



CLINIGENE CURRENT GENE THERAPY WEEKLY

From April 12th to April 19th 2010

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20400962

Nat Med. 2010 Apr 18. [Epub ahead of print]

Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy.

Bendle GM, Linnemann C, Hooijkaas AI, Bies L, de Witte MA, Jorritsma A, Kaiser AD, Pouw N, Debets R, Kieback E, Uckert W, Song JY, Haanen JB, Schumacher TN.

[1] Division of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

[2] These authors contributed equally to this work.

The transfer of T cell receptor (TCR) genes can be used to induce immune reactivity toward defined antigens to which endogenous T cells are insufficiently reactive. This approach, which is called TCR gene therapy, is being developed to target tumors and pathogens, and its clinical testing has commenced in patients with cancer. In this study we show that lethal cytokine-driven autoimmune pathology can occur in mouse models of TCR gene therapy under conditions that closely mimic the clinical setting. We show that the pairing of introduced and endogenous TCR chains in TCR gene-modified T cells leads to the formation of self-reactive TCRs that are responsible for the observed autoimmunity. Furthermore, we demonstrate that adjustments in the design of gene therapy vectors and target T cell populations can be used to reduce the risk of TCR gene therapy-induced autoimmune pathology.

PMID:
20399883

Prog Retin Eye Res. 2010 Apr 15. [Epub ahead of print]

Leber Congenital Amaurosis due to RPE65 Mutations and its Treatment with Gene Therapy.

Cideciyan AV.

Scheie Eye Institute, University of Pennsylvania, 51 North 39(th) St, Philadelphia, PA 19104, USA.

Leber congenital amaurosis (LCA) is a rare hereditary retinal degeneration caused by mutations in more than a dozen genes. RPE65, one of these mutated genes, is highly expressed in the retinal pigment epithelium where it encodes the retinoid isomerase enzyme essential for the production of chromophore which forms the visual pigment in rod and cone photoreceptors of the retina. Congenital loss of chromophore production due to RPE65-deficiency together with progressive photoreceptor degeneration cause severe and progressive loss of vision. RPE65-associated LCA recently gained recognition outside of specialty ophthalmic circles due to early success achieved by three clinical trials of gene therapy using recombinant adeno-associated virus (AAV) vectors. The trials were built on multitude of basic, pre-clinical and clinical research defining the pathophysiology of the disease in human subjects and animal models, and demonstrating the proof-of-concept of gene (augmentation) therapy. Substantial gains in visual function of clinical trial participants provided evidence for physiologically relevant biological activity resulting from a newly introduced gene. This article reviews the current knowledge on retinal degeneration and visual dysfunction in animal models and human patients with RPE65 disease, and examines the consequences of gene therapy in terms of improvement of vision reported.

PMID:
20399497

Biomaterials. 2010 Apr 15. [Epub ahead of print]

Effects of the nanostructure of dendrimer/DNA complexes on their endocytosis and gene expression.

Peng SF, Su CJ, Wei MC, Chen CY, Liao ZX, Lee PW, Chen HL, Sung HW.

Department of Chemical Engineering, National Tsing Hua University, Hsinchu 30013, Taiwan, ROC.

Cationic dendrimers constitute a potential nonviral vector for gene therapy due to their ability of forming electrostatic complexes with DNA (dendriplexes). However, the supramolecular structure of dendriplexes and its impact on the cellular uptake and gene transfection remain largely unknown. Using synchrotron small angle X-ray scattering, here we show that DNA in complexes with poly(amidoamine) (PAMAM) G4 dendrimers exhibited three distinct packaging states modulated by the degree of their protonation (dp). Our structure characterization suggests that the nanostructure of DNA in dendriplexes transformed from square-packed straightened chains (dp/0.1) to hexagonally-packed superhelices (dp/0.3) and eventually to a beads-on-string configuration (dp/0.6 and dp/0.9). The transfection efficiency in HT1080 cells significantly enhanced when the dp value was increased from 0.1 to 0.3. This enhancement was due to a higher positive surface charge of dendriplexes formed at higher dp, which facilitated adherence of test dendriplexes to the negatively charged cell membranes for the subsequent endocytosis. Although the surface charge of dendriplexes still increased accordingly, further increase of the dendrimer dp value to 0.9 reduced the transfection efficiency. This unexpected suppression of transfection may be attributed to the wrapping of DNA around dendrimers that frustrates the interaction between dendrimer and cholesterol in the membrane raft via the caveola-mediated endocytosis. These results can be used for the rational design of dendrimer-based gene delivery devices.

PMID:
20399239

Adv Drug Deliv Rev. 2010 Apr 14. [Epub ahead of print]

Development of recombinant cationic polymers for gene therapy research.

Canine BF, Hatefi A.

Department of Pharmaceutical Sciences, Center for Integrated Biotechnology, Washington State University, Pullman, WA 99164, USA.

Cationic polymers created through recombinant DNA technology have the potential to fill a void in the area of gene delivery. The recombinant cationic polymers to be discussed here are amino acid based polymers synthesized in E.coli with the purpose to not only address the major barriers to efficient gene delivery but offer safety, biodegradability, targetability and cost-effectiveness. This review helps the readers to get a better understanding about the evolution of recombinant cationic polymers; and the potential advantages that they could offer over viral and synthetic non-viral vectors for gene delivery. It also discusses some of the major challenges that must be addressed in future studies to turn recombinant polymers into clinically effective gene delivery systems. Recent advances with the biopolymer design suggest that this emerging new class of gene delivery systems has the potential to address some of the major barriers to efficient, safe and cost-effective gene therapy.

PMID:
20398929

Biomaterials. 2010 Apr 14. [Epub ahead of print]

Ex vivo expansion of human circulating myogenic progenitors on cluster-assembled nanostructured TiO₂.

Belicchi M, Erratico S, Razini P, Meregalli M, Cattaneo A, Jacchetti E, Farini A, Villa C, Bresolin N, Porretti L, Lenardi C, Milani P, Torrente Y.

Stem Cell Laboratory, Department of Neurological Sciences, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Centro Dino Ferrari, Università di Milano, Padiglione Ponti, Via F. Sforza 35, 20122 Milano, Italy.

Ex vivo expansion of hematopoietic stem cells has been explored in the fields of stem cell biology, gene therapy and clinical transplantation. Recently, we demonstrated the existence of a circulating myogenic progenitor expressing the CD133 antigen. The relative inability of circulating CD133⁺ stem cells to reproduce themselves ex vivo imposes substantial limitations on their use for clinical applications in muscular dystrophies. Here we report that the use of cluster-assembled nanostructured titanium dioxide (ns-TiO₂) substrates, in combination with cytokine enriched medium, enables high-level expansion of circulating CD133⁺ stem cells in vitro. Furthermore, we demonstrate that expanded circulating CD133⁺ stem cells retain their in vitro capacity to differentiate into myogenic cells. The exploitation of cluster-assembled ns-TiO₂ substrates for the expansion of CD133⁺ stem cells in vitro could therefore make the clinical application of these stem cells for the treatment of muscle diseases practical.

PMID:
20398712

Adv Drug Deliv Rev. 2010 Apr 13. [Epub ahead of print]

Cell delivery therapeutics for musculoskeletal regeneration.

