



CLINIGENE CURRENT GENE THERAPY WEEKLY

From June 07th to June 14th 2010

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**PMID:
20539913**

Thromb Haemost. 2010 Jun 10;104(2). [Epub ahead of print]

FIX-Triple, a gain-of-function factor IX variant, improves haemostasis in mouse models without increased risk of thrombosis.

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Engineered recombinant factor IX (FIX) with augmented clotting activity may prove useful for replacement therapy, but it has not been studied for risk of thrombosis. We used three mouse models to evaluate thrombosis risk associated with the FIX variant FIX-Triple, which has a 13-fold higher specific activity than wild-type FIX (FIX-WT). Protein infusion of FIX-Triple into haemophilia B mice was not thrombogenic, even at a dose of 13-fold higher than FIX-WT. Gene knock-in to generate mice that constitutively produce FIX-WT or FIX-Triple protein revealed that all mice expressed equal antigen levels. FIX-Triple knock-in mice that exhibited 10-fold higher FIX clotting activity did not show hypercoagulation. Adeno-associated viral (AAV) delivery of the FIX gene into mice was used to mimic gene therapy. Haemophilia B and inbred C57Bl/6 mice injected with different doses of virus particles carrying FIX-WT or FIX-Triple and expressing up to a nearly 13-fold excess (1289% of normal) of FIX clotting activity did not show increased risk of thrombosis compared with untreated wild-type mice in a normal haemostatic state. When challenged with ferric chloride (FeCl₃), the mesenteric venules of AAV-treated C57Bl/6 mice that gave a nearly five-fold excess (474%) of FIX clotting activity were not thrombotic; however, thrombosis became obvious in FeCl₃-challenged mice expressing extremely high FIX clotting activities (976-1289%) achieved by AAV delivery of FIX-Triple. These studies suggest that FIX-Triple is not thrombogenic at therapeutic levels and is a potential therapeutic substitute for FIX-WT.

**PMID:
20539323**

Cancer Gene Ther. 2010 Jun 11. [Epub ahead of print]

Inhibition of prostate cancer growth and metastasis using small interference RNA specific for minichromosome complex maintenance component 7.

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Minichromosome complex maintenance component 7 (MCM7) is a critical component of DNA replication licensing. Amplification and overexpression of MCM7 leads to high rate of prostate cancer metastasis. Recent studies indicate that MCM7 genome encodes a putative 'super-oncogene' cluster including MCM7 oncogene and a miRNA cluster that knocks down the expression of several critical tumor-suppressor genes. In this study, we constructed a vector that constitutively expresses small interference RNA (siRNA) specific for MCM7. Introduction of this vector into prostate cancer cell lines PC3 or Du145 decreases the expression of MCM7 by 80%. The vector inhibits DNA synthesis and generates growth arrest of these cancer cells. Severe combined immunodeficient mice were xenografted PC3 or Du145 tumors, and subsequently treated with this vector through tail vein injection with polyethylenimine. The animals had dramatically smaller tumor volume, less metastasis and better survival rate in comparison with the controls. As a result, intervention of MCM7 expression using siRNA approach may hold the promise for treating androgen refractory prostate cancer.

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20539322

Cancer Gene Ther. 2010 Jun 11. [Epub ahead of print]

Role of cell surface molecules and autologous ascitic fluid in determining efficiency of adenoviral transduction of ovarian cancer cells.

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Adenovirus is the most frequently used virus in gene therapy clinical trials. There have been conflicting reports on the ability of adenovirus to transduce primary ovarian cancer samples and the expression of relevant cell surface molecules. These factors were examined using primary ovarian cancer cells cultured from ascites and solid tumor to gain insights into the clinical use of adenovirus in ovarian cancer. The level of transduction of primary cultures was much higher than uncultured cells and established cell lines, and correlated with higher levels of coxsackie-adenovirus receptor (CAR) and integrin expression. Growth of primary cultures in autologous ascitic fluid prevented an increase in CAR expression and inhibited transduction compared with cells treated in supplemented RPMI. Cells at the periphery of solid tumor samples were transduced using a replication-incompetent virus and correlated with CAR expression. However, transduction was abolished by autologous ascitic fluid, despite the expression of CAR. We conclude that the use of adenoviruses for ovarian cancer gene therapy will require testing in the presence of inhibitory factors in ascitic fluid. The clinical use of adenoviral vectors may require circumvention of such inhibitory factors and the use of replication competent adenovirus to enable efficient viral penetration of the cancer.

