocellular carcinoma (HCC) cell line Hca-F in vitro. Three shRNA expression vectors (JNK1shRNA-1, JNK1shRNA-2, and JNK1shRNA-3) were constructed and transfected to Hca-F cells stably. The most effective shRNA was selected by detecting the expression levels of mRNA and protein. Transwell assay was performed to detect the ability of migration and invasion of cells. A negative control sequence (JNK1shRNA control) and non-transfected normal Hca-F cells were treated as control groups. The "Results" showed that the expression vectors of pSilencer-JNK1shRNA were constructed and transfected to Hca-F cells successfully. The most effective shRNA was JNK1shRNA-2. The expressions of mRNA and protein of JNK1 in Hca-F cells after transfection of JNK1shRNA-2 were decreased significantly compared with the other groups (all, $P < 0.01$; all, $P < 0.05$). The ability of migration and invasion was decreased after down-regulation of JNK1 expression (all, $P < 0.05$). These results suggest that the inhibition of JNK1 expression can decrease ability of migration and invasion of mouse hepatocellular carcinoma cell line in vitro. JNK1 plays an important role in lymphatic metastasis of HCC. It may be a new target for gene therapy of lymphatic metastasis of HCC.

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20490489**

Cancer Immunol Immunother. 2010 May 20. [Epub ahead of print]

Endostatin gene therapy enhances the efficacy of IL-2 in suppressing metastatic renal cell carcinoma in mice.

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Abstract

We investigated whether the administration of IL-2 combined with endostatin gene therapy was able to produce additive or even synergistic immunomodulatory activity in a mouse model of metastatic renal carcinoma. Renca cells were injected into the tail vein of BALB/c mice. After 24 h, the animals were randomly divided into four groups (5 mice/group). One group of mice was the control, the second group received treatment with 100,000 UI of Recombinant IL-2 (Proleukin, Chiron) twice a day, 1 day per week during 2 weeks (IL-2), the third group received treatment with a subcutaneous inoculation of 3.6×10^6 endostatin-producing cells, and the fourth group received both therapies (IL-2 + ES). Mice were treated for 2 weeks. In the survival studies, 10 mice/group daily, mice were monitored daily until they died. The presence of metastases led to a twofold increase in endostatin levels. Subcutaneous inoculation of NIH/3T3-LendSN cells resulted in a 2.75 and 2.78-fold increase in endostatin levels in the ES and IL-2 + ES group, respectively. At the end of the study, there was a significant decrease in lung wet weight, lung nodules area, and microvascular area (MVA) in all treated groups compared with the control group ($P < 0.001$). The significant difference in lung wet weight and lung nodules area between groups IL-2 and IL-2 + ES revealed a synergistic antitumor effect of the combined treatment ($P < 0.05$). The IL-2 + ES therapy Kaplan-Meier survival curves showed that the probability of survival was significantly higher for mice treated with the combined therapy (log-rank test, $P = 0.0028$). Conjugated therapy caused an increase in the infiltration of CD4, CD8 and CD49b lymphocytes. An increase in the amount of CD8 cells ($P < 0.01$) was observed when animals received both ES and IL-2, suggesting an additive effect of ES over IL-2 treatment. A synergistic effect of ES on the infiltration of CD4 ($P < 0.001$) and CD49b cells ($P < 0.01$) was also observed over the effect of IL-2. Here, we show that ES led to an increase in CD4 T helper cells as well as cytotoxic lymphocytes, such as NK cells and CD8 cells, within tumors of IL-2 treated mice. This means that ES plays a role in supporting the actions of T cells.

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20489789**

Cancer Gene Ther. 2010 May 21. [Epub ahead of print]

Autophagy is induced by adenoviral-mediated interferon alpha treatment in interferon resistant bladder cancer and normal urothelial cells as a cell death protective mechanism but not by the bystander factors produced.

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We have previously shown that adenoviral-mediated interferon alpha (Ad-IFNalpha) treatment is highly cytotoxic to tumor cells which are resistant to the IFNalpha protein. We now report that autophagy is produced after Ad-IFNalpha treatment of either IFN resistant bladder cancer cells (UC9 and KU7) or the normal urothelial cell line (TERT-NHUC). After Ad-IFNalpha infection autophagosomes, an early stage of autophagy, were seen in cancer cells whereas autophagolysosomes, a later stage of autophagy, were observed mostly in normal cells by electron microscopy. Conditioned medium from either normal or bladder cancer cells obtained after Ad-IFNalpha infection, however, produced no autophagy when placed on the bladder cancer cells, although again marked cytotoxicity was observed. This indicated that the autophagy seen was related to the direct effect of Ad-IFNalpha transfection and expression rather than to the bystander factors produced. In addition, autophagic changes were seen using LysoTracker Red DND-99 in both normal and cancer cells. We also documented that Ad-IFNalpha treatment produces the autophagic protein form, light chain 3 (LC3)-II, in cancer cells but not normal cells, which in turn was inhibited by the autophagic inhibitor, 3-methyladenine (3-MA). This inhibition of autophagy resulted in a significant increase in apoptotic cell death as measured by the sub-G1 population. We hypothesize that the autophagy seen in normal urothelial cells is a protective response and is allowed to be completed, providing a survival mechanism after Ad-IFN treatment, whereas the autophagy produced in IFN resistant cancer cells is not allowed to be completed and is insufficient to significantly suppress cytotoxicity.