Nöth U, Rackwitz L, Steinert AF, Tuan RS.

Orthopaedic Center for Musculoskeletal Research, Department of Orthopaedic Surgery, König-Ludwig-Haus, Julius-Maximilians-University, Würzburg, Germany.

The last decade has witnessed the development of cell-based therapy as a major biomedical research area, including the treatment of musculoskeletal diseases. Both differentiated and undifferentiated stem cells have been used as starting cell sources. In particular, the use of multipotent adult mesenchymal stem cells holds great promise for future therapeutic strategies. In addition to the cell type used, the cell delivery system is also of critical importance in cell-based therapy. Cell delivery may be achieved by direct cell injection or by grafting engineered constructs derived by cell seeding into natural or synthetic biomaterial scaffolds. While direct injection is the most direct and convenient means of cell delivery, the latter approach is capable of producing three-dimensional engineered tissues with mechanical properties compatible with those of various musculoskeletal tissues. This review will focus on the functional approach of using biomaterial scaffold materials as cell carriers for musculoskeletal applications, as well as the use of cell-based gene therapy for tissue engineering and regeneration.

PMID:
20398675

J Mol Biol. 2010 Apr 13. [Epub ahead of print]

Molecular gene therapy: Overexpression of the alternative NADH dehydrogenase NDI1 restores overall physiology in a fungal model of respiratory complex I deficiency.

Maas MF, Sellem CH, Krause F, Dencher NA, Sainsard-Chanet A.

Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91198 Gif sur Yvette Cedex, France; Groningen University, 9751NN Haren, The Netherlands.

Defects in oxidative phosphorylation lie at the heart of a wide variety of degenerative disorders, cancer as well as aging. Here we show, using the fungal model *Podospora anserina*, that the overexpression of the native mitochondrial matrix-faced type II NADH dehydrogenase NDI1, paralogue of the human apoptosis inducing factor AIF1, can fully restore all physiological consequences of respiratory complex I deficiency. We disrupted the 19.3 kDa subunit of the complex I catalytic core, orthologue of the human PSST subunit, leading to a complete absence of the complex without affecting the assembly and/or stability of the rest of the respiratory chain. This disruption caused a several-fold life span extension at the expense of both male and female fertility. The effect was generally similar but markedly milder than that caused by defects in the complex III/IV-dependent pathway, and not associated with a clear reduction in the steady-state level of mitochondrial reactive oxygen species (ROS). Whereas the native expression of NDI1 was sufficient to overcome lethality, only the artificial, constitutive overexpression of NDI1 could fully remedy this deficiency: The latter strikingly restored both life span and fertility to levels indistinguishable from wild-type, thus demonstrating its unique potential in molecular gene therapy.

PMID:
20398385

J Biomed Sci. 2010 Apr 16;17(1):26. [Epub ahead of print]

Characterization of Fabry mice treated with recombinant adeno-associated virus 2/8-mediated gene transfer.

Choi JO, Lee MH, Park HY, Jung SC.

ABSTRACT: BACKGROUND: Enzyme replacement therapy (ERT) with alpha-galactosidase A (alpha-Gal A) is currently the most effective therapeutic strategy for patients with Fabry disease, a lysosomal storage disease. However, ERT has limitations of a short half-life, requirement for frequent administration, and limited efficacy for patients with renal failure. Therefore, we investigated the efficacy of recombinant adeno-associated virus (rAAV) vector-mediated gene therapy for a Fabry disease mouse model and compared it with that of ERT. **METHODS:** A pseudotyped rAAV2/8 vector encoding alpha-Gal A cDNA (rAAV2/8-hAGA) was prepared and injected into 18-week-old male Fabry mice through the tail vein. The alpha-Gal A expression level and globotriaosylceramide (Gb3) levels in the Fabry mice were examined and compared with Fabry mice with ERT. Immunohistochemical and ultrastructural studies were conducted. **RESULTS:** Treatment of Fabry mice with rAAV2/8-hAGA resulted in the clearance of accumulated Gb3 in tissues such as liver, spleen, kidney, heart, and brain with concomitant elevation of alpha-Gal A enzyme activity. Enzyme activity was elevated for up to 60 weeks. In addition, expression of the alpha-Gal A protein was identified in the presence of rAAV2/8-hAGA at 6, 12, and 24 weeks after treatment. alpha-Gal A activity was significantly higher in the mice treated with rAAV2/8-hAGA than in Fabry mice that received ERT. Along with higher alpha-Gal A activity in the kidney of the Fabry mice treated with gene therapy, immunohistochemical studies showed more alpha-Gal A expression in the proximal tubules and glomerulus, and less Gb3 deposition in Fabry mice treated with this gene therapy than in mice given ERT. The alpha-gal A gene transfer significantly reduced the accumulation of Gb3 in the tubules and podocytes of the kidney. Electron microscopic analysis of the kidneys of Fabry mice also showed that gene therapy was more effective than ERT. **CONCLUSIONS:** The rAAV2/8-hAGA mediated alpha-Gal A gene therapy provided improved efficiency over ERT in the Fabry disease mouse model. Furthermore, rAAV2/8-hAGA-mediated expression showed a greater effect in the kidney than ERT.

Structures and Functions of Parvovirus Capsids and the Process of Cell Infection.

Parrish CR.

Baker Institute for Animal Health, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853, USA, crp3@cornell.edu.

To infect a cell, the parvovirus or adeno-associated virus (AAV) genome must be delivered from outside the plasma membrane to the nucleus, and in the process, the capsid must follow a series of binding and trafficking steps and also undergo necessary changes that result in exposure or release the ssDNA genome at the appropriate time and place within the cell. The 25 nm parvovirus capsid is comprised of two or three forms of a single protein, and although it is robust and stable, it is still sufficiently flexible to allow the exposure of several internal components at appropriate times during cell infection. The capsid can also accommodate insertion of peptides into surface loops, and capsid proteins from different viral serotypes can be shuffled to create novel functional variants. The capsids of the different viruses bind to one or more cell receptors, and for at least some viruses, the insertion of additional or alternative receptor binding sequences or structures into the capsid can expand or redirect its tropism. The infection process after cell binding involves receptor-mediated endocytosis followed by viral trafficking through the endosomal systems. That endosomal trafficking may be complex and prolonged for hours or be relatively brief. Generally only a small proportion of the particles taken up enter the cytoplasm after altering the endosomal membrane through the activity of a VP1-encoded phospholipase A2 domain that becomes released to the outside of the viral particle. Modifications to the capsid that can occur within the endosome or cytoplasm include structural changes to expose internal components, ubiquitination and proteosomal processing, and possible trafficking of particles on molecular motors. It is still not clear how the genomes enter the nucleus, but nuclear pore-dependent entry of particles or permeabilization of nuclear membranes have been proposed. Those processes control the infection, pathogenesis, and host ranges of the autonomous viruses and determine the effectiveness of gene therapy using AAV capsids.

PMID:
20397061

Appl Biochem Biotechnol. 2010 Apr 16. [Epub ahead of print]

A Study of the Expression of Functional Human Coagulation Factor IX in Keratinocytes Using a Nonviral Vector Regulated by K14 Promoter.

Hosseini SJ, Zomorodipour A, Jalal R, Sabouni F, Ataei F.