PMID:
20539321

Cancer Gene Ther. 2010 Jun 11. [Epub ahead of print]

Baculovirus-transduced bone marrow mesenchymal stem cells for systemic cancer therapy.

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Adult stem cells may serve as powerful cellular vehicles to deliver therapeutic genes for cancer therapy. In such applications, effective and safe transduction to load stem cells with genes of interest is essential. To examine whether baculovirus can be used to fulfill this task, we tested a range of baculoviral vectors in human bone marrow mesenchymal stem cells (MSCs). A vector using the human cytomegalovirus immediate-early gene promoter to drive transgene expression and the woodchuck hepatitis virus posttranscriptional regulatory element to enhance translation was able to transduce MSCs with efficiency close to 80%. Following the observation that baculoviral transduction did not significantly affect surface marker expression of the stem cells, we tested the feasibility of using baculovirus-transduced MSCs for targeted cancer therapy. We transduced cells with a baculoviral vector harboring the herpes simplex virus thymidine kinase gene, and performed tail vein injection of the transduced cells into mice preinoculated subcutaneously with human U87 glioma cells. After ganciclovir prodrug injection, we observed inhibition of tumor growth and significantly prolonged survival of tumor-inoculated animals. Our findings suggest that baculoviral transduction of MSCs is an attractive option to generate targeting vehicles for systemic cancer therapy.

**PMID:
20539320**

Cancer Gene Ther. 2010 Jun 11. [Epub ahead of print]

Eliciting protective immune responses against murine myeloma challenge in lymphopenia mice through adoptive transfer of tumor antigen-specific lymphocytes and immunization of tumor vaccine secreting mIL-21.

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Previous studies have indicated that the cytokine interleukin (IL)-21 may induce both innate and adaptive immune responses against tumors. The goal of this study was to evaluate a new adoptive immunotherapy strategy that combined lymphocytes from mice immunized with a murine myeloma vaccine secreting murine IL-21 (mIL-21-Sp2/0) in lymphopenic mice induced by cyclophosphamide. The data indicate that effective antitumor immunity was induced in mice receiving syngeneic murine lymphocytes from the mice immunized with the mIL-21-Sp2/0. More importantly, the efficacy against the Sp2/0 cell challenge was enhanced after the lymphocytes were activated and proliferated *ex vivo* before administration into the lymphopenic mice. We conclude that the adoptive transfer of tumor antigen-specific lymphocytes into mice immunized with mIL-21-Sp2/0 induced protective immune responses against myeloma challenge.

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20539319**

Cancer Gene Ther. 2010 Jun 11. [Epub ahead of print]

Hepatitis B surface antigen fusions delivered by DNA vaccination elicit CTL responses to human papillomavirus oncoproteins associated with tumor protection.

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We describe the construction and evaluation of a recombinant hepatitis B surface antigen (HBsAg)-vectored DNA vaccine encoding the E7 and E6 tumor-associated oncoproteins of human papillomavirus (HPV) type 16. We show the induction of effector and memory cytotoxic T lymphocyte responses to E7 and E6 class I-restricted epitopes after a single immunization, which were associated with tumor prevention and therapy. The findings vindicate the use of a HBsAg-based DNA vaccine as a vehicle to elicit responses to co-encoded tumor antigens, and have specific implications for the development of a therapeutic vaccine for HPV-associated squamous carcinomas.

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20539318

Cancer Gene Ther. 2010 Jun 11. [Epub ahead of print]

Suppression of tumor growth in xenograft model mice by programmed cell death 4 gene delivery using folate-PEG-baculovirus.

