CLINIGENE CURRENT GENE THERAPY WEEKLY

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Impact of lentiviral vector-mediated transduction on the tightness of a polarized model of airway epithelium and effect of cationic polymer polyethylenimine.

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Lentiviral (LV) vectors are promising agents for efficient and long-lasting gene transfer into the lung and for gene therapy of genetically determined pulmonary diseases, such as cystic fibrosis, however, they have not been evaluated for cytotoxicity and impact on the tightness of the airway epithelium. In this study, we evaluated the transduction efficiency of a last-generation LV vector bearing Green Fluorescent Protein (GFP) gene as well as cytotoxicity and tight junction (TJ) integrity in a polarized model of airway epithelial cells. High multiplicities of infection (MOI) showed to be cytotoxic, as assessed by increase in propidium iodide staining and decrease in cell viability, and harmful for the epithelial tightness, as demonstrated by the decrease of transepithelial resistance (TER) and delocalization of occludin from the TJs. To increase LV efficiency at low LV:cell ratio, we employed noncovalent association with the polycation branched 25 kDa polyethylenimine (PEI). Transduction of cells with PEI/LV particles resulted in 2.5-3.6-fold increase of percentage of GFP-positive cells only at the highest PEI:LV ratios (1 x 10(7) PEI molecules/transducing units with 50 MOI LV) as compared to plain LV. At this dose PEI/LV transduction resulted in 6.5 +/- 2.4% of propidium iodide-positive cells. On the other hand, PEI/LV particles did not determine any alteration of TER and occludin localization. We conclude that PEI may be useful for improving the efficiency of gene transfer mediated by LV vectors in airway epithelial cells, in the absence of high acute cytotoxicity and alteration in epithelial tightness.

PMID: 20616678

Retina. 2010 Jul-Aug;30(7):983-1001.

Progress toward the maintenance and repair of degenerating retinal circuitry.

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BACKGROUND: Retinal diseases such as age-related macular degeneration and retinitis pigmentosa remain major causes of severe vision loss in humans. Clinical trials for treatment of retinal degenerations are underway and advancements in our understanding of retinal biology in health/disease have implications for novel therapies. METHODS: A review of retinal biology is used to inform a discussion of current strategies to maintain/repair neural circuitry in age-related macular degeneration, retinitis pigmentosa, and Type 2 Leber congenital amaurosis. RESULTS: In age-related macular degeneration/retinitis pigmentosa, a progressive loss of rods/cones results in corruption of bipolar cell circuitry, although retinal output neurons/photoreceptive melanopsin cells survive. Visual function can be stabilized/enhanced after treatment in age-related macular degeneration, but in advanced degenerations, reorganization of retinal circuitry may preclude attempts to restore cone function. In Type 2 Leber congenital amaurosis, useful vision can be restored by gene therapy where central cones survive. Remarkable progress has been made in restoring vision to rodents using light-responsive ion channels inserted into bipolar cells/retinal ganglion cells. CONCLUSION: Advances in genetic, cellular, and prosthetic therapies show varying degrees of promise for treating retinal degenerations. While functional benefits can be obtained after early therapeutic interventions, efforts should be made to minimize circuitry changes as soon as possible after rod/cone loss. Advances in retinal anatomy/physiology and genetic technologies should allow refinement of future reparative strategies.

PMID: 20616221

Blood. 2010 Jul 8. [Epub ahead of print]

TAT-mediated transduction of NF-Ya peptide induces the ex vivo proliferation and engraftment potential of human hematopoietic progenitor cells.

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Retroviral overexpression of NF-Ya, the regulatory subunit of the transcription factor NF-Y, activates the transcription of multiple genes implicated in hematopoietic stem cells (HSC) self-renewal and differentiation and directs HSC toward self-renewal. We asked whether TAT-NF-Ya fusion protein could be employed to transduce human CD34+ cells as a safer, more regulated alternative approach to gene therapy. Here we show that externally added recombinant protein was able to enter the cell nucleus and activate HoxB4, a target gene of NF-Ya, using real-time PCR RNA and luciferase-based protein assays. Following TAT-NF-Ya transduction, the proliferation of human CD34+ cells in the presence of myeloid cytokines was increased 4-fold. Moreover, TAT-NF-Ya treated human primary bone marrow cells showed a 4-fold increase in the percentage of huCD45+ cells recovered from the bone marrow of sublethally irradiated, transplanted NOD-scid IL2Rgamma (null) mice. These data demonstrate that TAT-peptide therapies are an alternative approach to retroviral stem cell therapies, and suggest that NF-Ya peptide delivery should be further evaluated as a therapeutic tool for HSC/progenitors ex vivo expansion and therapy.

PMID: 20615691

Bioorg Med Chem Lett. 2010 Apr 14. [Epub ahead of print]

Cancer cell specific gene delivery by laminin-derived peptide AG73-labeled liposomes.

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We developed laminin-derived AG73 peptide labeled liposomes for cancer specific gene therapy. AG73 peptide is well known as a ligand for syndecan-2 which is highly expressed in various cancer cells. Liposomes labeled with AG73 showed high efficient transfection efficiency in syndecan-2 overexpressing cells, and found that AG73 could be a superior molecule in the development of non-viral vector using liposomes for the gene delivery to syndecan-2 overexpressing cancer cells. Copyright © 2010 Elsevier Ltd. All rights reserved.