**PMID:
20489626**

Curr Opin Organ Transplant. 2010 Jun;15(3):269-76.

Liver transplantation for inherited metabolic disorders of the liver.

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PURPOSE OF REVIEW: Liver transplantation is curative, life saving or both for a range of inherited diseases affecting the liver. Indications, timing and outcome of transplantation for these diseases are the focus of this review. **RECENT FINDINGS:** Liver transplant represents a mode of gene replacement therapy for several disorders, including Wilson disease, hemochromatosis, tyrosinemia, urea cycle defects and hypercholesterolemia in which the primary defect residing in the liver results in hepatic complications or severe extrahepatic disease. Liver transplant is also an important therapeutic modality in multisystemic genetic disorders with major hepatic disease such as glycogen storage disease types I, III and IV and porphyria. For familial amyloidosis and primary hyperoxaluria, liver replacement eliminates the source of the injurious products that results in extrahepatic disease. Innovations in medical and surgical management of these patients have led to improved outcomes providing an important benchmark for future gene therapy of these disorders. **SUMMARY:** Recent developments have refined the indications for liver transplant in the treatment of inherited metabolic diseases. The full potential of liver transplant in these disorders can be harnessed by careful patient selection, optimizing timing and perioperative metabolic management of these patients.

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20488531

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In vivo restoration of full-thickness cartilage defects by poly(lactide-co-glycolide) sponges filled with fibrin gel, bone marrow mesenchymal stem cells and DNA complexes.

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A composite construct comprising of bone marrow mesenchymal stem cells (BMSCs), plasmid DNA encoding transforming growth factor-beta1 (pDNA-TGF-beta1), fibrin gel and poly (lactide-co-glycolide) (PLGA) sponge was designed and employed to repair articular cartilage defects. To improve the gene transfection efficiency, a cationized chitosan derivative N,N,N-trimethyl chitosan chloride (TMC) was employed as the vector. The TMC/DNA complexes had a transfection efficiency of 9% to BMSCs and showed heterogeneous TGF-beta1 expression in a 10-day culture period in vitro. In vivo culture of the composite constructs was performed by implantation into full-thickness cartilage defects of New Zealand white rabbit joints, using the constructs absence of pDNA-TGF-beta1 or BMSCs as controls. Heterogeneous expression of TGF-beta1 in vivo was detected at 4 weeks, but its level was decreased in comparison with that of 2 weeks. After implantation for 12 weeks, the cartilage defects were successfully repaired by the composite constructs of the experimental group, and the neo-cartilage integrated well with its surrounding tissue and subchondral bone. Immunohistochemical and glycosaminoglycans (GAGs) staining confirmed the similar amount and distribution of collagen type II and GAGs in the regenerated cartilage as that of hyaline cartilage. The cartilage special genes expressed in the neo-tissue were closer to those of the normal cartilage. An overall score of 2.83 was obtained according to Wakitani's standard. By contrast, only part of the defects was repaired by the pDNA-TGF-beta1 absence constructs, and no cartilage repair but fibrous tissue was found for the BMSCs absence constructs. Therefore, combination of the PLGA sponge/fibrin gel scaffold with BMSCs and gene therapy is an effective method to restore cartilage defects and may have a great potential for practical applications in the near future.

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A transient increase of intra-hepatic pressure mediates successful treatment of the Gunn rat with reduced doses of lentiviral vector.

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Lentiviral vectors can stably transduce hepatocytes and are promising tools for gene therapy of hepatic diseases. Although hepatocytes are accessible to blood-borne viral vectors through fenestrations of the hepatic endothelium, improved liver transduction after delivery of vectors to the blood stream is needed. As the normal endothelial fenestration and lentiviral vectors have a similar size (150 nm), we hypothesized that a transient increase in hepatic blood pressure may enhance in-vivo gene transfer to hepatocytes. We designed a simple surgical procedure, by which the liver is temporarily excluded from the blood flow. Lentiviral vectors were injected in a large volume to increase intra-hepatic pressure. We demonstrated that in the Gunn rat, a model of Crigler-Najjar disease, the administration of low vector doses (corresponding to a multiplicity of infection of 0.2) using this procedure resulted in therapeutic correction of hyperbilirubinemia, without toxicity. The correction was sustained for 10 months (end of study). The same vector amounts yielded only partial correction after intra-portal delivery. We believe that this new and clinically-applicable strategy may broaden the range of genetic liver diseases accessible to gene therapy.

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20486773

Hum Gene Ther. 2010 May 20. [Epub ahead of print]

Liver-Directed rAAV Gene Delivery Rescues a Lethal Mouse Model of Methylmalonic Acidemia and Provides Long-Term Phenotypic Correction.