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Cancer gene therapy using tumor suppressor genes is considered to be an attractive approach for arresting cell growth and inducing apoptosis. Programmed cell death 4 (Pcd4) is a tumor suppressor gene, which prevents tumorigenesis and tumor progression. To address the issue of whether expression of PDCD4 protein induces apoptosis in cancerous cells, the Pcd4 gene was delivered using folate-PEG-baculovirus. Folate-PEG-baculovirus containing Pcd4 gene (F-P-Bac-Pcd4) was constructed by attachment of F-PEG to the baculovirus surface using chemical modification. The F-P-Bac-Pcd4 showed enhanced transduction efficiency, efficiently expressed PDCD4 protein, and induced apoptosis in human epidermal carcinoma (KB) cells as compared with an unmodified baculovirus. In a tumor xenograft study, injection of F-P-Bac-Pcd4 into tumors established from the KB cell line by subcutaneous implantation significantly suppressed tumor growth and induced apoptosis. Thus, this study shows a new baculovirus-mediated tumor suppressor gene delivery system for cancer therapy.

PMID:
20538619

Sci Transl Med. 2010 Jun 9;2(35):35ra42.

Systemic Delivery of scAAV9 Expressing SMN Prolongs Survival in a Model of Spinal Muscular Atrophy.

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Spinal muscular atrophy is one of the most common genetic causes of death in childhood, and there is currently no effective treatment. The disease is caused by mutations in the survival motor neuron gene. Gene therapy aimed at restoring the protein encoded by this gene is a rational therapeutic approach to ameliorate the disease phenotype. We previously reported that intramuscular delivery of a lentiviral vector expressing survival motor neuron increased the life expectancy of transgenic mice with spinal muscular atrophy. The marginal efficacy of this therapeutic approach, however, prompted us to explore different strategies for gene therapy delivery to motor neurons to achieve a more clinically relevant effect. Here, we report that a single injection of self-complementary adeno-associated virus serotype 9 expressing green fluorescent protein or of a codon-optimized version of the survival motor neuron protein into the facial vein 1 day after birth in mice carrying a defective survival motor neuron gene led to widespread gene transfer. Furthermore, this gene therapy resulted in a substantial extension of life span in these animals. These data demonstrate a significant increase in survival in a mouse model of spinal muscular atrophy and provide evidence for effective therapy.

PMID:
20538153

Pediatr Clin North Am. 2010 Jun;57(3):719-727.

Gene Doping: The Hype and the Harm.

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"Gene doping" is the term used to describe the potential abuse of gene therapy as a performance-enhancing agent. Gene doping would apply the techniques used in gene therapy to provide altered expression of genes that would promote physical superiority. For example, insulin-like growth factor 1 (IGF-1) is a primary target for growth hormone; overexpression of IGF-1 can lead to increased muscle mass and power. Although gene doping is still largely theoretical, its implications for sports, health, ethics, and medical genetics are significant. Copyright © 2010 Elsevier Inc. All rights reserved.

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20537569

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The Role of ex-vivo Gene Therapy of Vein Grafts with Egr-1 Decoy in the Suppression of Intimal Hyperplasia.

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OBJECTIVES: To test the hypothesis that vein graft intimal hyperplasia can be significantly suppressed by a single intra-operative transfection of the graft with a decoy oligonucleotide (ODN) binding the transcription factor Egr-1. **DESIGN:** Experimental study. **MATERIALS AND METHODS:** Jugular vein to carotid artery interposition grafts in rabbits were treated with Egr-1 decoy, mutant decoy ODN, vehicle alone, using a non-distending pressure of 300mmHg for 20min, or were left untreated. All animals were fed a 2% cholesterol diet. The animals were sacrificed after 48h, 6 weeks and 12 weeks. Paraffin-embedded vein sections were subjected to angiometric analysis. **RESULTS:** Successful delivery of the ODN was confirmed by DAPI staining. Quantitative real-time PCR revealed a 60% decrease of the Egr-1 gene expression in the animals in which the Egr-1 decoy ODN was delivered. Cellular proliferation was also significantly decreased as indicated by the Ki-67 labelling index. An increase in intimal and medial thickness was found in all vein grafts. However, intimal thickness was significantly reduced in the grafts treated with Egr-1 decoy ODN, whereas luminal area was significantly increased. **CONCLUSION:** A single intra-operative pressure-mediated transfection of vein grafts with Egr-1 decoy ODN significantly suppresses intimal hyperplasia in a rabbit hypercholesterolaemic model.

