



CLINIGENE CURRENT GENE THERAPY WEEKLY

From February 22nd to March 01st 2010

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PMID:
20187132

Adeno-associated virus type 5-mediated intraarticular administration of tumor necrosis factor small interfering RNA improves collagen-induced arthritis.

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OBJECTIVE: RNA interference (RNAi) is a powerful tool for sequence-specific gene silencing, and interest in its application in human diseases is growing. Given the success of recent strategies for administering gene therapy in rheumatoid arthritis using recombinant vectors such as adeno-associated virus type 5 (rAAV5) for optimized intraarticular gene transfer, we undertook the present study to determine the feasibility of using rAAV5-mediated RNAi-based therapy in arthritis. **METHODS:** We developed rAAV5 vectors expressing short hairpin small interfering RNA (shRNA) against tumor necrosis factor alpha (TNFalpha) under H1 promoter, and carrying the enhanced green fluorescent protein (eGFP) reporter gene under cytomegalovirus promoter (rAAV5-shTNF). TNFalpha gene silencing was validated in vitro with mouse macrophages. Mice with collagen-induced arthritis were injected in the ankle and knee joints, at disease onset, with either rAAV5-shTNF or control rAAV5-eGFP vectors (5 x 10⁹ particles). Arthritis severity was assessed clinically and histologically, and immunologic response was examined. Local and systemic transgene expression was monitored using quantitative reverse transcriptase-polymerase chain reaction, immunohistochemical analysis, and enzyme-linked immunosorbent assay. **RESULTS:** After a single injection of rAAV5-shTNF into inflamed joints, local TNFalpha gene silencing provided rapid and long-term suppression of arthritis progression and reduced joint damage compared with that observed in control groups. Treatment with rAAV5-shTNF was associated with decreased proliferation and interferon-gamma production by antigen-stimulated T cells from draining lymph nodes, and the potency of this treatment was similar to that observed with other treatment strategies targeting TNFalpha at the protein level, either locally or systemically. **CONCLUSION:** Our data present the first proof-of-concept for the application of rAAV5-mediated RNAi-based gene therapy for local blockade of inflammation in experimental arthritis.

PMID:
20186514

In Vitro Cell Dev Biol Anim. 2010 Feb 26. [Epub ahead of print]

Human embryonic stem cells carrying mutations for severe genetic disorders.

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Human embryonic stem cells (HESCs) carrying specific mutations potentially provide a valuable tool for studying genetic disorders in humans. One preferable approach for obtaining these cell lines is by deriving them from affected preimplantation genetically diagnosed embryos. These unique cells are especially important for modeling human genetic disorders for which there are no adequate research models. They can be further used to gain new insights into developmentally regulated events that occur during human embryo development and that are responsible for the manifestation of genetically inherited disorders. They also have great value for the exploration of new therapeutic protocols, including gene-therapy-based treatments and disease-oriented drug screening and discovery. Here, we report the establishment of 15 different mutant human embryonic stem cell lines derived from genetically affected embryos, all donated by couples undergoing preimplantation genetic diagnosis in our in vitro fertilization unit. For further information regarding access to HESC lines from our repository, for research purposes, please email dalitb@tasmc.health.gov.il.

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20186445

An HDAC inhibitor increases AcMNPV gene expression in mammalian cells.

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The baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is used as a safer viral vector in mammalian cells with potential applications in gene therapy. However, the mechanism for the insusceptibility of mammalian cells to proliferative infection by entomopathogenic viruses is not well understood. Here, we studied the significance of epigenetic modifications such as histone acetylation, histone methylation and HP1 accumulation for AcMNPV gene expression in mammalian BHK cells. Real-time PCR and chromatin immunoprecipitation with sodium butyrate revealed an important relationship between viral gene expression and histone acetylation, with implications for a mechanism of suppression of AcMNPV gene expression in BHK cells.