PMID: 20614615

In: Melton D, Girard L, editors. StemBook [Internet]. Cambridge (MA): Harvard Stem Cell Institute; 2008-.2008 Sep 30.

The role of bone marrow-derived stem cells in lung regeneration and repair.

Sage EK, Loebinger MR, Polak J, Janes SM.

Adult stem cells posses the ability to undergo both self-renewal and differentiation in multiple lineages. A recent body of work has utilised exogenous mesenchymal stem cells from the bone marrow compartment to attenuate lung injury. Initial studies suggested that bone marrow-derived stem cells (BMSCs) could repair damaged tissue by differentiating into epithelial cells in disparate sites. However this has been challenged and is now felt to be of limited clinical significance. What is clearer is that in chronic lung injury these cells are activated in response to tissue damage, migrate to the site of injury and contribute to both structural and functional repair. They are relatively non-immunogenic allowing them to be expanded and engineered ex vivo and re-introduced without immunomodulation. In acute lung injury BMSCs have been shown to reduce the pulmonary inflammatory response via a number of mechanisms to cause down-regulation of pro-inflammatory cytokines and a reduction in pathological lung damage. This chapter examines the role of exogenous bone marrow-derived cells, and in particular mesenchymal stem cells in both repair of chronic lung disease and acute lung injury, and their suitability as vectors for gene therapy.

PMID: 20614603

In: Melton D, Girard L, editors. StemBook [Internet]. Cambridge (MA): Harvard Stem Cell Institute; 2008-.2009 Jan 15.

Mesenchymal stromal cells as a drug delivery system.

Menon LG, Shi VJ, Carroll RS.

Mesenchymal stromal cells (MSC) are multipotent cells that can self-renew and at the same time differentiate into multiple lineages with specific surface marker expression. During the past several years, MSCs have generated a great deal of interest in many clinical settings, including regenerative medicine, immune modulation, and tissue engineering. Many studies have demonstrated their remarkable tumor tropic properties. Several pre-clinical and clinical studies have demonstrated the efficacy of genetically modified MSC to express and release therapeutic factors, confirming their ability to serve as an excellent base for cell-mediated gene therapy. This chapter will review the literature on the use of MSCs as a therapeutic drug delivery system.

PMID: 20614008

PLoS One. 2010 Jun 29;5(6):e11367.

Impact of hydrodynamic injection and phiC31 integrase on tumor latency in a mouse model of MYC-induced hepatocellular carcinoma.

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BACKGROUND: Hydrodynamic injection is an effective method for DNA delivery in mouse liver and is being translated to larger animals for possible clinical use. Similarly, phiC31 integrase has proven effective in mediating long-term gene therapy in mice when delivered by hydrodynamic injection and is being considered for clinical gene therapy applications. However, chromosomal aberrations have been associated with phiC31 integrase expression in tissue culture, leading to questions about safety. METHODOLOGY/PRINCIPAL FINDINGS: To study whether hydrodynamic delivery alone, or in conjunction with delivery of phiC31 integrase for long-term transgene expression, could facilitate tumor formation, we used a transgenic mouse model in which sustained induction of the human C-MYC oncogene in the liver was followed by hydrodynamic injection. Without injection, mice had a median tumor latency of 154 days. With hydrodynamic injection of saline alone, the median tumor latency was significantly reduced, to 105 days. The median tumor latency was similar, 106 days, when a luciferase donor plasmid and backbone plasmid without integrase were administered. In contrast, when active or inactive phiC31 integrase and donor plasmid were supplied to the mouse liver, the median tumor latency was 153 days, similar to mice receiving no injection. CONCLUSIONS/SIGNIFICANCE: Our data suggest that phiC31 integrase does not facilitate tumor formation in this C-MYC transgenic mouse model. However, in groups lacking phiC31 integrase, hydrodynamic injection appeared to contribute to C-MYC-induced hepatocellular carcinoma in adult mice. Although it remains to be seen to what extent these findings may be extrapolated to catheter-mediated hydrodynamic delivery in larger species, they suggest that caution should be used during translation of hydrodynamic injection to clinical applications.

PMID: 20613637

J Neuropathol Exp Neurol. 2010 Jul 7. [Epub ahead of print]

Targeting Fibrosis in Duchenne Muscular Dystrophy.

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Duchenne muscular dystrophy (DMD) is the most common genetic muscle disease affecting 1 in 3,500 live male births. It is an X-linked recessive disease caused by a defective dystrophin gene. The disease is characterized by progressive limb weakness, respiratory and cardiac failure, and premature death. Fibrosis is a prominent pathological feature of muscle biopsies from patients with DMD. It directly causes muscle dysfunction and contributes to the lethal DMD phenotype. Although gene therapy and cell therapy may ultimately provide a cure for DMD, currently the disease is devastating, with no effective therapies. Recent studies have demonstrated that amelioratingmuscle fibrosis may represent a viable therapeutic approach for DMD. By reducing scar formation, antifibrotic therapies may not onlyimprove muscle function but also enhance muscle regeneration and promote gene and stem cell engraftment. Antifibrotic therapy mayserve as a necessary addition to gene and cell therapies to treat DMD in the future. Therefore, understanding cellular and molecular mechanisms underlying muscle fibrogenesis associated with dystrophin deficiency is key to the development of effective antifibrotic therapies for DMD.