**PMID:
20537174**

J Transl Med. 2010 Jun 10;8(1):55. [Epub ahead of print]

Strategy Escalation: An emerging paradigm for safe clinical development of T cell gene therapies.

Junghans RP.

ABSTRACT: Gene therapy techniques are being applied to modify T cells with chimeric antigen receptors (CARs) for therapeutic ends. The versatility of this platform has spawned multiple options for their application with new permutations in strategies continually being invented, a testimony to the creative energies of many investigators. The field is rapidly expanding with immense potential for impact against diverse cancers. But this rapid expansion, like the Big Bang, comes with a somewhat chaotic evolution of its therapeutic universe that can also be dangerous, as seen by recently publicized deaths. Time-honored methods for new drug testing embodied in Dose Escalation that were suitable for traditional inert agents are now inadequate for these novel "living drugs". In the following, I propose an approach to escalating risk for patient exposures with these new immuno-gene therapy agents, termed Strategy Escalation, that accounts for the molecular and biological features of the modified cells and the methods of their administration. This proposal is offered not as a prescriptive but as a discussion framework that investigators may wish to consider in configuring their intended clinical applications.

**PMID:
20536991**

Haemophilia. 2010 May;16(102):89-94.

Gene therapy for immunological tolerance: using 'transgenic' B cells to treat inhibitor formation.

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B cells have been shown to function as tolerogenic antigen presenting cells (APCs) both in vivo and in vitro. We have taken advantage of this property, as well as the ability of IgG carriers to be potent 'schleppers' for tolerogenic entities, to develop a gene therapy approach to induce unresponsiveness in a number of systems, including the elimination of haemophilia inhibitors. Thus, peptide-IgG constructs have been engineered into retroviral vectors to create 'transgenic' B cells for tolerance applications. In this paper, we discuss our gene therapy approach mediated by B cells (as well as bone marrow cells) for tolerance acquisition in various mouse models for autoimmune disease and haemophilia A. The mechanisms that are the underpinning of this effort and role of regulatory T cells are discussed herein. Our results indicate that gene therapy strategies can successfully reduce the incidence and or onset of autoimmune diseases and prevent/reverse inhibitor formation in haemophilia A mice. Based on recent success with a model for tolerance with human T cell clones in vitro, plans for future application in patients are discussed.

**PMID:
20535218**

Gene Ther. 2010 Jun 10. [Epub ahead of print]

MVA-nef induces HIV-1-specific polyfunctional and proliferative T-cell responses revealed by the combination of short- and long-term immune assays.

Kutscher S, Allgayer S, Dembek CJ, Bogner JR, Protzer U, Goebel FD, Erfle V, Cosma A. Institute of Virology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany.

Several vaccination trials are evaluating the modified vaccinia virus Ankara (MVA) as a delivery vector in various clinical settings. In this paper, we present the reevaluation of a therapeutic vaccination trial in human immunodeficiency virus (HIV)-1-infected individuals treated with highly active antiretroviral therapy using MVA-expressing HIV-1 nef. Immunogenicity of MVA-nef was assessed using multicolor flow cytometry. Vaccine-induced polyfunctionality and proliferative capacity, which are associated with nonprogressive HIV-1 infection, were detectable by combining two immune assays. By means of short-term polychromatic intracellular cytokine staining, we observed a significant increase in polyfunctional Nef-specific CD4 T cells expressing interferon-gamma, interleukin (IL)-2 and CD154 after vaccination, whereas changes in the quality of CD8 T-cell response could not be observed. Only the additional use of a long-term polychromatic Carboxyfluorescein succinimidyl ester (CFSE)-based proliferation assay revealed vaccine-induced Nef-specific CD8, as well as CD4 T cells with proliferative capacity. The correlation between vaccine-induced IL-2 production by CD4 T cells and the increase in proliferating Nef-specific CD8 T cells suggests a causal link between these two functions. These results highlight the importance of combining sophisticated immunomonitoring tools to unravel concealed effects of immunological interventions and support the use of the poxvirus-derived MVA vector to stimulate highly functional HIV-1-specific T-cell responses. However, the clinical benefit of these functional T cells remains to be determined.