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Methylmalonic acidemia is a severe metabolic disorder caused by a deficiency of the ubiquitously expressed mitochondrial enzyme, methylmalonyl-CoA mutase (MUT). Liver transplantation has been used to treat a small number of patients with variable success, and whether liver-directed gene therapy might be employed in such a pleiotropic metabolic disorder is uncertain. In this study, we examined the therapeutic effects of hepatocyte-directed delivery of the Mut gene to mice with a severe form of methylmalonic acidemia. We show that a single intrahepatic injection of a recombinant adeno-associated virus serotype 8 (rAAV8) expressing the Mut gene under the control of the liver-specific thyroxine-binding globulin (TBG) promoter is sufficient to rescue Mut^{-/-} mice from neonatal lethality and provide long-term phenotypic correction. Treated Mut^{-/-} mice lived beyond a year of age, had improved growth, lower plasma methylmalonic acid levels and an increased capacity to oxidize 1-¹³C-propionate in vivo. The older treated mice showed increased Mut transcription, presumably mediated by up-regulation of the TBG promoter during senescence. The results indicate that the stable transduction of a small number of hepatocytes with the Mut gene can be efficacious in the phenotypic correction of an inborn error of organic acid metabolism and support the rapid translation of liver-directed gene therapy vectors already optimized for human subjects to patients with methylmalonic acidemia.

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20486772

Hum Gene Ther. 2010 May 20. [Epub ahead of print]

Enhanced Long-term Transduction and Multilineage Engraftment of Human Hematopoietic Stem Cells Transduced with Tyrosine-Modified rAAV2.

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The search for the ideal stem cell gene therapy vector continues as recognized problems persist. Although recombinant AAV (rAAV2) mediates gene transfer into hematopoietic stem cells, identified restrictions to transgene expression reduce overall efficiency. Recent studies have shown that transduction efficiencies are significantly improved by preventing early proteasomal degradation following mutation of surface-exposed tyrosine residues on the capsid to phenylalanine. Here, we report that transduction of human cord blood (CB) CD34⁺ stem cells by tyrosine-modified rAAV2 is significantly enhanced both in vitro and in vivo. Serial long-term in vivo bioluminescent imaging of immune-deficient recipients following xenotransplantation of CD34⁺ cells transduced with tyrosine-modified rAAV2-luciferase revealed that modification of rAAV2 capsids led to a significant increase in the transduction of human CD34⁺ cells, without adversely affecting engraftment capacity, or the ability to undergo multilineage differentiation and self-renewal. Together with observations of sustained high level transgene expression in vivo and efficient persistence of rAAV genomes in human hematopoietic cells, these results suggest that due to their ability to bypass restrictions to transduction, tyrosine-modified rAAV vectors, particularly Y500F, Y700F, Y444F and Y704F, represent highly promising candidates for therapeutic evaluation for diseases of human hematopoietic stem cells.

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20486664

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Molecular Restructuring of Water and Lipids upon the Interaction of DNA with Lipid Monolayers.

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Understanding the molecular mechanism of DNA/lipid interaction is critical in optimizing the use of lipid cofactors in gene therapy. Here, we address this question by employing label-free vibrational sum frequency (VSF) spectroscopy to study the interaction of DNA with lipid monolayers of the cationic lipids DPTAP(1,2-dipalmitoyl-3-trimethylammonium-propane) and diC14-amidine as well as the zwitterionic lipid DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) in the presence and absence of calcium. Our approach has the advantage both of allowing us to explicitly probe intermolecular interactions and of providing insight into the structure of water and lipids around DNA at the lipid interface. We find, by examination of the OD stretch of interfacial D(2)O, that water structure differs markedly between systems containing DNA adsorbed to cationic and those that contain DNA adsorbed to zwitterionic lipid monolayers (in the presence or absence of Ca(2+)). The spectral response of interfacial water in the cationic system is consistent with a highly structured, undercoordinated, structural 'type' of water. Further, by investigation of CH stretch modes of the diC14-amidine lipid tails, we demonstrate that the adsorption of DNA to this lipid leads to increased ordering of lipid tails.

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20485384

Gene Ther. 2010 May 20. [Epub ahead of print]

Mifepristone increases gamma-retroviral infection efficiency by enhancing the integration of virus into the genome of infected cells.

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Gamma-retroviruses are commonly used to deliver genes to cells. Previously, we demonstrated that the synthetic anti-glucocorticoid and anti-progestin agent, mifepristone, increased gamma-retroviral infection efficiency in different target cells, independent of viral titer. In this study, we examine how this occurs. We studied the effect of mifepristone on different steps of viral infection (viral entry, viral survival, viral DNA synthesis and retrovirus integration into the host genome) in three distinct retroviral backbones using different virus recognition receptors. We also tested the potential role of glucocorticoid and progesterone receptors in mediating mifepristone's ability to increase gamma-retroviral infectivity. We show that mifepristone increases gamma-retroviral infection efficiency by facilitating viral integration into the host genome and that this effect seems to be due to mifepristone's anti-glucocorticoid, but not its anti-progestin, activity. These results suggest that inhibition of the glucocorticoid receptor enhances retroviral integration into the host genome and indicates that cells may have a natural protection against retroviral infection that may be reduced by glucocorticoid receptor antagonists.

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20485383**

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Gene delivery by surface immobilization of plasmid to tissue-engineering scaffolds.