Department of Molecular Genetics, National Institute of Genetic Engineering and Biotechnology, P.O. Box 14965/161, Tehran, Iran.

Ex vivo gene therapy requires a suitable bioreactor for production and delivery of the gene products into a target tissue, and keratinocyte is suitable model in this regard because of its potential for systemic release of proteins. To establish a keratinocyte-specific expression system, a mammalian-based expression plasmid equipped with a 2,240-bp fragment from the human keratin 14 (k14) gene enhancer/promoter region was constructed and used for the insertion of the human coagulation factor IX (hFIX)-cDNA downstream the K14-derived regulatory elements. The human epidermal keratinocytes isolated from neonatal foreskin were cultivated in keratinocyte serum-free media and transfected with the recombinant plasmid. The K14-promoter-driven expression of recombinant hFIX (rhFIX) was evaluated by performing coagulation test as well as enzyme-linked immunosorbent assay on the cultured media collected from the transfected cells at various stages. The rhFIX corresponding transcript and protein were confirmed by performing reverse transcription PCR as well as immunoblotting experiments, respectively. Based on the coagulation activities obtained from the conditioned media of nine isolated clones, the hFIX expression levels vary from 5% to 39% of normal human plasma. Expression levels of the hFIX obtained in this study are comparable to those reported for viral systems. The obtained data supported the potential of keratinocyte for the expression and secretion of biologically active rhFIX and underscore the importance of the examined cis sequences for enhancing gene expression in a mammalian expression system. Besides, it has provided means for further bioengineering strategies to improve the expression efficiency of the hFIX in keratinocytes and other mammalian host cells.

PMID:
20396957

Mol Imaging Biol. 2010 Apr 16. [Epub ahead of print]

Irradiation, Cisplatin, and 5-Azacytidine Upregulate Cytomegalovirus Promoter in Tumors and Muscles: Implementation of Non-invasive Fluorescence Imaging.

Kamensek U, Sersa G, Vidic S, Tevc G, Kranjc S, Cemazar M.

Department of Experimental Oncology, Institute of Oncology Ljubljana, Zaloska 2, SI-1000, Ljubljana, Slovenia.

PURPOSE: The cytomegalovirus (CMV) promoter is one of the most commonly used promoters for expression of transgenes in mammalian cells. The aim of our study was to evaluate the role of methylation and upregulation of the CMV promoter by irradiation and the chemotherapeutic agent cisplatin in vivo using non-invasive fluorescence in vivo imaging. **PROCEDURES:** Murine fibrosarcoma LPB and mammary carcinoma TS/A cells were stably transfected with plasmids encoding CMV and p21 promoter-driven green fluorescent protein (GFP) gene. Solid TS/A tumors were induced by subcutaneous injection of fluorescent tumor cells, while leg muscles were transiently transfected with plasmid encoding GFP under the control of the CMV promoter. Cells, tumors, and legs were treated either by DNA methylation inhibitor 5-azacytidine, irradiation, or cisplatin. GFP expression was determined using a fluorescence microplate reader in vitro and by non-invasive fluorescence imaging in vivo. **RESULTS:** Treatment of cells, tumors, and legs with 5-azacytidine (re)activated the CMV promoter. Furthermore, treatment with irradiation or cisplatin resulted in significant upregulation of GFP expression both in vitro and in vivo. **CONCLUSIONS:** Observed alterations in the activity of the CMV promoter limit the usefulness of this widely used promoter as a constitutive promoter. On the other hand, inducibility of CMV promoters can be beneficially used in gene therapy when combined with standard cancer treatment, such as radiotherapy and chemotherapy.

PMID:
20395979

Cancer Gene Ther. 2010 Apr 16. [Epub ahead of print]

Gemcitabine synergistically enhances the effect of adenovirus gene therapy through activation of the CMV promoter in pancreatic cancer cells.

Onimaru M, Ohuchida K, Egami T, Mizumoto K, Nagai E, Cui L, Toma H, Matsumoto K, Hashizume M, Tanaka M.

[1] Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Maidashi, Fukuoka, Japan [2] Department of Advanced Medical Initiatives, Graduate School of Medical Sciences, Kyushu University, Maidashi, Fukuoka, Japan.

Adenovirus-mediated gene therapy shows remarkable promise as a new strategy for advanced pancreatic cancer, but satisfactory clinical results have not yet been obtained. To improve this gene therapy, we investigated the effects of gemcitabine (GEM) on transgene expression by adenoviral vectors and their biological effects. We used Ad-lacZ and adenoviral vector-expressing NK4 (Ad-NK4) as representative adenoviral vectors. These vectors express beta-galactosidase (beta-gal) and NK4 (which inhibits the invasion of cancer cells), respectively, under the control of the CMV promoter. Cells were infected with the individual adenoviruses and then treated with GEM. GEM increased beta-gal mRNA expression and beta-gal activity, and increased NK4 expression in both culture media and within infected cells, in dose-dependent manners. The increased expression of NK4 delivered by Ad-NK4 had biological effects by inhibiting the invasion of cancer cells. GEM also enhanced NK4 expression in SUIT-2 cells transfected with an NK4-expressing plasmid, suggesting that GEM enhanced CMV promoter activity. In in vivo experiments, NK4 expression within subcutaneously implanted tumors was increased in GEM-treated mice compared with control mice. These results suggest that adenovirus-mediated gene therapy with GEM may be a promising approach for treating pancreatic cancer, and that this combination therapy may decrease the risks of side effects.

PMID:
20393738

Basic Res Cardiol. 2010 Apr 15. [Epub ahead of print]

Hepatocyte growth factor/Met gene transfer in cardiac stem cells-potential for cardiac repair.

Madonna R, Rokosh G, De Caterina R, Bolli R.

Institute of Cardiology, Center of Excellence on Aging, "G. d'Annunzio" University Chieti, Via dei Vestini, 66013, Chieti, Italy, rmadonna@unich.it.

The adult heart has been recently recognized as a self-renewing organ that contains a pool of committed resident cardiac stem cells (CSCs) and cardiac progenitor cells (CPCs). These adult CSCs and CPCs can be induced by cytokines and growth factors to migrate, differentiate, and proliferate in situ and potentially replace lost cardiomyocytes. Ligand-receptor systems, such as the tyrosine kinase receptor mesenchymal-epithelial transition factor (Met) and its ligand hepatocyte growth factor (HGF), are potential candidates for boosting migration, engraftment and commitment of CSCs. Here, we discuss the possible application of HGF/Met gene therapy to enhance the ability of CSCs to promote myocardial regeneration.

PMID:
20393511

Gene Ther. 2010 Apr 15. [Epub ahead of print]

Affinity maturation of an anti-V antigen IgG expressed in situ through adenovirus gene delivery confers enhanced protection against *Yersinia pestis* challenge.

Van Blarcom TJ, Sofer-Podesta C, Ang J, Boyer JL, Crystal RG, Georgiou G.

[1] Department of Chemical Engineering, The University of Texas at Austin, Austin, TX, USA

[2] Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX, USA.