PMID: 20586798

Haemophilia. 2010 May;16 Suppl 3:24-8.

Gene therapy in haemophilia--going for cure?

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Traditional treatment for haemophilia consists of bolus infusion of the missing coagulation factor, either prophylactically or on demand, but is complicated by the development of inhibitory antibodies to the infused factor. In those cases, as well as in patients with platelet defects or factor VII (FVII) deficiency, recombinant human activated FVII has been successfully used, but carries the disadvantage of a short plasma half-life. As an alternative, emerging methodology based on gene transfer may be utilized to provide effective haemostasis in patients with coagulation defects. The goal of this article is to introduce the novel concept of continuous expression of activated FVII from a donated gene for the treatment of haemophilia, and to review the safety and efficacy data that have been produced so far by this approach in small and large animal models.

PMID: 20607625

Mol Biotechnol. 2010 Jul 8. [Epub ahead of print]

Vector Insert-Targeted Integrative Antisense Expression System for Plasmid Stabilization.

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Some DNA vaccine and gene therapy vector-encoded transgenes are toxic to the E. coli plasmid production host resulting in poor production yields. For plasmid products undergoing clinical evaluation, sequence modification to eliminate toxicity is undesirable because an altered vector is a new chemical entity. We hypothesized that: (1) insert-encoded toxicity is mediated by unintended expression of a toxic insert-encoded protein from spurious bacterial promoters; and (2) that toxicity could be eliminated with antisense RNA-mediated translation inhibition. We developed the pINT PR PL vector, a chromosomally integrable RNA expression vector, and utilized it to express insert-complementary (anti-insert) RNA from a single defined site in the bacterial chromosome. Anti-insert RNA eliminated leaky fluorescent protein expression from a target plasmid. A toxic retroviral gag pol helper plasmid produced in a gag pol anti-insert strain had fourfold improved plasmid fermentation yields. Plasmid fermentation yields were also fourfold improved when a DNA vaccine plasmid containing a toxic Influenza serotype H1 hemagglutinin transgene was grown in an H1 sense strand anti-insert production strain, suggesting that in this case toxicity was mediated by an antisense alternative reading frame-encoded peptide. This anti-insert chromosomal RNA expression technology is a general approach to improve production yields with plasmid-based vectors that encode toxic transgenes, or toxic alternative frame peptides.

PMID: 20519210

J R Soc Interface. 2010 Aug 6;7 Suppl 4:S393-402. Epub 2010 Jun 2.

Size mapping of electric field-assisted production of polycaprolactone particles.

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In this investigation, biodegradable polycaprolactone polymeric particles (300-4500 nm in diameter) were prepared by jetting a solution in an electric field. An extensive study has been carried out to determine how the size and size distribution of the particles generated can be controlled by systematically varying the polymer concentration in solution (and thereby its viscosity and electrical conductivity), and also the selected flow rate (2-50 microl min(-1)) and applied voltage (0-15 kV) during particle generation. Change in these parameters affects the mode of jetting, and within the stable cone-jet mode window, an increase in the applied voltage (approx. 15 kV) resulted in a reduction in particle size and this was more pronounced at high flow rates (such as; 30, 40 and 50 microl min(-1)) in the same region. The carrier particles were more polydisperse at the peripheral regions of the stable cone-jet mode, as defined in the applied voltage-flow rate parametric map. The effect of loading a drug on the particle size, size distribution and encapsulation efficiency was also studied. Release from drug-loaded particles was investigated using UV spectrophotometry over 45 days. This work demonstrates a powerful method of generating drug-loaded polymeric particles, with the ability to control size and polydispersivity, which has great potential in several categories of biotechnology requiring carrier particles, such as drug delivery and gene therapy.

PMID: 20607367

Pharm Res. 2010 Jul 7. [Epub ahead of print]

Hypoxia-Inducible Vascular Endothelial Growth Factor Gene Therapy Using the Oxygen-Dependent Degradation Domain in Myocardial Ischemia.

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PURPOSE: A hypoxia-inducible VEGF expression system with the oxygen-dependent degradation (ODD) domain was constructed and tested to be used in gene therapy for ischemic myocardial disease. METHODS: Luciferase and VEGF expression vector systems were constructed with or without the ODD domain: pEpo-SV-Luc (or pEpo-SV-VEGF) and pEpo-SV-Luc-ODD (or pEpo-SV-VEGF-ODD). In vitro gene expression efficiency of each vector type was evaluated in HEK 293 cells under both hypoxic and normoxic conditions. The amount of VEGF protein was estimated by ELISA. The VEGF expression vectors with or without the ODD domain were injected into ischemic rat myocardium. Fibrosis, neovascularization, and cardiomyocyte apoptosis were assessed using Masson's trichrome staining, alpha-smooth muscle actin (alpha-SMA) immunostaining, and the TUNEL assay, respectively. RESULTS: The plasmid vectors containing ODD significantly improved the expression level of VEGF protein in hypoxic conditions. The enhancement of VEGF protein production was attributed to increased protein stability due to oxygen deficiency. In a rat model of myocardial ischemia, the pEpo-SV-VEGF-ODD group exhibited less myocardial fibrosis, higher microvessel density, and less cardiomyocyte apoptosis compared to the control groups (saline and pEpo-SV-VEGF treatments). CONCLUSION: An ODD-mediated VEGF expression system that facilitates VEGF-production under hypoxia may be useful in the treatment of ischemic heart disease.