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Biomaterial scaffolds that serve as vehicles for gene delivery to promote expression of inductive factors have numerous regenerative medicine applications. In this report, we investigate plasmid delivery from biomaterial scaffolds using a surface immobilization strategy. Porous scaffolds were fabricated from poly(D,L-lactide-co-glycolide) (PLG), and plasmids were immobilized by drying. In vitro plasmid release indicated that the majority (>70%) of adsorbed plasmids were released within 24 h and >98% within 3 days; however, in vivo implantation of the scaffolds at the subcutaneous site yielded transgene expression that persisted for at least 28 weeks and was localized to the site of implantation. Histological analysis of DNA-adsorbed scaffolds indicated that macrophages at the scaffold were transfected in the first 2 weeks after implantation, whereas muscle cells adjacent to the implant primarily expressed the transgene at 4 weeks. In addition to localized gene expression, a secreted protein (human factor IX) was retained at the implant site and not available systemically after 3 days, indicating minimal off-target effects. These findings show that surface immobilization of plasmid onto microporous PLG scaffolds can produce localized and long-term gene expression in vivo, which may be used to enhance the bioactivity of scaffolds used for regenerative medicine.

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20485382**

Gene Ther. 2010 May 20. [Epub ahead of print]

Preclinical correction of human Fanconi anemia complementation group A bone marrow cells using a safety-modified lentiviral vector.

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One of the major hurdles for the development of gene therapy for Fanconi anemia (FA) is the increased sensitivity of FA stem cells to free radical-induced DNA damage during ex vivo culture and manipulation. To minimize this damage, we have developed a brief transduction procedure for lentivirus vector-mediated transduction of hematopoietic progenitor cells from patients with Fanconi anemia complementation group A (FANCA). The lentiviral vector FancA-sW contains the phosphoglycerate kinase promoter, the FANCA cDNA, and a synthetic, safety-modified woodchuck post transcriptional regulatory element (sW). Bone marrow mononuclear cells or purified CD34(+) cells from patients with FANCA were transduced in an overnight culture on recombinant fibronectin peptide CH-296, in low (5%) oxygen, with the reducing agent, N-acetyl-L-cysteine (NAC), and a combination of growth factors, granulocyte colony-stimulating factor (G-CSF), Flt3 ligand, stem cell factor, and thrombopoietin. Transduced cells plated in methylcellulose in hypoxia with NAC showed increased colony formation compared with 21% oxygen without NAC ($P < 0.03$), showed increased resistance to mitomycin C compared with green fluorescent protein (GFP) vector-transduced controls ($P < 0.007$), and increased survival. Thus, combining short transduction and reducing oxidative stress may enhance the viability and engraftment of gene-corrected cells in patients with FANCA.

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Gene Ther. 2010 May 20. [Epub ahead of print]

Branched oligomerization of cell-permeable peptides markedly enhances the transduction efficiency of adenovirus into mesenchymal stem cells.

Park SH, Doh J, Park SI, Lim JY, Kim SM, Youn JI, Jin HT, Seo SH, Song MY, Sung SY, Kim M, Hwang SJ, Choi JM, Lee SK, Lee HY, Lim CL, Chung YJ, Yang D, Kim HN, Lee ZH, Choi KY, Jeun SS, Sung YC.

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Cell-permeable peptides (CPPs) promote the transduction of nonpermissive cells by recombinant adenovirus (rAd) to improve the therapeutic efficacy of rAd. In this study, branched oligomerization of CPPs significantly enhanced the transduction of human mesenchymal stem cells (MSCs) by rAd in a CPP type-independent manner. In particular, tetrameric CPPs increased transduction efficiency at 3000-5000-fold lower concentrations than did monomeric CPPs. Although branched oligomerization of CPPs also increases cytotoxicity, optimal concentrations of tetrameric CPPs required for maximum transduction are at least 300-1000-fold lower than those causing 50% cytotoxicity. Furthermore, although only approximately 60% of MSCs were maximally transduced at 500 μ M of monomeric CPPs, >95% of MSCs were transduced with 0.1 μ M of tetrameric CPPs. Tetrameric CPPs also significantly increased the formation and net surface charge of CPP/rAd complexes, as well as the binding of rAd to cell membranes at a greater degree than did monomeric CPPs, followed by rapid internalization into MSCs. In a critical-size calvarial defect model, the inclusion of tetrameric CPPs in ex vivo transduction of rAd expressing bone morphogenetic protein 2 into MSCs promoted highly mineralized bone formation. In addition, MSCs that were transduced with rAd expressing brain-derived neurotrophic factor in the presence of tetrameric CPPs improved functional recovery in a spinal cord injury model. These results demonstrated the potential for tetrameric CPPs to provide an innovative tool for MSC-based gene therapy and for in vitro gene delivery to MSCs.

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20485380**

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Local arterial nanoparticle delivery of siRNA for NOX2 knockdown to prevent restenosis in an atherosclerotic rat model.

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Both atherosclerosis and arterial interventions induce oxidative stress mediated in part by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases that have a pivotal role in the development of neointimal hyperplasia and restenosis. For small interfering RNA (siRNA) targeting of the NOX2 (Cybb) component of the NADPH oxidase to prevent restenosis, gene transfer with viral vectors is effective, but raises safety issues in humans. We developed a new approach using the amino-acid-based nanoparticle HB-OLD7 for local delivery of siRNA targeting NOX2 to the arterial wall. siRNA-nanoparticle complexes were transferred into the regional carotid artery walls after angioplasty in an atherosclerotic rat model. Compared with angioplasty controls, Cybb gene expression (measured by quantitative reverse transcriptase-PCR) in the experimental arterial wall 2 weeks after siRNA was reduced by >87%. The neointima-to-media-area ratio was decreased by >83%, and the lumen-to-whole-artery area ratio was increased by >89%. Vital organs showed no abnormalities and splenic Cybb gene expression showed no detectable change. Thus, local arterial wall gene transfer with HB-OLD7 nanoparticles provides an effective, nonviral system for efficient and safe local gene transfer in a clinically applicable approach to knock down an NADPH oxidase gene. Local arterial knockdown of the Cybb gene significantly inhibited neointimal hyperplasia and preserved the vessel lumen without systemic toxicity.