Genetic transfer of neutralizing antibodies (Abs) has been shown to confer strong and persistent protection against bacterial and viral infectious agents. Although it is well established that for many exogenous neutralizing Abs increased antigen affinity correlates with protection, the effect of antigen affinity on Abs produced in situ after adenoviral gene transfer has not been examined. The mouse IgG2b monoclonal Ab, 2C12.4, recognizes the *Yersinia pestis* type III secretion apparatus protein, LcrV (V antigen), and confers protection in mice when administered as an IgG intraperitoneally or after genetic immunization with engineered, replication-defective serotype 5 human adenovirus (Ad). The 2C12.4 Ab was expressed as a single-chain variable fragment (scFv) in *Escherichia coli* and was shown to display an equilibrium dissociation constant ($K(D)$)=3.5 nM by surface plasmon resonance analysis. The 2C12.4 scFv was subjected to random mutagenesis, and variants with increased affinity were isolated by flow cytometry using the anchored periplasmic expression bacterial display system. After a single round of mutagenesis, variants displaying up to 35-fold lower $K(D)$ values (H8, $K(D)$ =100 pM) were isolated. The variable domains of the H8 scFv were used to replace those of the parental 2C12.4 IgG encoded in the Ad vector, AdalphaV, giving rise to AdalphaV.H8. The two adenoviral vectors resulted in similar titers of anti-V antigen Abs 3 days after immunization, with 10(9), 10(10) or 10(11) particle units (pu). After intranasal challenge with 363 LD(50) (lethal dose, 50%) of *Y. pestis* CO92, 54% of the mice immunized with 10(10) pu of AdalphaV.H8 survived through the 14 day end point compared with only 15% survivors for the group immunized with AdalphaV expressing the lower-affinity 2C12.4 ($P<0.04$; AdalphaV versus AdalphaV.H8). These results indicate that affinity maturation of a neutralizing Ab delivered by genetic transfer may confer increased protection not only for *Y. pestis* challenge but also possibly for other pathogens.

PMID:
20393510

Gene Ther. 2010 Apr 15. [Epub ahead of print]

Heart-targeted adeno-associated viral vectors selected by in vivo biopanning of a random viral display peptide library.

Ying Y, Müller OJ, Goehringer C, Leuchs B, Trepel M, Katus HA, Kleinschmidt JA.

Department of Tumorigenesis, German Cancer Research Center, Im Neuenheimer Feld 242, Heidelberg, Germany.

Selection of targeted vectors from virus display peptide libraries is a versatile and efficient approach to improve vector specificity and efficiency. This strategy has been used to target various cell types in vitro. Here, we report the screening of an adeno-associated virus type 2 (AAV2) display peptide library in vivo to select vectors specifically homing to heart tissue after systemic application in mice. Selected library clones indicated superior specificity of gene transfer compared with wild-type AAV2, AAV9 and a heparin binding-deficient AAV2 mutant. Such targeted vectors were able to reconstitute expression of delta-sarcoglycan in the heart of adult delta-sarcoglycan knockout mice after systemic gene transfer in vivo, attesting to the therapeutic potential of this approach.

PMID:
20393509

Gene Ther. 2010 Apr 15. [Epub ahead of print]

Meganucleases can restore the reading frame of a mutated dystrophin.

Chapdelaine P, Pichavant C, Rousseau J, Pâques F, Tremblay JP.
CHUL, Centre de Recherche du CHUQ, Québec, Canada.

Mutations in Duchenne muscular dystrophy (DMD) are either inducing a nonsense codon or a frameshift. Meganucleases (MGNs) can be engineered to induce double-strand breaks (DSBs) at specific DNA sequences. These breaks are repaired by homologous recombination or by non-homologous end joining (NHEJ), which results in insertions or deletions (indels) of a few base pairs. To verify whether MGNs could be used to restore the normal reading frame of a dystrophin gene with a frameshift mutation, we inserted in a plasmid coding for the dog mu-dystrophin sequences containing a MGN target. The number of base pairs in these inserted sequences changed the reading frame. One of these modified target mu-dystrophin plasmids and an appropriate MGN were then transfected in 293FT cells. The MGN induced micro-deletion or micro-insertion in the mu-dystrophin that restored dystrophin expression. MGNs also restored mu-dystrophin expression in myoblasts in vitro and in muscle fibers in vivo. The mutation of the targeted mu-dystrophin was confirmed by PCR amplification followed by digestion with the Surveyor enzyme and by cloning and sequencing of the amplicons. These experiments are thus a proof of principle that MGNs that are adequately engineered to target appropriate sequences in the human dystrophin gene should be able to restore the normal reading frame of that gene in DMD patients with an out-of-frame deletion. New MGNs engineered to target a sequence including or near nonsense mutation could also be used to delete it. Gene Therapy advance online publication, 15 April 2010; doi:10.1038/gt.2010.26.

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20393508

Gene Ther. 2010 Apr 15. [Epub ahead of print]

Randomized, double-blind, placebo-controlled clinical trial of hepatocyte growth factor plasmid for critical limb ischemia.

Shigematsu H, Yasuda K, Iwai T, Sasajima T, Ishimaru S, Ohashi Y, Yamaguchi T, Ogihara T, Morishita R.

Department of Vascular Surgery, Tokyo Medical University, Tokyo, Japan.

Hepatocyte growth factor (HGF) is a potent angiogenic factor. The efficacy and safety of intramuscular injection of a naked plasmid encoding human HGF gene (bepermingene perplasmid, Collategene) was investigated in patients with critical limb ischemia (CLI) in a multicenter, randomized, double-blind, placebo-controlled trial. The randomization ratio for plasmid to placebo was 2:1. Injection sites were selected in each patient limb based on angiographic findings. Placebo or plasmid was injected on days 0 and 28. Evaluation of efficacy was carried out after 12 weeks. The primary end point was the improvement of rest pain in patients without ulcers (Rutherford 4) or the reduction of ulcer size in patients with ulcer(s) (Rutherford 5). Secondary end points were ankle-brachial pressure index, amputation, and quality of life (QOL). Forty-four patients were treated, and we performed interim analysis of efficacy in 40 patients. The overall improvement rate of the primary end point was 70.4% (19/27) in HGF group and 30.8% (4/13) in placebo group, showing a significant difference ($P=0.014$). In Rutherford 5 patients, HGF achieved a significantly higher improvement rate (100% [11/11]) than placebo (40% [2/5]; $P=0.018$). HGF plasmid also improved QOL. There were no major safety problems. HGF gene therapy is safe and effective for CLI. Gene Therapy advance online publication, 15 April 2010; doi:10.1038/gt.2010.51.

PMID:
20393507

Gene Ther. 2010 Apr 15. [Epub ahead of print]

Increased perfusion and angiogenesis in a hindlimb ischemia model with plasmid FGF-2 delivered by noninvasive electroporation.

Ferraro B, Cruz YL, Baldwin M, Coppola D, Heller R.

Department of Molecular Medicine, University of South Florida, Tampa, FL, USA.