PMID: 20606864

Oman J Ophthalmol. 2010 Jan;3(1):2-6.

Gene therapy in glaucoma-part I: Basic mechanisms and molecular genetics.

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Glaucoma is the second most common cause of blindness in the world as determined by the World Health Organization (WHO). Glaucoma diagnosis, identification of people at risk, initiation of treatment and timing of surgical intervention remains a problem. Despite new and improving diagnostic and therapeutic options for glaucoma, blindness from glaucoma remains a major public health problem. The role of heredity in ocular disease is attracting greater attention as knowledge and recent advances of Human Genome Project and the HapMap Project have made genetic analysis of many human disorders possible.Glaucoma offers a variety of potential targets for gene therapy. All risk factors for glaucoma and their underlying causes are potentially susceptible to modulation by gene transfer. The discovery of genes responsible for glaucoma has led to the development of new methods of Deoxyribonucleic acid (DNA)-based diagnosis and treatment. As genetic defects responsible for glaucoma are identified and the biochemical mechanisms underlying the disease are recognized, new methods of therapy can be developed. It is of utmost importance for ophthalmologists and glaucoma specialists to be familiar with and understand the basic molecular mechanisms, genes responsible for glaucoma and the ways of genetic treatment. METHOD OF LITERATURE SEARCH: The literature was searched on the Medline database using the Pubmed interface.

PMID: 20606642

Mol Ther. 2010 Jul 6. [Epub ahead of print]

A Phase I Study of Aromatic L-Amino Acid Decarboxylase Gene Therapy for Parkinson's Disease.

Muramatsu SI, Fujimoto KI, Kato S, Mizukami H, Asari S, Ikeguchi K, Kawakami T, Urabe M, Kume A, Sato T, Watanabe E, Ozawa K, Nakano I.
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Gene transfer of dopamine-synthesizing enzymes into the striatal neurons has led to behavioral recovery in animal models of Parkinson's disease (PD). We evaluated the safety, tolerability, and potential efficacy of adeno-associated virus (AAV) vector-mediated gene delivery of aromatic L-amino acid decarboxylase (AADC) into the putamen of PD patients. Six PD patients were evaluated at baseline and at 6 months, using multiple measures, including the Unified Parkinson's Disease Rating Scale (UPDRS), motor state diaries, and positron emission tomography (PET) with 6-[(18)F]fluoro-L-m-tyrosine (FMT), a tracer for AADC. The short-duration response to levodopa was measured in three patients. The procedure was well tolerated. Six months after surgery, motor functions in the OFF-medication state improved an average of 46% based on the UPDRS scores, without apparent changes in the short-duration response to levodopa. PET revealed a 56% increase in FMT activity, which persisted up to 96 weeks. Our findings provide class IV evidence regarding the safety and efficacy of AADC gene therapy and warrant further evaluation in a randomized, controlled, phase 2 setting.

PMID: 20606371

Yakugaku Zasshi. 2010 Jul;130(7):917-23.

Development of Non-viral Vector for Cancer Gene Therapy.

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Cancer gene therapy has been intensively developed using non-viral vectors, among which cationic liposomes and nanoparticles are the most investigated. Optimal gene therapy for tumors must deliver plasmid DNA (pDNA) or synthetic small interfering RNA (siRNA) to tumor cells with high efficiency and minimal toxicity. We developed new cationic nanoparticles (NP) composed of cholesteryl-3beta-carboxyamidoethylene-N-hydroxyethylamine (OH-Chol) and Tween 80, and evaluated the transfection efficiencies of pDNA and siRNA into human prostate tumor PC-3 xenografts. NP showed effective transfection of pDNA and siRNA when directly injected into the xenografts. For targeted delivery to tumors, vitamin folic acid has been utilized for folate receptor (FR)-mediated drug delivery since FR is frequently overexpressed on many types of human tumors. We developed folate-linked nanoparticles (NP-F) composed of OH-Chol, Tween 80 and folate-poly(ethylene glycol)-distearoylphosphatidylethanolamine conjugate. Tumor growth of FR-positive human nasopharyngeal tumor KB xenografts was significantly inhibited when a complex of NP-F and a therapeutic gene was intratumorally injected. These findings suggested that cationic cholesterol-based nanoparticles are potential non-viral pDNA and siRNA vectors for local tumor treatment.