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20485267**

Mol Ther. 2010 May 18. [Epub ahead of print]

B-Cell-Delivered Gene Therapy Induces Functional T Regulatory Cells and Leads to a Loss of Antigen-Specific Effector Cells.

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Previous reports have shown that B-cell-mediated gene therapy can induce tolerance in several animal models for autoimmune diseases and inhibitory antibody formation in hemophilia A mice. We know from our previous work that the induction of tolerance following B-cell therapy is dependent upon CD25(+) regulatory T cells (Tregs). To extend these studies and identify the effects of this gene therapy protocol on the target CD4 T cells, we have adapted in vitro suppression assays using Tregs isolated from treated and control mice. Using carboxyfluorescein succinimidyl ester (CFSE) dilution as a measure of T-cell responsiveness to FVIII, we show that CD25(+) Tregs from treated mice are more suppressive than those from control animals. To monitor the induction of antigen-specific Tregs, we repeated these studies in ovalbumin (OVA) peptide-specific DO11.10 T-cell receptor (TCR) transgenic mice. Tregs from DO11.10 mice treated with a tolerogenic OVA-Ig construct are better than polyclonal Tregs at suppressing the proliferation of responder cells stimulated with OVA peptide 323-339 (pOVA). Furthermore, we show that following B-cell therapy, there is an increase in antigen-specific FoxP3(+) Tregs, and there is also a distinct decrease in antigen-specific CD4(+) effector T cells. These changes in the lymphocyte population shift the balance away from effector function toward a tolerogenic phenotype.

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20485266**

Mol Ther. 2010 May 18. [Epub ahead of print]

SIN Retroviral Vectors Expressing COL7A1 Under Human Promoters for Ex Vivo Gene Therapy of Recessive Dystrophic Epidermolysis Bullosa.

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Recessive dystrophic epidermolysis bullosa (RDEB) is caused by loss-of-function mutations in COL7A1 encoding type VII collagen which forms key structures (anchoring fibrils) for dermal-epidermal adherence. Patients suffer since birth from skin blistering, and develop severe local and systemic complications resulting in poor prognosis. We lack a specific treatment for RDEB, but ex vivo gene transfer to epidermal stem cells shows a therapeutic potential. To minimize the risk of oncogenic events, we have developed new minimal self-inactivating (SIN) retroviral vectors in which the COL7A1 complementary DNA (cDNA) is under the control of the human elongation factor 1alpha (EF1alpha) or COL7A1 promoters. We show efficient ex vivo genetic correction of primary RDEB keratinocytes and fibroblasts without antibiotic selection, and use either of these genetically corrected cells to generate human skin equivalents (SEs) which were grafted onto immunodeficient mice. We achieved long-term expression of recombinant type VII collagen with restored dermal-epidermal adherence and anchoring fibril formation, demonstrating in vivo functional correction. In few cases, rearranged proviruses were detected, which were probably generated during the retrotranscription process. Despite this observation which should be taken under consideration for clinical application, this preclinical study paves the way for a therapy based on grafting the most severely affected skin areas of patients with fully autologous SEs genetically corrected using a SIN COL7A1 retroviral vector.

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20484599

Invest Ophthalmol Vis Sci. 2010 May 19. [Epub ahead of print]

Evaluation of AAV-Mediated Expression of Chop2-GFP in the Marmoset Retina.

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Purpose: Converting inner retinal neurons to photosensitive cells by expressing channelrhodopsin-2 (ChR2) offers a novel approach for treating blindness caused by retinal degenerative diseases. We evaluated the recombinant adeno-associated virus serotype 2 (rAAV2)-mediated expression and function of a fusion construct of channelrhodopsin-2 (Chop2) and green fluorescent protein (GFP) (Chop2-GFP) in inner retinal neurons in the common marmoset *Callithrix jacchus*. **Methods:** rAAV2 vectors carrying ubiquitous promoters were injected into the vitreous chamber. Expression of Chop2-GFP and functional properties of ChR2 were examined 3 months after injection with immunocytochemical and electrophysiological **METHODS:** **Results:** The percentage of Chop2-GFP-expressing cells in the ganglion cell layer was found to be retinal region- and animal age-dependent. The highest percentage was observed in the far-peripheral region. Chop2-GFP expression was also found in foveal and para-foveal region. In the peripheral retina in young animals with high viral concentrations, the expression of Chop2-GFP was observed in all major classes of retinal neurons, including all major types of ganglion cells. The morphological properties of Chop2-GFP-positive cells were normal for at least three months; and ChR2-mediated light responses were demonstrated by electrophysiological recordings. **Conclusions:** We reported the rAAV2-mediated expression of ChR2 in the inner retinal neurons in the marmoset retina through intravitreal delivery. The marmoset could be a valuable non-human primate model for developing ChR2-based gene therapy for treating blinding retinal degenerative diseases.

Redirecting lentiviral vectors pseudotyped with Sindbis virus-derived envelope proteins to DC-SIGN by modification of N-linked glycans of envelope proteins.