**PMID:
20535217**

Gene Ther. 2010 Jun 10. [Epub ahead of print]

Improvement of the mdx mouse dystrophic phenotype by systemic in utero AAV8 delivery of a minidystrophin gene.

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Duchenne muscular dystrophy (DMD) is a devastating primary muscle disease with pathological changes in skeletal muscle that are ongoing at the time of birth. Progressive deterioration in striated muscle function in affected individuals ultimately results in early death due to cardio-pulmonary failure. As affected individuals can be identified before birth by prenatal genetic testing for DMD, gene replacement treatment can be started in utero. This approach offers the possibility of preventing pathological changes in muscle that begin early in life. To test in utero gene transfer in the mdx mouse model of DMD, a minidystrophin gene driven by the human cytomegalovirus promoter was delivered systemically by an intraperitoneal injection to the fetus at embryonic day 16. Treated mdx mice studied at 9 weeks after birth showed widespread expression of recombinant dystrophin in skeletal muscle, restoration of the dystrophin-associated glycoprotein complex in dystrophin-expressing muscle fibers, improved muscle pathology, and functional benefit to the transduced diaphragm compared with untreated littermate controls. These results support the potential of the AAV8 vector to efficiently cross the blood vessel barrier to achieve systemic gene transfer to skeletal muscle in utero in a mouse model of muscular dystrophy, to significantly improve the dystrophic phenotype and to ameliorate the processes that lead to exhaustion of the skeletal muscle regenerative capacity.

**PMID:
20535216**

Gene Ther. 2010 Jun 10. [Epub ahead of print]

CFTR expression and activity from the human CFTR locus in BAC vectors, with regulatory regions, isolated by a single-step procedure.

Auriche C, Di Domenico EG, Pierandrei S, Lucarelli M, Castellani S, Conese M, Melani R, Zegarra-Moran O, Ascenzioni F.

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We have assembled two BAC vectors containing a single fragment spanning the entire CFTR locus and including the upstream and downstream regions. The two vectors differ in size of the upstream region, and were recovered in *Escherichia coli*, with intact BAC DNAs prepared for structural and functional analyses. Sequence analysis allowed precise mapping of the inserts. We show that the CFTR gene was wild type and is categorized as the most frequent haplotype in Caucasian populations, identified by the following polymorphisms: (GATT)(7) in intron 6a; (TG)(11)T(7) in intron 8; V470 at position 470. CFTR expression and activity were analyzed in model cells by RT-PCR, quantitative real-time PCR, western blotting, indirect immunofluorescence and electrophysiological methods, which show the presence of an active CFTR Cl⁻ channel. Finally, and supporting the hypothesis that CFTR functions as a receptor for *Pseudomonas aeruginosa*, we show that CFTR-expressing cells internalized more bacteria than parental cells that do not express CFTR. Overall, these data demonstrate that the BAC vectors contain a functional CFTR fragment and have unique features, including derivation from a single fragment, availability of a detailed genomic map and the possibility to use standard extraction procedures for BAC DNA preparations.

**PMID:
20533091**

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Downregulation of ornithine decarboxylase by pcDNA-ODCr inhibits gastric cancer cell growth in vitro.

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Ornithine decarboxylase (ODC), the first rate-limiting enzyme of polyamine biosynthesis, was found to be associated with cell growth, proliferation and transformation. ODC gene expression in gastric cancer was increased and its level was positively correlated with the degree of malignancy of gastric mucosa and development of gastric lesions. In order to evaluate the therapeutic effects of antisense RNA of ODC on gastric cancer, an antisense RNA of ODC expressing plasmid pcDNA-ODCr which delivered a 120 bp fragment complementary to the initiation codon of ODC gene was constructed and transfected to gastric cancer cells SGC7901 and MGC803. Expression of ODC in gastric cancer cells was determined by western blot. Cell proliferation was assessed by MTS assay. Cell cycle was analyzed by flow cytometry and Matrigel assay was performed to assess the ability of gastric cancer cell invasiveness. The results showed that the ODC gene expression in gastric cancer cells transfected with the pcDNA-ODCr was downregulated efficiently. Tumor cell proliferation was suppressed significantly, and cell cycle was arrested at G1 phase. Gastric cancer cells had reduced invasiveness after gene transfer. Our study suggested that antisense RNA of ODC expressing plasmid pcDNA-ODCr had antitumor activity by inhibiting the expression of ODC, and downregulation of ODC expression using a gene therapy approach might be a novel therapeutic strategy for gastric cancer.