PMID: 20605584

J Surg Res. 2010 Apr 18. [Epub ahead of print]

Overexpression of STAT3 Potentiates Growth, Survival, and Radioresistance of Non-Small-Cell Lung Cancer (NSCLC) cells.

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OBJECTIVE: Activation of signal transducer and activator of transcription 3 (STAT3) play important roles in tumorigenesis and tumor progression. The overexpression of STAT3 has been found in various malignancies including non-small-cell lung carcinoma (NSCLC). The purpose of this study was to explore the correlation between overexpression of STAT3 gene and growth, survival, and radiosensitivity of NSCLC cells. METHODS: Subclones using vector-based short hairpin RNA (shRNA) were established. RT-PCR and Western blot assays were performed to detect the expression of STAT3 mRNA and protein in untransfected or stably transfected NSCLC cells. Then, MTT and soft agar colony assays were performed to determine the effect of STAT3 inhibition on in vitro growth of NSCLC cells. Hoechst staining assay was performed to analyze the effect of STAT3 inhibition on apoptosis of NSCLC cells. Additionally, clonogenic survival assays were performed to detect the effect of STAT3 inhibition on in vitro radiosensitivity of NSCLC cells. Finally, to examine the effect of pSUPER-shSTAT3 on proliferation and radiosensitivity in vivo, a subcutaneous (s.c.) tumor formation assay in nude mice was performed. RESULTS: We successfully established two stable transfected cell lines (A549/shSTAT3 and SK-MES-1/shSTAT3) in which the expression of STAT3 mRNA and protein was down-regulated. Those two stable subclones showed a significantly dramatic reduction in colony-forming ability and proliferation not only in vitro but also in vivo. The apoptotic rates of A549/shSTAT3 and SK-MES-1/shSTAT3 cells increased to 19.2% and 16.4%, respectively. Moreover, shRNA-mediated STAT3 inhibition could also significantly enhance radiosensitivity of NSCLC cells both in vitro and in vivo. CONCLUSION: Together, the overexpression of STAT3 is correlated with growth, survival, and radioresistance of NSCLC cells, and STAT3 might be a molecular therapeutic target for gene therapy or radiosensitization of NSCLC. Copyright © 2010 Elsevier Inc. All rights reserved.

PMID: 20604683

Hum Gene Ther. 2010 Jul 6. [Epub ahead of print]

Molecular Anthropology meets Genetic Medicine to Treat Blindness in the North African Jewish Population: Human Gene Therapy Initiated in Israel.

Banin E, Bandah-Rosenfeld D, Obolensky A, Cideciyan AV, Aleman TS, Marks-Ohana D, Sela M, Boye SL, Sumaroka A, Roman AJ, Schwartz SB, Hauswirth W, Jacobson SG, Hemo I, Sharon D.
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The history of the North African Jewish community is ancient and complicated with a number of immigration waves and persecutions dramatically affecting its population size. A decade-long process in Israel of clinical-molecular screening of North African Jews with incurable autosomal recessive blindness led to the identification of a homozygous splicing mutation (c.95-2A>T; IVS2-2A>T) in RPE65, the gene encoding the isomerase that catalyzes a key step in the retinoid-visual cycle, in patients from 10 unrelated families. A total of 33 patients (4 now deceased) had the severe childhood blindness known as Leber congenital amaurosis (LCA), making it the most common cause of retinal degeneration in this population. Haplotype analysis in seven of the patients revealed a shared homozygous region, indicating a population-specific founder mutation. The age of the RPE65 founder mutation was estimated to have emerged 100-230 (mean, 153) generations ago, suggesting it originated before the establishment of the Jewish community in North Africa. Individuals with this RPE65 mutation were characterized with retinal studies to determine if they were candidates for gene replacement, the recent and only therapy to date for this otherwise incurable blindness. The step from molecular anthropological studies to application of genetic medicine was then taken and a representative of this patient subgroup was treated with subretinal rAAV2-RPE65 gene therapy. An increase in vision was present in the treated area as early as 15 days after the intervention. This process of genetically analyzing affected isolated populations as a screen for gene-based therapy suggests a new paradigm for disease diagnosis and treatment.

PMID: 20603862

J Gene Med. 2010 Jul;12(7):572-9.

Silk-elastinlike protein polymers improve the efficacy of adenovirus thymidine kinase enzyme prodrug therapy of head and neck tumors.

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BACKGROUND: Adenoviral-directed enzyme prodrug therapy is a promising approach for head and neck cancer gene therapy. The challenges faced by this approach, however, comprise transient gene expression and dissemination of viruses to distant organs. METHODS: We used recombinant silk-elastinlike protein polymer (SELP) matrices for intratumoral delivery of adenoviruses containing both thymidine kinase-1 and luciferase genes in a nude mouse model of JHU-022 head and neck tumor. Hydrogels made from two SELP analogues (47K and 815K), with similar silk to elastinlike block ratios but different block lengths, were studied for intratumoral viral delivery. Tumor-bearing mice were followed up for tumor progression and luciferase gene expression concomitantly for 5 weeks. Polymer safety was evaluated through body weight change, blood count, and liver and kidney functions, in addition to gross and microscopic histological examination. RESULTS: SELP-815K analogues efficiently controlled the duration and extent of transfection in tumors for up to 5 weeks with no detectable spread to the liver. An approximately five-fold greater reduction in tumor volume was obtained with matrix-mediated delivery compared to intra-tumoral injection of adenoviruses in saline. SELP matrix proved safe in all injected mice compared to the control group. CONCLUSIONS: The SELP-controlled gene delivery approach could potentially improve the anticancer activity of virus-mediated gene therapy at the same time as limiting viral spread to normal organs.