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Redirecting the tropism of viral vectors enables specific transduction of selected cells by direct administration of vectors. We previously developed targeting lentiviral vectors by pseudotyping with modified Sindbis virus envelope proteins. These modified Sindbis virus envelope proteins have mutations in their original receptor-binding regions to eliminate their natural tropisms, and they are conjugated with targeting proteins, including antibodies and peptides, to confer their tropisms to target cells. We investigated whether our targeting vectors interact with DC-SIGN, which traps many types of viruses and gene therapy vectors by binding to the N-glycans of their envelope proteins. We found that these vectors do not interact with DC-SIGN. When these vectors were produced in the presence of deoxymannojirimycin (DMNJ), which alters the structures of N-glycans from complex to high-mannose, these vectors used DC-SIGN as their receptor. Genetic analysis demonstrated that the N-glycans at E2 a.a. 196 and E1 a.a. 139 mediate binding to DC-SIGN, which supports the results of a previous report of cryo-electron microscopy analysis. In addition, we investigated whether modification of the N-glycan structures could activate serum complement activity, possibly by the lectin pathway of complement activation. DC-SIGN-targeted transduction occurs in the presence of human serum complement, demonstrating that high-mannose-structure N-glycans of the envelope proteins do not activate human serum complement. These results indicate that the strategy of redirecting viral vectors according to alterations of their N-glycan structures would enable the vectors to target specific cells types expressing particular types of lectins.

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Delivery of Na/I Symporter Gene into Skeletal Muscle Using Nanobubbles and Ultrasound: Visualization of Gene Expression by PET.

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The development of nonviral gene delivery systems is essential in gene therapy, and the use of a minimally invasive imaging methodology can provide important clinical endpoints. In the current study, we present a new methodology for gene therapy—a delivery system using nanobubbles and ultrasound as a nonviral gene delivery method. We assessed whether the gene transfer allowed by this methodology was detectable by PET and bioluminescence imaging. **METHODS:** Two kinds of reported vectors (luciferase and human Na/I symporter [hNIS]) were transfected or cotransfected into the skeletal muscles of normal mice (BALB/c) using the ultrasound-nanobubbles method. The kinetics of luciferase gene expression were analyzed in vivo using bioluminescence imaging. At the peak of gene transfer, PET of hNIS expression was performed using our recently developed PET scanner, after (^{124}I) injection. The imaging data were confirmed using reverse-transcriptase polymerase chain reaction amplification, biodistribution, and a blocking study. The imaging potential of the 2 methodologies was evaluated in 2 mouse models of human pathology (McH/lpr-RA1 mice showing vascular disease and C57BL/10-mdx Jic mice showing muscular dystrophy). **RESULTS:** Peak luciferase gene activity was observed in the skeletal muscle 4 d after transfection. On day 2 after hNIS and luciferase cotransfection, the expression of these genes was confirmed by reverse-transcriptase polymerase chain reaction on a muscle biopsy. PET of the hNIS gene, biodistribution, the blocking study, and autoradiography were performed on day 4 after transfection, and it was indicated that hNIS expression was restricted to the site of plasmid administration (skeletal muscle). Similar localized PET and (^{124}I) accumulation were successfully obtained in the disease-model mice. **CONCLUSION:** The hNIS gene was delivered into the skeletal muscle of healthy and disease-model mice by the ultrasound-nanobubbles method, and gene expression was successfully visualized with PET. The combination of ultrasound-nanobubble gene transfer and PET may be applied to gene therapy clinical protocols.

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Minimal amino acid exchange in human TCR constant regions fosters improved function of TCR gene-modified T cells.

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TCR gene therapy using adoptive transfer of TCR gene-modified T cells is a new strategy for treatment of cancer. One critical prerequisite for TCR gene therapy is sufficient expression of transferred TCRs. Several strategies to achieve optimal expression were developed, including "murinization," which replaces the human TCRalpha and TCRbeta constant regions by their murine counterparts. Using a series of mouse-human hybrid constructs, we have identified nine amino acids responsible for the improved expression of murinized TCRs. Five essential amino acid exchanges were identified in the TCRbeta C region, with exchange of a glutamic acid (human) for a basic lysine (mouse) at position 18 of the C region, being most important. For the TCRalpha C region, an area of four amino acids was sufficient for improved expression. The minimally murinized TCR variants (harboring only nine residues of the mouse sequence) enhanced expression of human TCRs by supporting preferential pairing of transferred TCR chains and a more stable association with the CD3 proteins. Most important, usage of minimally murinized TCR chains improved the function of transduced primary human T cells in comparison with cells transduced with wild-type TCRs. For TCR gene therapy, the utilization of minimally instead of completely murinized constant regions dramatically reduces the number of foreign residues and thereby the risk for immunogenicity of therapeutic TCRs.

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20483370

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Gene therapy, gene targeting and induced pluripotent stem cells: Applications in monogenic disease treatment.

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Monogenic diseases are often severe, life-threatening disorders for which lifelong palliative treatment is the only option. Over the last two decades, a number of strategies have been devised with the aim to treat these diseases with a genetic approach. Gene therapy has been under development for many years, yet suffers from the lack of an effective and safe vector for the delivery of genetic material into cells. More recently, gene targeting by homologous recombination has been proposed as a safer treatment, by specifically correcting disease-causing mutations. However, low efficiency is a major drawback. The emergence of two technologies could overcome some of these obstacles. Terminally differentiated somatic cells can be reprogrammed, using defined factors, to become induced pluripotent stem cells (iPSCs), which can undergo efficient gene mutation correction with the aid of fusion proteins known as zinc finger nucleases (ZFNs). The amalgamation of these two technologies has the potential to break through the current bottleneck in gene therapy and gene targeting.