Gene therapy approaches delivering fibroblast growth factor-2 (FGF-2) have shown promise as a potential treatment for increasing blood flow to ischemic limbs. Currently, effective noninvasive techniques to deliver plasmids encoding genes of therapeutic interest, such as FGF-2, are limited. We sought to determine if intradermal injection of plasmid DNA encoding FGF-2 (pFGF) followed by noninvasive cutaneous electroporation (pFGFE+) could increase blood flow and angiogenesis in a rat model of hindlimb ischemia. pFGFE+ or control treatments were administered on postoperative day 0. Compared to injection of pFGF alone (pFGFE-), delivery of pFGFE+ significantly increased FGF-2 expression for 10 days. Further, the increase in FGF-2 expression with pFGFE+ was sufficient to significantly increase ischemic limb blood flow, measured by laser Doppler perfusion imaging, beginning on postoperative day 3. Ischemic limb blood flow in the pFGFE+ treatment group remained significantly higher than all control groups through the end point of the study, postoperative day 14. Immunohistochemical staining of gastrocnemius cross sections determined there was a twofold increase in capillary density in the pFGFE+ treatment group. Our results suggest that pFGFE+ is a potential noninvasive, nonviral therapeutic approach to increase perfusion and angiogenesis for the treatment of limb ischemia.

PMID:
20393506

Gene Ther. 2010 Apr 15. [Epub ahead of print]

Influence of chimeric human-bovine fibers on adenoviral uptake by liver cells and the antiviral immune response.

Rogée S, Grellier E, Bernard C, Jouy N, Loyens A, Beauvillain JC, Fender P, Corjon S, Hong SS, Boulanger P, Quesnel B, D'Halluin JC, Colin M.

[1] Inserm (Institut National de la Santé et de la Recherche Médicale), U837, Place de Verdun, Lille, France [2] University Lille Nord de France, Lille, France [3] Institut de Recherches sur le Cancer de Lille, Place de Verdun, Lille, France.

Human adenoviruses (HAdV) are widely used for in vitro and in vivo gene transfer. Viral hepatotropism, inflammatory responses and neutralization by pre-existing antibodies (NAbs) are obstacles for clinical applications of HAdV vectors. Although the multifactorial events leading to innate HAdV toxicity are far from being elucidated, there is a consensus that the majority of intravenously injected-HAdV vectors is sequestered by Kupffer cells, probably independently of coagulation factors. In this study, we show that the adenoviral-associated humoral and innate cytokine immune responses are significantly reduced when HAdV-5 vector carrying human bovine chimeric fibers (HAdV-5-F2/BAdV-4) is intravenously injected into mice. Fiber pseudotyping modified its interaction with blood coagulation factors, as FIX and FX no longer mediate the infection of liver cells by HAdV-5-F2/BAdV-4. As a consequence, at early time points post-infection, several cytokines and chemokines (IFN-gamma, IL-6, IP-10, MCP-1, RANTES and MP1beta) were found to be present at lower levels in the plasma of mice that had been intravenously injected with HAdV-5-F2/BAdV-4 compared with mice injected with the parental vector HAdV-5. Moreover, genetic modification of the fiber allowed HAdV-5-F2/BAdV-4 to partially escape neutralization by NAbs.

PMID:
20390416

Cancer Immunol Immunother. 2010 Apr 14. [Epub ahead of print]

DNA vaccination strategies for anti-tumour effective gene therapy protocols.

Signori E, Iurescia S, Massi E, Fioretti D, Chiarella P, De Robertis M, Rinaldi M, Tonon G, Fazio VM.

CNR-Institute of Neurobiology and Molecular Medicine, Via Fosso del Cavaliere 100, 00133, Rome, Italy, emanuela.signori@cnr.it.

After more than 15 years of experimentation, DNA vaccines have become a promising perspective for tumour diseases, and animal models are widely used to study the biological features of human cancer progression and to test the efficacy of vaccination protocols. In recent years, immunisation with naked plasmid DNA encoding tumour-associated antigens or tumour-specific antigens has revealed a number of advantages: antigen-specific DNA vaccination stimulates both cellular and humoral immune responses; multiple or multi-gene vectors encoding several antigens/determinants and immune-modulatory molecules can be delivered as single administration; DNA vaccination does not induce autoimmune disease in normal animals; DNA vaccines based on plasmid vectors can be produced and tested rapidly and economically. However, DNA vaccines have shown low immunogenicity when tested in human clinical trials, and compared with traditional vaccines, they induce weak immune responses. Therefore, the improvement of vaccine efficacy has become a critical goal in the development of effective DNA vaccination protocols for anti-tumour therapy. Several strategies are taken into account for improving the DNA vaccination efficacy, such as antigen optimisation, use of adjuvants and delivery systems like electroporation, co-expression of cytokines and co-stimulatory molecules in the same vector, different vaccination protocols. In this review we discuss how the combination of these approaches may contribute to the development of more effective DNA vaccination protocols for the therapy of lymphoma in a mouse model.

PMID:
20389291

Mol Ther. 2010 Apr 13. [Epub ahead of print]

Gene Correction by Homologous Recombination With Zinc Finger Nucleases in Primary Cells From a Mouse Model of a Generic Recessive Genetic Disease.

Connelly JP, Barker JC, Pruett-Miller S, Porteus MH.

[1] Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, Texas, USA [2] Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas, USA.

Zinc Finger nucleases (ZFNs) have been used to create precise genome modifications at frequencies that might be therapeutically useful in gene therapy. We created a mouse model of a generic recessive genetic disease to establish a preclinical system to develop the use of ZFN-mediated gene correction for gene therapy. We knocked a mutated GFP gene into the ROSA26 locus in murine embryonic stem (ES) cells and used these cells to create a transgenic mouse. We used ZFNs to determine the frequency of gene correction by gene targeting in different primary cells from this model. We achieved targeting frequencies from 0.17 to 6% in different cell types, including primary fibroblasts and astrocytes. We demonstrate that ex vivo gene-corrected fibroblasts can be transplanted back into a mouse where they retained the corrected phenotype. In addition, we achieved targeting frequencies of over 1% in ES cells, and the targeted ES cells retained the ability to differentiate into cell types from all three germline lineages. In summary, potentially therapeutically relevant frequencies of ZFN-mediated gene targeting can be achieved in a variety of primary cells and these cells can then be transplanted back into a recipient.

PMID:
20389290

Mol Ther. 2010 Apr 13. [Epub ahead of print]

Complete Normalization of Hepatic G6PC Deficiency in Murine Glycogen Storage Disease Type Ia Using Gene Therapy.

Yiu WH, Lee YM, Peng WT, Pan CJ, Mead PA, Mansfield BC, Chou JY.

Section on Cellular Differentiation, Program on Developmental Endocrinology and Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA.

Glycogen storage disease type Ia (GSD-Ia) patients deficient in glucose-6-phosphatase-alpha (G6Pase-alpha or G6PC) manifest disturbed glucose homeostasis. We examined the efficacy of liver G6Pase-alpha delivery mediated by AAV-GPE, an adeno-associated virus (AAV) serotype 8 vector expressing human G6Pase-alpha directed by the human G6PC promoter/enhancer (GPE), and compared it to AAV-CBA, that directed murine G6Pase-alpha expression using a hybrid chicken beta-actin (CBA) promoter/cytomegalovirus (CMV) enhancer. The AAV-GPE directed hepatic G6Pase-alpha expression in the infused G6pc(-/-) mice declined 12-fold from age 2 to 6 weeks but stabilized at wild-type levels from age 6 to 24 weeks. In contrast, the expression directed by AAV-CBA declined 95-fold over 24 weeks, demonstrating that the GPE is more effective in directing persistent in vivo hepatic transgene expression. We further show that the rapid decline in transgene expression directed by AAV-CBA results from an inflammatory immune response elicited by the AAV-CBA vector. The AAV-GPE-treated G6pc(-/-) mice exhibit normal levels of blood glucose, blood metabolites, hepatic glycogen, and hepatic fat. Moreover, the mice maintained normal blood glucose levels even after 6 hours of fasting. The complete normalization of hepatic G6Pase-alpha deficiency by the G6PC promoter/enhancer holds promise for the future of gene therapy in human GSD-Ia patients.