PMID: 20603861

J Gene Med. 2010 Jul;12(7):580-5.

Long-term human growth hormone expression and partial phenotypic correction by plasmid-based gene therapy in an animal model of isolated growth hormone deficiency.

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Biotechnology Department, National Nuclear Energy Commission (IPEN-CNEN), Cidade Universitária, São Paulo, SP, Brazil.

BACKGROUND: A model for in vivo gene therapy based on electroporation of human growth hormone (hGH)-coding naked DNA in the muscle of dwarf (lit/lit) and immunodeficient dwarf (lit/scid) mice is described. METHODS: A plasmid containing the ubiquitin C promoter and the genomic hGH sequence was administered to the exposed quadriceps muscle, followed by electrotransfer using eight 50-V pulses of 20 ms at a 0.5-s interval. Serum hGH levels were determined after various days of DNA administration and a long-term body weight gain experiment was carried out. RESULTS: Serum hGH, determined 3 days after DNA administration, revealed a significant dose-response curve ($p < 0.01$) in the 0-50 microg range. Because 50 microg of plasmid DNA produced circulating hGH levels of 2-3 ng/ml for at least 12 days, a long-term body weight gain assay was carried out. After 60 days, the weight of treated lit/scid mice increased 33.1% compared to a 4.2% weight decrease for the control group. hGH circulating levels were of the order of 1.5-3 ng/ml throughout the experiment and the average weight increase during the first 10 days was comparable to that obtained upon regular daily injection of 10 microg of recombinant hGH per mouse, producing comparable circulating levels of the hormone. A lower, but still significant increase in body weight was obtained upon repeating the experiment in immunocompetent dwarf mice (lit/lit). CONCLUSIONS: We report for the first time sustained levels of circulating hGH after intramuscular naked DNA administration and, consequently, a highly significant weight increase of dwarf 'little' mice.

PMID: 20603651

Drug News Perspect. 2010 Jun;23(5):281-6.

The future of epilepsy treatment: Focus on adeno-associated virus vector gene therapy.

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Adeno-associated virus (AAV) vectors support long-term, nontoxic gene expression in the central nervous system, and these AAV properties prove particularly applicable to the treatment of focal epilepsies, especially intractable temporal lobe epilepsy. A number of clinical studies have employed AAV vectors and to date, no known adverse effects have been directly associated with these treatments, particularly AAV serotype 2 (AAV2). Although other AAV serotypes may confer an advantage in the future, extensive studies on the inhibitory neuropeptides, galanin and neuropeptide Y, have generated enough preclinical evidence of efficacy to warrant AAV2-based clinical trials in the near future. Beyond these trials, emerging evidence suggests that AAV-mediated manipulation of adenosine can significantly impact limbic seizure activity. Thus, with appropriate nonhuman primate transduction patterns and favorable overall toxicology studies, AAV-based manipulation of adenosine could follow the AAV-neuropeptide clinical studies. Finally, recent findings using AAV capsid shuffling and directed evolution have identified a hybrid AAV vector that can selectively cross the seizure compromised blood-brain barrier and transduce cells after peripheral, intravenous administration. Thus, in the more distant future, AAV therapeutics for focal epilepsies may be delivered without any neurosurgical interventions.

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Protein trans-splicing based dual-vector delivery of the coagulation factor VIII gene.

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A dual-vector system was explored for the delivery of the coagulation factor VIII gene, using intein-mediated protein trans-splicing as a means to produce intact functional factor VIII post-translationally. A pair of eukaryotic expression vectors, expressing Ssp DnaB intein-fused heavy and light chain genes of B-domain deleted factor VIII (BDD-FVIII), was constructed. With transient co-transfection of the two vectors into 293 and COS-7 cells, the culture supernatants contained (137+/-23) and (109+/-22) ng mL(-1) spliced BDD-FVIII antigen with an activity of (1.05+/-0.16) and (0.79+/-0.23) IU mL(-1) for 293 and COS-7 cells, respectively. The spliced BDD-FVIII was also detected in supernatants from a mixture of cells transfected with inteinfused heavy and light chain genes. The spliced BDD-FVIII protein bands from cell lysates were visualized by Western blotting. The data demonstrated that intein could be used to transfer the split factor VIII gene and provided valuable information on factor VIII gene delivery by dual-adeno-associated virus in hemophilia A gene therapy.

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Increased Cellular Uptake of Biocompatible Superparamagnetic Iron Oxide Nanoparticles into Malignant Cells by an External Magnetic Field.