PMID:
20531394

Mol Ther. 2010 Jun 8. [Epub ahead of print]

Eight Years of Clinical Improvement in MPTP-Lesioned Primates After Gene Therapy With AAV2-hAADC.

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This study completes the longest known in vivo monitoring of adeno-associated virus (AAV)-mediated gene expression in nonhuman primate (NHP) brain. Although six of the eight parkinsonian NHP originally on study have undergone postmortem analysis, as described previously, we monitored the remaining two animals for a total of 8 years. In this study, NHP received AAV2-human L-amino acid decarboxylase (hAADC) infusions into the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-lesioned putamen. Restoration of AADC activity restored normal response to levodopa and gene expression could be quantitated repeatedly over many years by 6-[(18)F]fluoro-meta-tyrosine (FMT)-positron emission tomography (PET) and confirm that AADC transgene expression remained unchanged at the 8-year point. Behavioral assessments confirmed continued, normalized response to levodopa (improvement by 35% over historical controls). Postmortem analysis showed that, although only 5.6 +/- 1% and 6.6 +/- 1% of neurons within the transduced volumes of the striatum were transduced, this still secured robust clinical improvement. Importantly, there were no signs of neuroinflammation or reactive gliosis at the 8-year point, indicative of the safety of this treatment. The present data suggest that the improvement in the L-3,4-dihydroxyphenylalanine (L-Dopa) therapeutic window brought about by AADC gene therapy is pronounced and persistent for many years.

PMID:
20528736

Crit Rev Eukaryot Gene Expr. 2010;20(1):35-50.

Strategies for regeneration of heart muscle.

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Regenerative medicine has emerged to the forefront of cardiac research, marrying discoveries in both basic science and engineering to develop viable therapeutic approaches for treating the diseased heart. Significant advancements in gene therapy, stem cell biology, and cardiomyoplasty provide new optimism for regenerating damaged myocardium. Exciting new strategies for endogenous and exogenous regeneration have been proposed. However, questions remain as to whether these approaches can provide enough new myocyte mass to sufficiently restore mechanical function to the heart. In this article, we consider the mechanisms of endogenous cardiomyocyte regeneration and exogenous cell differentiation (with respect to myoblasts, stem cells, and induced pluripotent cells being researched for cell therapies). We begin by reviewing some of the cues that are being harnessed in strategies of gene/cell therapy for regenerating myocardium. We also consider some of the technical challenges that remain in determining new myocyte generation, tracking delivered cells in vivo, and correlating new myocyte contractility with cardiac function. Strategies for regenerating the heart are being realized as both animal and clinical trials suggest that these new approaches provide short-term improvement of cardiac function. However, a more complete understanding of the underlying mechanisms and applications is necessary to sustain longer-term therapeutic success.

PMID:
20528679

Hum Gene Ther. 2010 Jun 9. [Epub ahead of print]

Functional expression of secreted proteins from a bicistronic retroviral cassette based on FMDV 2A can be position-dependent.

Rothwell DG, Crossley R, Bridgeman JS, Sheard V, Zhang Y, Sharp T, Hawkins RE, Gilham D, McKay TR.

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The expression of two or more genes from a single viral vector has been widely utilised to label or select for cells containing the transgenic element. The recent identification of the Foot and Mouth Disease Virus (FMDV) 2A cleavage peptide as a polycistronic linker capable of producing equivalent levels of transgene expression has greatly improved this approach in the field of gene therapy. However, as a consequence of 2A post-translational cleavage the upstream protein is left with a residual 19 amino-acids from the 2A sequence on its carboxy-terminus, while the downstream protein is left with an additional 2-5 amino-acids on its amino-terminus. Here we have assessed the functional consequences of the FMDV 2A cleavage motif upon two secreted proteins (IL-2 and TGF) when expressed from a retroviral bicistronic vector. While IL-2 expression and function was found to be unaffected by the 2A motif in either orientation, functional expression of secreted TGFbeta was significantly abrogated when the transgene was expressed upstream of the 2A sequence. We believe this is a consequence of aberrant cleavage and intracellular trafficking of the TGF polyprotein. These results highlight that to achieve functional expression of secreted proteins consideration must be taken of the transgenic protein's post-translational modification and trafficking when using 2A-based bicistronic cassettes.