PMID:
20388844

Clin Cancer Res. 2010 Apr 13. [Epub ahead of print]

Targeted Chemotherapy for Head and Neck Cancer with a Chimeric Oncolytic Adenovirus Coding for Bifunctional Suicide Protein FCU1.

Dias JD, Liikanen I, Guse K, Foloppe J, Sloniecka M, Diaconu I, Rantanen V, Eriksson M, Hakkarainen T, Lusky M, Erbs P, Escutenaire S, Kanerva A, Pesonen S, Cerullo V, Hemminki A.

Authors' Affiliations: Cancer Gene Therapy Group, Molecular Cancer Biology Program & Transplantation Laboratory & Haartman Institute & Finnish Institute for Molecular Medicine; Genome-Scale Biology Program, Institute of Biomedicine, University of Helsinki; HUSLAB; and Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland; and Transgene SA, boulevard Gonthier d'Andernach, Illkirch-Graffenstaden, France.

PURPOSE: Transfer of prodrug activation systems into tumors by using replication-deficient viruses has been suggested to be an effective method for achieving high local and low systemic anticancer drug concentrations. However, most current suicide gene therapy strategies are still hindered by poor efficiency of in vivo gene transfer, inefficient tumor penetration, limited bystander cell killing effect, and need of large prodrug doses. We hypothesized that local amplification provided by a replication competent platform would help overcome these limitations. **EXPERIMENTAL DESIGN:** We generated a transductionally and transcriptionally targeted oncolytic adenovirus Ad5/3-Delta24FCU1 expressing the fusion suicide gene FCU1. FCU1 encodes a bifunctional fusion protein that efficiently catalyzes the direct conversion of 5-FC, a relatively nontoxic antifungal agent, into the toxic metabolites 5-fluorouracil and 5-fluorouridine monophosphate, bypassing the natural resistance of certain human tumor cells to 5-fluorouracil. **RESULTS:** We examined the efficacy of Ad5/3-Delta24FCU1 and the replication-defective control Ad5/3-FCU1 with and without 5-FC. FCU1 expression was confirmed by Western blot, whereas enzymatic conversion levels in vitro and in vivo were determined by high-performance liquid chromatography separation. Significant antitumor effect was observed in vitro and in vivo in a murine model of head and neck squamous cell carcinoma. Although we observed a decrease in viral DNA copy number in vitro and in tumors treated with Ad5/3-Delta24FCU1 and 5-FC, suggesting an effect on virus replication, the highest antitumor effect was observed for this combination. **CONCLUSIONS:** It seems feasible and efficacious to combine adenovirus replication to the FCU1 prodrug activation system.

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20388825

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Adenovirus Infection Results in Alterations of Insulin Signaling and Glucose Homeostasis.

Jiang S, Gavrikova TA, Pereboev A, Messina JL.
University of Alabama at Birmingham.

Recombinant adenovirus (Ad) vectors can initiate an inflammatory response, limiting its use in gene therapy and basic research. Despite increased efforts to better understand adenovirus infection, little is known about how it affects cellular metabolic responses. In the current studies, we explored the effects of Ad vectors on insulin signaling molecules and glucose homeostasis. Non-replicative Ad vectors were injected into rats through the tail vein and at 4 - 13 days post-injection, insulin signaling and glucose tolerance were examined. Ad vector infection significantly reduced total levels of the insulin receptor (IR), and insulin receptor substrates 1 and 2 (IRS1, IRS2) in the liver of rats, resulting in decreased insulin-induced tyrosine phosphorylation of IR, IRS1 and IRS2, and decreased interaction of IRS1 and IRS2 with phosphoinositide 3-kinase (PI3K). In addition, adenovirus infection resulted in impaired systemic glucose homeostasis, which recovered by 13 days, after the protein levels of IR, IRS1 and IRS2 had started to normalize. Expression of a TNF inhibitor or Kupffer cell depletion attenuated the Ad vector-induced decreases of insulin signaling molecules, indicating a potential role of Kupffer cell activation in this process. These studies provide evidence that systemic administration of Ad vectors can impair insulin signaling in liver, resulting in altered systemic glucose metabolism. Thus, effects of adenoviral vector infection on insulin action and glucose metabolism need to be considered when adenovirus vectors are used in research or gene therapy and may be more broadly applicable to other viral agents.

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20388793

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Adipose-Derived Mesenchymal Stem Cells as Stable Source of Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Delivery for Cancer Therapy.

Grisendi G, Bussolari R, Cafarelli L, Petak I, Rasini V, Veronesi E, De Santis G, Spano C, Tagliazzucchi M, Barti-Juhasz H, Scarabelli L, Bambi F, Frassoldati A, Rossi G, Casali C, Morandi U, Horwitz EM, Paolucci P, Conte P, Dominici M.

Authors' Affiliations: Department of Oncology, Hematology and Respiratory Diseases, Plastic Surgery Unit, Department of Laboratory and Pathology, Department of Mother and Child, Department of Pathologic Anatomy and Forensic Medicine, Section of Pathology, and Chest Surgery Division, University-Hospital of Modena and Reggio Emilia, Modena, Italy; Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary; Blood Bank and Cellular Therapy Unit, Meyer Hospital, Firenze, Italy; and Division of Oncology/Blood and Marrow Transplantation, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Adipose-derived mesenchymal stromal/stem cells (AD-MSC) may offer efficient tools for cell-based gene therapy approaches. In this study, we evaluated whether AD-MSC could deliver proapoptotic molecules for cancer treatment. Human AD-MSCs were isolated and transduced with a retroviral vector encoding full-length human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a proapoptotic ligand that induces apoptosis in a variety of human cancers but not normal tissues. Although several studies have documented the antitumor activity of recombinant human TRAIL, its use in vivo is limited by a short half-life in plasma due to a rapid clearance by the kidney. We found that these limitations can be overcome using stably transduced AD-MSC, which could serve as a constant source of TRAIL production. AD-MSC armed with TRAIL targeted a variety of tumor cell lines in vitro, including human cervical carcinoma, pancreatic cancer, colon cancer, and, in combination with bortezomib, TRAIL-resistant breast cancer cells. Killing activity was associated with activation of caspase-8 as expected. When injected i.v. or s.c. into mice, AD-MSC armed with TRAIL localized into tumors and mediated apoptosis without significant apparent toxicities to normal tissues. Collectively, our results provide preclinical support for a model of TRAIL-based cancer therapy relying on the use of adipose-derived mesenchymal progenitors as cellular vectors.

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20387096

Pharm Res. 2010 Apr 13. [Epub ahead of print]

Gene Therapy: A Pharmacokinetic/Pharmacodynamic Modelling Overview.