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Superparamagnetic iron oxide nanoparticles (SPIONs) are used as delivery systems for different therapeutics including nucleic acids for magnetofection-mediated gene therapy. The aim of our study was to evaluate physicochemical properties, biocompatibility, cellular uptake and trafficking pathways of the custom-synthesized SPIONs for their potential use in magnetofection. Custom-synthesized SPIONs were tested for size, shape, crystalline composition and magnetic behavior using a transmission electron microscope, X-ray diffractometer and magnetometer. SPIONs were dispersed in different aqueous media to obtain ferrofluids, which were tested for pH and stability using a pH meter and zetameter. Cytotoxicity was determined using the MTS and clonogenic assays. Cellular uptake and trafficking pathways were qualitatively evaluated by transmission electron microscopy and quantitatively by inductively coupled plasma atomic emission spectrometry. SPIONs were composed of an iron oxide core with a diameter of 8-9 nm, coated with a 2-nm-thick layer of silica. SPIONs, dispersed in 0.9% NaCl solution, resulted in a stable ferrofluid at physiological pH for several months. SPIONs were not cytotoxic in a broad range of concentrations and were readily internalized into different cells by endocytosis. Exposure to neodymium-iron-boron magnets significantly increased the cellular uptake of SPIONs, predominantly into malignant cells. The prepared SPIONs displayed adequate physicochemical and biomedical properties for potential use in magnetofection. Their cellular uptake was dependent on the cell type, and their accumulation within the cells was dependent on the duration of exposure to an external magnetic field.

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Anti-tumor effect of (125)I-UdR in combination with Egr-1 promoter-based IFNgamma gene therapy in vivo.

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Although (125)I-UdR treatment of malignant tumors in animal models and patients has achieved a certain effect, the short half-life of (125)I-UdR in vivo and its cellular uptake only in S phase of the cell cycle are limiting factors with regard to tumor eradication, and therefore its combination with other applications is a promising strategy in cancer therapy. In this study, we show that (125)I-UdR radionuclide therapy in combination with Egr-1 promoter-based IFNgamma gene therapy is more effective than (125)I-UdR therapy alone in suppressing tumor growth and extending survival duration in mice bearing H22 hepatomas. Combined therapy could significantly inhibit cell proliferation and tumor angiogenesis, induce apoptosis and enhance cytotoxic activities of splenic CTL of the mice. Our results suggest that (125)I-UdR in combination with Egr-1 promoter-based IFNgamma gene therapy may provide novel approaches for cancer treatment.

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Construction, expression and characterization of a chimeric multi-domain protein mediating specific DNA transfer.

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The delivery of plasmid DNA to target cells using a simple, defined, non-viral system is an area of intense research in gene therapy. Here, we describe a novel DNA carrier protein termed TG, consisting of the DNA-binding domain of the yeast transcriptional activator GAL4 and human immunodeficiency virus type 1 Tat protein, which can transfer modified naked plasmid DNA into target cells to express foreign genes of interest. The TG protein was expressed in Escherichia coli (E. coli), refolded and purified on an immobilized Ni(2+) affinity chromatography column. SDS-PAGE and Western blotting revealed that the fusion protein was highly expressed with a yield of approximately 275 mg/L. We also constructed the pIRES-UAS-EGFP DNA vector, consisting of upstream activating sequences (UASs) for the specific binding of the DNA-binding protein and the enhanced green fluorescent protein (EGFP) gene. The TG protein could bind specifically to pIRES-UAS-EGFP, forming a complex which could efficiently transfect target cells and result in detectable EGFP protein expression. Thus, these results provide a basis for development of efficient non-viral DNA transfer vectors for further improvements of gene therapy strategies. Copyright © 2010. Published by Elsevier Inc.

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The genomic risk of somatic gene therapy.

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Gene vectors with an untargeted insertion profile have been explored in preclinical models and clinical trials for the transfer of potentially therapeutic genetic information into somatic cells that have a high replicative potential. The gene-modified cell population can be viewed as a genetic mosaic whose complexity depends upon the number of transduced cells, the average number of insertions per cell, the target cell type, the genetic stability and composition of the transgene, and the integration pattern of the vector. Selection by the environment encountered in the patient may support the preferential survival of clones with insertional deregulation of genes that are involved in the control of engraftment, proliferation or differentiation, in the worst case initiating oncogenic progression. Rapid scientific and technological progress has shed much light onto this dark side of untargeted vector integration. New approaches to unbiased and highly sensitive "integromics" promise a precise documentation of stable polyclonality, clonal fluctuation or clonal imbalance of gene-modified cell populations. Evidence has been obtained for a number of approaches to potentially reduce the genomic risk of gene therapy: targeting cells that lack sustained replicative potential, using vectors with a more neutral integration spectrum, reducing the number of vector copies per cell, designing gene expression cassettes that avoid long-distance enhancer interactions or fusion transcripts, and reducing, as far as possible, the risk of secondary mutations. The genomic risk of gene therapy can thus be prevented by the collective targeting of all contributing factors. Copyright © 2010. Published by Elsevier Ltd.

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Gene therapy for ischemic heart disease.