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20481633

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Solid-State NMR Reveals the Hydrophobic-Core Location of Poly(amidoamine) Dendrimers in Biomembranes.

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Poly(amidoamine) (PAMAM) dendrimer nanobiotechnology shows great promise in targeted drug delivery and gene therapy. Because of the involvement of cell membrane lipids with the pharmacological activity of dendrimer nanomedicines, the interactions between dendrimers and lipids are of particular relevance to the pharmaceutical applications of dendrimers. In this study, solid-state NMR was used to obtain a molecular image of the complex of generation-5 (G5) PAMAM dendrimer with the lipid bilayer. Using ¹H radio frequency driven dipolar recoupling (RFDR) and ¹H magic angle spinning (MAS) nuclear Overhauser effect spectroscopy (NOESY) techniques, we show that dendrimers are thermodynamically stable when inserted into zwitterionic lipid bilayers. ¹⁴N and ³¹P NMR experiments on static samples and measurements of the mobility of C-H bonds using a 2D proton detected local field protocol under MAS corroborate these results. The localization of dendrimers in the hydrophobic core of lipid bilayers restricts the motion of bilayer lipid tails, with the smaller G5 dendrimer having more of an effect than the larger G7 dendrimer. Fragmentation of the membrane does not occur at low dendrimer concentrations in zwitterionic membranes. Because these results show that the amphipathic dendrimer molecule can be stably incorporated in the interior of the bilayer (as opposed to electrostatic binding at the surface), they are expected to be useful in the design of dendrimer-based nanobiotechnologies.

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20479154

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Selective Molecular Potassium Channel Blockade Prevents Atrial Fibrillation.

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BACKGROUND: -Safety and efficacy limit currently available atrial fibrillation (AF) therapies. We hypothesized that atrial gene transfer would allow focal manipulation of atrial electrophysiology and, by eliminating reentry, would prevent AF. **Methods and Results-**In a porcine AF model, we compared control animals to animals receiving adenovirus that encoded KCNH2-G628S, a dominant negative mutant of the IKr potassium channel alpha-subunit (G628S animals). After epicardial atrial gene transfer and pacemaker implantation for burst atrial pacing, animals were evaluated daily for cardiac rhythm. Electrophysiological and molecular studies were performed at baseline and when animals were euthanized on either postoperative day 7 or 21. By day 10, none of the control animals and all of the G628S animals were in sinus rhythm. After day 10, the percentage of G628S animals in sinus rhythm gradually declined until all animals were in AF by day 21. The relative risk of AF throughout the study was 0.44 (95% confidence interval 0.33 to 0.59, P<0.01) among the G628S group versus controls. Atrial monophasic action potential was considerably longer in G628S animals than in controls at day 7, and KCNH2 protein levels were 61% higher in the G628S group than in control animals (P<0.01). Loss of gene expression at day 21 correlated with loss of action potential prolongation and therapeutic efficacy. **Conclusions-**Gene therapy with KCNH2-G628S eliminated AF by prolonging atrial action potential duration. The effect duration correlated with transgene expression.

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20477694**

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Novel gene therapeutic strategies for the induction of tolerance in cornea transplantation.

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With more than 100,000 procedures each year, corneal transplantation (keratoplasty) is the most frequent transplantation procedure in humans. Technical advances in microsurgery have moved forward to transplant isolated layers of the cornea; however, immune-mediated allograft rejection is still a key problem and continued therapeutic efforts are required to improve the prognosis of keratoplasty. New treatment protocols need to be introduced to prevent the rejection of allogeneic grafts. The genetic modification of the graft or cells prior to transplantation is an attractive approach to protect the graft from allogeneic rejection. The transplant setting offers the unique advantage for gene therapy to modify allografts ex vivo prior to transplantation. In this review, novel therapeutic strategies using recombinant viruses as gene-transfer vehicles and, more recently, the use of gene-modified dendritic cells or regulatory T cells to protect the graft from immune-mediated rejection will be discussed.

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20477328**

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Cystic fibrosis gene therapy: successes, failures and hopes for the future.

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Cystic fibrosis (CF) is a single-gene disorder with insufficient treatment options and a target organ, the lung that is relatively easily accessible. Thus, it is not surprising that in the early years of gene therapy, CF was at the forefront of this field. Since cloning of the CF gene in 1989, 25 Phase I/II clinical trials involving approximately 420 CF patients have been carried out using a variety of viral and nonviral gene transfer agents. Most early trials focused on the nasal epithelium as a surrogate for the lung to allow for easy access and sampling, and, importantly, to ensure safety. Once an acceptable safety profile had been established, gene transfer agents were administered directly into the lung. Although many of these trials established proof-of-principle for gene transfer in the airways, a gene therapy-based treatment has not yet been developed. Here, we will summarize the key findings of these clinical studies and describe current preclinical and clinical research aimed at further developing gene therapy for CF.

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20473873

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The growth of brain tumors can be suppressed by multiple transplantation of mesenchymal stem cells expressing cytosine deaminase.