Parra-Guillén ZP, González-Aseguinolaza G, Berraondo P, Trocóniz IF.

Department of Pharmacy and Pharmaceutical Technology School of Pharmacy, University of Navarra, Pamplona, Spain.

Since gene therapy started over 20 years ago, more than one-thousand clinical trials have been carried out. Nonviral vectors present interesting properties for their clinical application, but their efficiency in vivo is relatively low, and further improvements in these vectors are needed. Elucidating how nonviral vectors behave at the intracellular level is enlightening for vector improvement and optimization. Model-based approach is a powerful tool to understand and describe the different processes that gene transfer systems should overcome inside the body. Model-based approach allows for proposing and predicting the effect of parameter changes on the overall gene therapy response, as well as the known application of the pharmacokinetic/pharmacodynamic modelling in conventional therapies. The objective of this paper is to critically review the works in which the time-course of naked or formulated DNA have been quantitatively studied or modelled.

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20385789

Blood. 2010 Apr 12. [Epub ahead of print]

Lentiviral gene therapy of murine hematopoietic stem cells ameliorates the Pompe disease phenotype.

van Til NP, Stok M, Aerts Kaya FS, de Waard MC, Farahbakhshian E, Visser TP, Kroos MA, Jacobs EH, Willart MA, van der Wegen P, Scholte BJ, Lambrecht BN, Duncker DJ, van der Ploeg AT, Reuser AJ, Verstegen MM, Wagemaker G.

Department of Hematology, Erasmus University Medical Center, Rotterdam, Netherlands;

Pompe disease (acid alpha-glucosidase deficiency) is a lysosomal glycogen storage disorder characterized in its most severe early-onset form by rapidly progressive muscle weakness and mortality within the first year of life due to cardiac and respiratory failure. Enzyme replacement therapy prolongs the life of affected infants and supports the condition of older children and adults but entails life-long treatment and can be counteracted by immune responses to the recombinant enzyme. We have explored the potential of lentiviral vector mediated expression of human acid alpha-glucosidase in hematopoietic stem cells (HSC) in a Pompe mouse model. After mild conditioning, transplantation of genetically engineered HSC resulted in stable chimerism of ~35% hematopoietic cells that over express acid alpha-glucosidase and in major clearance of glycogen in heart, diaphragm, spleen and liver. Cardiac remodeling was reversed and respiratory function, skeletal muscle strength and motor performance improved. Over expression of acid alpha-glucosidase did not affect overall hematopoietic cell function and led to immune tolerance as shown by challenge with the human recombinant protein. Based on the prominent and sustained therapeutic efficacy without adverse events in mice we conclude that ex vivo HSC gene therapy is a treatment option worthwhile to pursue.

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20384524

Rexin-G, a targeted genetic medicine for cancer.

Gordon EM, Hall FL.

Epeius Biotechnologies Corporation, 475 Huntington Drive, San Marino, CA 91108, USA.
egordon@epeiusbiotech.com

IMPORTANCE OF THE FIELD: Rexin-G, a tumor-targeted retrovector bearing a cytotoxic cyclin G1 construct, is the first targeted gene therapy vector to gain fast track designation and orphan drug priorities for multiple cancer indications in the US. **AREAS COVERED IN THIS REVIEW:** This review describes the major milestones in the clinical development of Rexin-G: from the molecular cloning and characterization of the human cyclin G1 proto-oncogene in 1994, to the design of the first knockout constructs and genetic engineering of the targeted delivery system from 1995 to 1997, through the initial proofs-of-concept, molecular pharmacology and toxicology studies of Rexin-G in preclinical cancer models from 1997 to 2001, to the pioneering clinical studies in humans from 2002 to 2004, which--together with the advancements in bioprocess development of high-potency clinical grade vectors circa 2005 - 2006--led to the accelerated approval of Rexin-G for all solid tumors by the Philippine FDA in 2007 and the rapid progression of clinical studies from 2007 to 2009 to the cusp of pivotal Phase III trials in the US. **WHAT THE READER WILL GAIN:** In recording the development of Rexin-G as a novel form of targeted biological therapy, this review also highlights important aspects of vector design engineering which served to overcome the physiological barriers to gene delivery as it addresses the key regulatory issues involved in the development of a targeted gene therapy product. **TAKE HOME MESSAGE:** Progressive clinical development of Rexin-G demonstrates the potential safety and efficacy of targeted genetic medicine, while validating the design engineering of the molecular biotechnology platform.

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20384482

Capsomere-specific fluorescent labeling of adenovirus vector particles allows for detailed analysis of intracellular particle trafficking and of the performance of bioresponsive bonds for vector capsid modifications.

Espenlaub S, Corjon S, Engler T, Fella C, Ogris M, Wagner E, Kochanek S, Kreppel F.

University of Ulm, Division of Gene Therapy, Ulm, Germany; sigrid.espenlaub@uni-ulm.de.

Adenovirus vectors are widely used for gene therapy approaches. Due to the high abundance of the natural adenovirus receptors CAR/integrins on a wide variety of cells numerous methods have been developed to redirect the virions to specific receptors on target cell surfaces. Importantly, an increasing number of recent publications evidenced that the success of targeting does not only depend on receptor binding and cellular uptake, but also on intracellular trafficking processes. Therefore, improved knowledge on the intracellular fate of targeted Ad vector particles is mandatory for a rational design of targeted Ad vectors. However, the technologies currently available for fluorescent labeling of Ad vectors have significant limitations: (i) using chemistry capsids can only be labeled all over the particle surface and this imposes the risk of interference with particle infectivity, (ii) capsomere-specific labeling requires extensive genetic modifications and has only been demonstrated at protein IX, (iii) two-color labeling approaches are not available. Here we present a novel, robust and straight forward labeling procedure that overcomes these limitations. It allows for specific labeling of the capsomeres fiber, protein IX, or hexon and permits two-color labeling. We demonstrate the potential of this labeling technology by analyzing two different bioresponsive bonds that can be used for the attachment of shielding or targeting moieties to the capsids: disulfide and hydrazone bonds. We demonstrate that in contrast to disulfide bonds, hydrazone bonds become quickly hydrolyzed after uptake of the virions and are thus favourable for the generation of bioresponsive vectors.

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20384479

Hum Gene Ther. 2010 Apr 12. [Epub ahead of print]

Replacement Gene Therapy with a Human RPGRIP1 Sequence Slows Photoreceptor Degeneration in a Murine Model of Leber Congenital Amaurosis.

Pawlyk BS, Bulgakov OV, Liu X, Xu X, Adamian M, Sun X, Khani SC, Berson EL, Sandberg MA, Li T.

Mass. Eye & Ear Infirmary, Boston, Massachusetts, United States; basil_pawlyk@meei.harvard.edu.