PMID:
20527047

J Gene Med. 2010 Jun;12(6):545-54.

Carrier cell-mediated cell lysis of squamous cell carcinoma cells by squamous cell carcinoma antigen 1 promoter-driven oncolytic adenovirus.

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BACKGROUND: The squamous cell carcinoma antigen (SCCA) serves as a serological marker for squamous cell carcinomas. Molecular cloning of the SCCA genomic region has revealed the presence of two tandemly arrayed genes: SCCA1 and SCCA2. SCCA1 gene is up-regulated in squamous cell carcinoma cells. We analyzed the proximal region of the SCCA1 promoter and the antitumor effect of oncolytic adenovirus driven by the SCCA1 promoter in squamous cell carcinoma cells. **METHODS:** The SCCA1 promoter was analyzed by dual luciferase assay and substituted with the E1A promoter to construct the oncolytic adenovirus to determine the squamous cell carcinoma-specific cell lysis. **RESULTS:** Deletion analysis of SCCA1 promoter identified a 175-bp core promoter region and an enhancer region at -525 to -475 bp upstream of the transcription start site. The transcriptional activity of the SCCA1 promoter was up-regulated in squamous cell carcinoma cells. Five tandem repeats of enhancer increased SCCA1 promoter activity by four-fold. Oncolytic adenovirus driven by this SCCA1 enhancer-promoter complex specifically killed squamous cell carcinoma cells in vitro and in vivo. A549 carrier cells infected with the oncolytic adenovirus induced complete regression of syngeneic squamous cell carcinoma cell tumor by overcoming immunogenicity and adenovirus-mGM-CSF augmented the antitumor effect of carrier cells. **CONCLUSIONS:** SCCA1 was up-regulated in squamous cell carcinoma cells and oncolytic adenovirus driven by SCCA1 promoter specifically killed these cells. These findings suggest that SCCA1 promoter is a potential target of gene therapy for squamous cell carcinoma.

PMID:
20527042

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Cationic and anionic lipoplexes inhibit gene transfection by electroporation in vivo.

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BACKGROUND: Nonviral gene therapy still suffers from low efficiency. Methods that would lead to higher gene expression level of longer duration would be a major advance in this field. Lipidic vectors and physical methods have been investigated separately, and both induced gene expression improvement. **METHODS:** We sought to combine both chemical and physical methods. Cationic or anionic lipids can potentially destabilize the cell membrane and could consequently enhance gene delivery by a physical method such as electrotransfer. A plasmid model encoding luciferase was used, either free or associated with differently-charged lipoplexes before electrotransfer. **RESULTS:** Electrotransfer alone strongly enhanced gene expression after intramuscular and intradermal injection of naked DNA. On the other hand, cationic and anionic lipoplex formulations decreased gene expression after electrotransfer, whereas poorly-charged thiourea-based complexes, brought no benefit. Pre-injection of the lipids, followed by administration of naked DNA, did not modified gene expression induced by electroporation in the skin. **CONCLUSIONS:** The results obtained in the present study suggest that packing of DNA plasmid in lipoplexes strongly decreases the efficiency of gene electrotransfer, independently of the lipoplex charge. Non-aggregating complexes, such as poorly-charged thiourea-based complexes, should be preferred to increase DNA release.

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Gene therapy for psoriasis in the K14-VEGF transgenic mouse model by topical transdermal delivery of interleukin-4 using ultradeformable cationic liposome.