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Suicide genes have recently emerged as an attractive alternative therapy for the treatment of various types of intractable cancers. The efficacy of suicide gene therapy relies on efficient gene delivery to target tissues and the localized concentration of final gene products. Here, we showed a potential ex-vivo therapy that utilized mesenchymal stem cells (MSCs) as cellular vehicles to deliver a bacterial suicide gene, cytosine deaminase (CD) to brain tumors. MSCs were engineered to produce CD enzymes at various levels using different promoters. When co-cultured, CD-expressing MSCs had a bystander, anti-cancer effect on neighboring C6 glioma cells in proportion to the levels of CD enzymes that could convert a nontoxic prodrug, 5-fluorocytosine (5-FC) into cytotoxic 5-fluorouracil (5-FU) in vitro. Consistent with the in vitro results, for early stage brain tumors induced by intracranial inoculation of C6 cells, transplantation of CD-expressing MSCs reduced tumor mass in proportion to 5-FC dosages. However, for later stage, established tumors, a single treatment was insufficient, but only multiple transplantations were able to successfully repress tumor growth. Our findings indicate that the level of total CD enzyme activity is a critical parameter that is likely to affect the clinical efficacy for CD gene therapy. Our results also highlight the potential advantages of autograftable MSCs compared to other types of allogeneic stem cells for the treatment of recurrent glioblastomas through repetitive treatments.

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20473388

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Stimulation of functional vessel growth by gene therapy.

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The process of growing new blood vessels through gene therapy may be difficult but is certainly possible. This review will discuss the most important factors determining the efficacy of angiogenic gene therapy.

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20473307

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Epidermal growth factor receptor is a co-receptor for adeno-associated virus serotype 6.

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A key step in gene therapy is the efficient transfer of genes in a cell type- and tissue-specific manner. To better understand the mechanism of adeno-associated virus serotype 6 (AAV6) transduction, we used comparative gene analysis (CGA) combined with pathway visualization software to identify a positive correlation between AAV6 transduction and epidermal growth factor receptor (EGFR) expression. Subsequent experiments suggested that EGFR is necessary for vector internalization and probably functions as a co-receptor for AAV6.

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20471652**

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Quantitative, structural, and image-based mechanical analysis of nonunion fracture repaired by genetically engineered mesenchymal stem cells.

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Stem cell-mediated gene therapy for fracture repair, utilizes genetically engineered mesenchymal stem cells (MSCs) for the induction of bone growth and is considered a promising approach in skeletal tissue regeneration. Previous studies have shown that murine nonunion fractures can be repaired by implanting MSCs over-expressing recombinant human bone morphogenetic protein-2 (rhBMP-2). Nanoindentation studies of bone tissue induced by MSCs in a radius fracture site indicated similar elastic modulus compared to intact murine bone, eight weeks post-treatment. In the present study we sought to investigate temporal changes in microarchitecture and biomechanical properties of repaired murine radius bones, following the implantation of MSCs. High-resolution micro-computed tomography (micro-CT) was performed 10 and 35 weeks post MSC implantation, followed by micro-finite element (micro-FE) analysis. The results have shown that the regenerated bone tissue remodels over time, as indicated by a significant decrease in bone volume, total volume, and connectivity density combined with an increase in mineral density. In addition, the axial stiffness of limbs repaired with MSCs was 2-1.5 times higher compared to the contralateral intact limbs, at 10 and 35 weeks post-treatment. These results could be attributed to the fusion that occurred in between the ulna and radius bones. In conclusion, although MSCs induce bone formation, which exceeds the fracture site, significant remodeling of the repair callus occurs over time. In addition, limbs treated with an MSC graft demonstrated superior biomechanical properties, which could indicate the clinical benefit of future MSC application in nonunion fracture repair.

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20470907**

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Caspase-9-dependent decrease of nuclear pore channel hydrophobicity is accompanied by nuclear envelope leakiness.

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Advances in nano-medicine require conceptual understanding of physiological processes. Apoptosis is a fundamental physiological process, which is characterised among other things by an increased permeability of the nuclear envelope (NE). The latter is a tight transport barrier, known to restrict nuclear delivery rate of therapeutic nano-particles. Therefore, an understanding of the underlying mechanism, which leads to the breakdown of the barrier during apoptosis, could stimulate the development of new approaches in gene therapy. We set out to elucidate this mechanism following induction of apoptosis on isolated cell nuclei. We tested the hypothesis whether caspases, mediators of apoptosis, trigger the NE leakiness at the level of the nuclear pore complexes (NPCs) using fluorescence techniques. As the permeability barrier inside the NPC channel is thought to be based on hydrophobic-hydrophobic protein interactions we further investigated the NPC channel hydrophobicity using atomic force microscopy (AFM). Caspase-9 was found to induce NE leakiness to large macromolecules. Leakiness was prevented by pretreatment of NPCs with an importin-beta mutant, which irreversibly binds and thereby obstructs the NPC channel. Utilizing an ultra sharp, hydrophobic AFM tip as a chemical nano-sensor, which reaches deep into the apoptotic NPC channel, a remarkable decrease of hydrophobic binding sites was detected therein. We conclude that caspase 9 gives rise to NE leakiness by perturbing the hydrophobicity-based barrier inside the NPC channel. This explains the high passive NE permeability in early apoptosis.