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Current pharmacologic therapy for ischemic heart disease suffers multiple limitations such as compliance issues and side effects of medications. Revascularization procedures often end with need for repeat procedures. Patients remain symptomatic despite maximal medical therapy. Gene therapy offers an attractive alternative to current pharmacologic therapies and may be beneficial in refractory disease. Gene therapy with isoforms of growth factors such as VEGF, FGF and HGF induces angiogenesis, decreases apoptosis and leads to protection in the ischemic heart. Stem cell therapy augmented with gene therapy used for myogenesis has proven to be beneficial in numerous animal models of myocardial ischemia. Gene therapy coding for antioxidants, eNOS, HSP, mitogen-activated protein kinase and numerous other anti apoptotic proteins have demonstrated significant cardioprotection in animal models. Clinical trials have demonstrated safety in humans apart from symptomatic and objective improvements in cardiac function. Current research efforts are aimed at refining various gene transfection techniques and regulation of gene expression in vivo in the heart and circulation to improve clinical outcomes in patients that suffer from ischemic heart disease. In this review article we will attempt to summarize the current state of both preclinical and clinical studies of gene therapy to combat myocardial ischemic disease.

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AAV serotype influences gene transfer in corneal stroma in vivo.

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This study evaluated the cellular tropism and relative transduction efficiency of three AAV serotypes, AAV6, AAV8 and AAV9, for corneal gene delivery using mouse cornea in vivo and donor human cornea ex vivo. The AAV6, AAV8 and AAV9 serotypes having AAV2 plasmid encoding for alkaline phosphatase (AP) gene were generated by transfecting HEK 293 cell line with pHelper, pARAP4 and pRep/Cap plasmids. Viral vectors (10(9) vg/mul) were topically applied onto mouse cornea in vivo and human cornea ex vivo after removing the epithelium. Human corneas were processed for transgene delivery at day 5 after viral vector application. Mouse corneas were harvested at 4, 14 and 30 days after vector application for AP staining. Transduction efficiency was calculated by quantifying pixels of AP-stained area using Image J software and also confirmed by functional AP enzyme activity in the corneal lysates. Cellular toxicity of the three AAV serotypes was tested with TUNEL assay. Inflammatory response was detected by immunostaining for CD11b and F4/80. All three AAV serotypes successfully transduced mouse and human corneas. The order of transduction efficiency was AAV9 > AAV8 > AAV6. The transduction efficiency of AAV9 was 1.1-1.4 fold higher ($p > 0.05$) as compared to AAV8 and 3.5-5.5 fold higher ($p < 0.01$) as compared to AAV6. The level of transgene expression for all the three serotypes was greater at 14 days compared to 4 days and this high level of transgene expression was maintained up to the tested time point of 30 days. Corneas exposed to any of the three AAV serotypes did not show significant TUNEL positive cells or any inflammatory response as tested by CD11b or F4/80 staining suggesting that tested AAV serotypes do not induce cell death or inflammation and are safe for corneal gene therapy. Copyright © 2010. Published by Elsevier Ltd.

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Affinity recovery of lentivirus by diaminopelargonic acid mediated desthiobiotin labelling.

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Desthiobiotin-tagged lentiviral vectors have been metabolically produced by DBL producer cells in a 7,8-diaminopelargonic acid (7-DAPA) dependent manner for envelope independent, single-step affinity purification. 7-DAPA, which has little or no affinity for avidin/streptavidin, was synthesised and verified by NMR spectroscopy and mass spectrometry. By expressing the biotin acceptor, biotin ligase and desthiobiotin synthase bioD, DBL cells converted exogenous 7-DAPA into membrane-bound desthiobiotin. Desthiobiotin on the DBL cell surface was visualised by confocal microscopy and the desthiobiotin density was quantified by HABA-avidin assay. Desthiobiotin was then spontaneously incorporated onto the surface of lentiviral vectors produced by the DBL cells. It has been demonstrated by flow cytometry that the desthiobiotinylated lentiviruses were captured from the crude 7-DAPA-containing viral supernatant by Streptavidin Magnespheres and eluted by biotin solution efficiently whilst retaining infectivity. The practical, high yielding virus purification using Pierce monomeric avidin coated columns indicates a highly efficient biotin-dependent recovery of infectious lentiviruses at 68%. The recovered lentiviral vectors had a high purity and the majority were eluted within 45 min. This 7-DAPA mediated desthiobiotinylation technology can be applied in scalable production of viral vectors for clinical gene therapy. 2010 Elsevier B.V. All rights reserved.

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Gaussia luciferase reporter assay for assessment of gene delivery systems in vivo.

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OBJECTIVE: To develop an alternative method for assessment of gene delivery systems in vivo. METHODS: Mouse primary spleen lymphocytes were genetically modified in vitro by a retroviral vector harboring a Gaussia luciferase (Gluc) expression cassette. After implantation of these cells into recipient mice, the expression of Gluc was detected in whole blood or plasma collected. RESULTS: As little as 10 muL whole blood drawn from the recipient mice could guarantee prompt reading of Gluc activity with a luminometer. And the reading was found in good correlation with the number of genetically modified spleen lymphocytes implanted to the mice. CONCLUSIONS: Gluc may be useful as an in vivo reporter for gene therapy researches, and Gluc blood assay could provide an alternative method for assessment of gene delivery systems in vivo.