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BACKGROUND: Topical transdermal gene delivery to the skin shows great potential for painless, non-invasive administration of vaccines and therapeutic agents. Interleukin (IL)-4 strategies have shown a good antipsoriatic effect in clinic trials. To date, no information has been acquired on the effectiveness of gene therapy for psoriasis in the K14-VEGF transgenic mouse model by topical transdermal penetration of murine IL-4 (mIL-4) using ultradeformable cationic liposome (UCL). **METHODS:** In the present study, we synthesized an UCL and determined a suitable formula for transdermally delivering plasmid DNA to mouse skin. We then tested the antipsoriatic efficacy in the K14-VEGF transgenic mouse model by transdermal delivery of mIL-4 using UCL. **RESULTS:** We found that plasmid DNA was transdermally delivered to vicinal sites of epidermis and hair follicles using this optimized formula. Plasmid DNA expression was detected in ear skin. Twenty-four hours after topical application, plasmid DNA was not detected in blood serum and liver, which may decrease the risk of insertion of promoter from plasmid to genomic DNA. Mice treated with UCL/mIL-4 displayed a mild psoriasis phenotype. Histological analysis of pathological score using the Baker scoring system revealed an antipsoriatic effect. Immunohistochemical analysis revealed that hyperplastic and inflamed vessels were suppressed. **CONCLUSIONS:** These observations provide evidence of antipsoriatic efficacy by topical transdermal delivery of mIL-4. Therefore, topical transdermal gene transfer is attractive and offers future potential for application in human patients with other dermatologic diseases.

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Antisense oligonucleotide targeting Livin induces apoptosis of human bladder cancer cell via a mechanism involving caspase 3.

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ABSTRACT: Background and Aim: In recent years, Livin, a new member of IAPs family, is found to be a key molecule in cancers. Researchers consider Livin may become a new target for tumor therapy; however, the role of it in bladder cancer is still unclear. The purpose of this article is to investigate Antisense Oligonucleotide (ASODN) of Livin on treating bladder cancer cell and underlying mechanisms. **METHODS:** Phosphorathioate modifying was used to synthesize antisense oligonucleotides targeting Livin, followed by transfection into human bladder cancer cell 5637. After transfection, Livin mRNA and protein level, cell proliferation and apoptosis changes, caspase3 level and its effect on human bladder cancer transplantable tumor in nude mice were measured. **Result:** Results showed Livin ASODN effectively inhibited Livin expression and tumor cell proliferation, and these effects probably through enhanced caspase3 activity and apoptosis of tumor cells. In nude mice transplantable tumor model, Livin expressions were inhibited meanwhile caspase3 expression was increased. Tumor growth slowed down and apoptosis was enhanced. **CONCLUSION:** Our data suggest that Livin plays an important role in inhibiting apoptosis of bladder cancer cells. Livin ASODN may promote cell apoptosis, inhibit bladder cancer growth, and become one of the methods of gene therapy for bladder cancer.

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Generation of human induced pluripotent stem cells bearing an anti-HIV transgene by a lentiviral vector carrying an internal murine leukemia virus promoter.

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The recent development of induced pluripotent stem cells (iPSCs) by ectopic expression of defined reprogramming factors offers enormous therapeutic opportunity. To deliver these factors, murine leukemia virus (MLV)-based vectors have been broadly used in the setting of hematopoietic stem cells transplantation. However, MLV vectors have been implicated in malignancy induced by insertional mutagenesis whereas lentiviral vectors have not. Furthermore, the infectivity of MLV vectors is limited to dividing cells whereas lentiviral vectors can also transduce non-dividing cells. One important characteristic of MLV vectors is a self-silencing property of the promoter element in pluripotent stem cells, allowing temporal transgene expression in a non-pluripotent state before iPSC derivation. Here we test iPSC generation using a novel chimeric vector carrying a mutant MLV promoter internal to a lentiviral vector backbone, thereby containing the useful properties of both types of vectors. Transgene expression of this chimeric vector was highly efficient compared to MLV vectors and was silenced specifically in human embryonic stem cells. Human fetal fibroblasts transduced with the vector encoding each factor were efficiently reprogrammed into a pluripotent state and these iPSCs had potential to differentiate into a variety of cell types. To explore the possibility of iPSC for gene therapy, we established iPSC clones expressing a short hairpin RNA (shRNA) targeting CCR5, the main coreceptor for HIV-1. Using a reporter construct for CCR5 expression, we confirmed that CCR5 shRNA was expressed and specifically knocked-down the reporter expression in iPSCs. These data indicate that our chimeric lentiviral vector is a valuable tool for generation of iPSCs and the combination with vectors encoding transgenes allows for rapid establishment of desired genetically engineered iPSC lines.