RPGR-interacting protein 1 (RPGRIP1) is localized in the photoreceptor connecting cilium where it anchors the RPGR (retinitis pigmentosa GTPase regulator) protein, and its function is essential for photoreceptor maintenance. Genetic defect in RPGRIP1 is a known cause of Leber congenital amaurosis (LCA), a severe, early-onset form of retinal degeneration. We evaluated the efficacy of replacement gene therapy in a murine model of LCA carrying a targeted disruption of RPGRIP1. The replacement construct, packaged in an AAV8 vector, utilized a rhodopsin kinase (RK) gene promoter to drive RPGRIP1 expression. Both promoter and transgene were of human origin. Following subretinal delivery of the replacement gene in the mutant mice, human RPGRIP1 was expressed specifically in photoreceptors, localized correctly in the connecting cilia, and it restored the normal localization of RPGR. Electroretinogram and histologic examinations showed better preservation of rod and cone photoreceptor function and improved survival in the treated eyes. This study demonstrates the efficacy of human gene replacement therapy and validates a gene therapy design for future clinical trials in patients afflicted with this condition. Our results also have therapeutic implications for other forms of retinal degenerations attributable to a ciliary defect.

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20384478

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Ocular Gene Therapy: An Evaluation of rAAV-mediated Gene Therapy Interventions for the Treatment of Ocular Disease.

Roy K, Stein L, Kaushal S.

University of Massachusetts Medical School, Dept. of Ophthalmology, Worcester, Massachusetts, United States; Kamolika.Roy@umassmed.edu.

Both gene replacement therapy and alteration of host gene expression are playing increasingly important roles in the treatment of ocular diseases. Ocular gene therapy may provide alternatives to current treatments for eye diseases that are either greatly invasive and thus run the risk of complications, that offer only short-term relief from disease symptoms, or that are unable to directly treat vision loss. The recent success of three separate Phase I clinical trials investigating a gene therapy intervention for the treatment of the retinal degenerative disorder Leber's congenital amaurosis (LCA) have unveiled the therapeutic potential of gene therapy. Preliminary results have demonstrated ocular gene transfer, using non-pathogenic recombinant adeno-associated viral (rAAV) vectors specifically, to be a safe, effective, and long-term treatment for LCA, a previously untreatable disorder. Non-pathogenic rAAV vectors offer the potential for long-term treatment. Many of the genes implicated in human ocular diseases have been identified, and animal models for such diseases have been developed, which has greatly facilitated the application of experimental rAAV-mediated gene therapy. This review highlights the key features of rAAV-mediated gene therapy that make it the most suitable gene therapy treatment approach for ocular diseases. Furthermore, it summarizes the current progress of rAAV-mediated gene therapy interventions/applications for a wide variety of ophthalmologic disorders.

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20384216

Regulatory considerations in application of encapsulated cell therapies.

van Zanten J, de Vos P.

Department of PLG/Medical Biology, University Medical Center Groningen, University of Groningen, PO Box 30001, 9700 RB Groningen, the Netherlands. j.van.zanten@med.umcg.nl

The encapsulation of tissue in semi-permeable membranes is a technology with high potential and in due time several new therapies based on this technology will be tested in clinical trials. Recent, new legislation requires that these investigational medicinal products used in clinical trials Phase I must be produced according to Good Manufacturing Practice (GMP). Consequently, the activities of GMP are expanding to the field of research and researchers might need to change developed protocols in order to meet GMP legislation. This chapters gives an overview of the overall guidelines covering GMP and more specific guidelines dealing with cell based therapies and gene therapy.

PMID: Acta Biochim Biophys Sin (Shanghai). 2010 Apr;42(4):274-80.
20383466

Target gene therapy of glioma: overexpression of BAX gene under the control of both tissue-specific promoter and hypoxia-inducible element.

Huang J, Gao J, Lv X, Li G, Hao D, Yao X, Zhou L, Liu D, Wang R.

Department of Neurosurgery, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China.

Glioma-specific transcription of tumor-killing genes has been exploited as a promising gene therapeutic modality in glioma patients. Musashi1 (Msi1) and GFAP gene promoters are both cancer-specific promoters. Optimized HIF-binding site (optHBS) sequence was newly found as efficient as EPO HREs used as enhancer in cancer gene therapy. We constructed 4optHBS-Msi1/GFAP promoters and tested their ability to mediate BAX expression to induce apoptosis in glioma cell lines. Our results demonstrated that 4optHBS-Msi1/GFAP promoters are apparently strong and glioma-selective promoters with potential application in targeted glioma gene therapy, and 4optHBS-Msi1/GFAPBAXa are valuable tools for glioma gene therapy.

PMID:
20382243

Vaccine. 2010 Apr 9. [Epub ahead of print]

Generation and characterization of a fusion protein of single-chain fragment variable antibody against hemagglutinin antigen of avian influenza virus and truncated protamine.

Zhang T, Wang CY, Zhang W, Gao YW, Yang ST, Wang TC, Zhang RZ, Qin C, Xia XZ. Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100021, China; Laboratory of Virology, Veterinary Institute, Academy of Military Medical Sciences, Changchun 130062, Jilin Province, China.

The hemagglutinin antigen (HA) of avian influenza virus (AIV) is an immunogen abundant on the surfaces of infected cells, and can be used as a target for specific antibodies to clear viral infection. Protamine has been demonstrated to deliver DNA into cells effectively. Accordingly, a fusion protein of anti-HA single-chain fragment variable (scFv) and truncated protamine (tP) may be used as a vehicle for delivering the anti-AIV siRNA into the AIV-infected cells for gene therapy. To test this hypothesis, we constructed a novel recombinant plasmid, pET28-scFv-tP, by connecting the genes for anti-H5N1 AIV HA-specific scFv with synthesized oligonucleotides encoding the 22 amino acids of human tP and a linker. Furthermore, the recombinant scFv-tP was expressed and purified, with a yield of 7-8mg of scFv-tP and a purity of >92% from 1L of bacterial culture. Characterization of its bioactivity revealed that scFv-tP recognized HA, similar to its scFv control, in a dose-dependent manner and that the scFv-tP, but not its scFv control, bound to DNA and delivered plasmid and oligonucleotide DNA into the AIV-infected MDCK cells effectively. More importantly, transfection with the mixture of the scFv-tP and plasmid for the NP-specific siRNA significantly inhibited the replication of AIV in MDCK cells, as compared with that transfection with the scFv-plasmid mixture, even with the plasmid in liposome. Our data demonstrated that the recombinant scFv-tP retained the functions of both scFv and tP, and might be potentially used for delivering genetic materials for targeting therapy of AIV infection in vivo.

PMID:
20381448

Biochim Biophys Acta. 2010 Apr 8. [Epub ahead of print]

Cationic carriers of genetic material and cell death: A mitochondrial tale.

Hunter AC, Moghimi SM.

Molecular Targeting and Polymer Toxicology Group, School of Pharmacy, University of Brighton, Cockcroft Building, Lewes Road, Brighton, East Sussex BN2 4GJ, United Kingdom.

Central to gene therapy technology has been the use of cationic polymers as vectors for DNA and RNA (polyfectins). These have been presumed to be safer than viral systems which, for example, have been found to switch on oncogenes. Two key polycations that have been intensively researched for use as synthetic vectors are poly(ethylenimine) and poly(L-lysine). A frequent stumbling block with these polyfectins is that long-term gene expression in cell lines has not been achieved. Recently it has transpired that both of these polycations can induce mitochondrially mediated apoptosis. It is the aim of this review to discuss the mechanisms behind the observed polycation toxicity including roles for little studied cellular organelles in the process such as the lysosome and endoplasmic reticulum.