PMID: Expert Rev Endocrinol Metab. 2009 Jul 1;4(4):359-370.
20186255

GENE THERAPY FOR THE TREATMENT OF PITUITARY TUMORS.

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Pituitary adenomas constitute the most frequent neuroendocrine pathology in humans. Current therapies include surgery, radiotherapy and pharmacological approaches. Although useful, none of them offers a permanent cure. Current research efforts to implement gene therapy in pituitary tumors include the treatment of experimental adenomas with adenoviral vector-mediated transfer of the suicide gene for thymidine kinase, which converts the prodrug ganciclovir into a toxic metabolite. In some cases, the suicide transgene has been placed under the control of pituitary cell-type specific promoters. Also, regulatable adenoviral vector systems are being assessed in gene therapy approaches for experimental pituitary tumors. Although the efficiency and safety of current viral vectors must be optimized before clinical use, they remain as highly promising therapeutic tools.

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20186173

Cancer Gene Ther. 2010 Feb 26. [Epub ahead of print]

Effective immunotherapy of weakly immunogenic solid tumours using a combined immunogene therapy and regulatory T-cell inactivation.

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Obstacles to effective immunotherapeutic anti-cancer approaches include poor immunogenicity of the tumour cells and the presence of tolerogenic mechanisms in the tumour microenvironment. We report an effective immune-based treatment of weakly immunogenic, growing solid tumours using a locally delivered immunogene therapy to promote development of immune effector responses in the tumour microenvironment and a systemic based T regulatory cell (Treg) inactivation strategy to potentiate these responses by elimination of tolerogenic or immune suppressor influences. As the JBS fibrosarcoma is weakly immunogenic and accumulates Treg in its microenvironment with progressive growth, we used this tumour model to test our combined immunotherapies. Plasmids encoding GM-CSF and B7-1 were electrically delivered into 100 mm³ tumours; Treg inactivation was accomplished by systemic administration of anti-CD25 antibody (Ab). Using this approach, we found that complete elimination of tumours was achieved at a level of 60% by immunogene therapy, 25% for Treg inactivation and 90% for combined therapies. Moreover, we found that these responses were immune transferable, systemic, tumour specific and durable. Combined gene-based immune effector therapy and Treg inactivation represents an effective treatment for weakly antigenic solid growing tumours and that could be considered for clinical development.

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20186172

Cancer Gene Ther. 2010 Feb 26. [Epub ahead of print]

Enhanced anti-tumor effects of combined MDR1 RNA interference and human sodium/iodide symporter (NIS) radioiodine gene therapy using an adenoviral system in a colon cancer model.

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Using an adenoviral system as a delivery mediator of therapeutic gene, we investigated the therapeutic effects of the use of combined MDR1 shRNA and human NIS (hNIS) radioiodine gene therapy in a mouse colon xenograft model. In vitro uptake of Tc-99m sestamibi was increased approximately two-fold in cells infected with an adenovirus vector that expressed MDR1 shRNA (Ad-shMDR1) and I-125 uptake was 25-fold higher in cells infected with an adenovirus vector that expressed human NIS (Ad-hNIS) as compared with control cells. As compared with doxorubicin or I-131 treatment alone, the combination of doxorubicin and I-131 resulted in enhanced cytotoxicity for both Ad-shMDR1- and Ad-hNIS-infected cells, but not for control cells. In vivo uptake of Tc-99m sestamibi and Tc-99m pertechnetate was twofold and 10-fold higher for Ad-shMDR1 and Ad-hNIS-infected tumors as compared with tumors infected with a control adenovirus construct that expressed beta-galactosidase (Ad-LacZ), respectively. In mice treated with either doxorubicin or I-131 alone, there was a slight delay in tumor growth as compared to mice treated with Ad-LacZ. However, combination therapy with doxorubicin and I-131 induced further significant inhibition of tumor growth as compared with mice treated with Ad-LacZ. We have shown successful therapeutic efficacy of combined MDR1 shRNA and hNIS radioiodine gene therapy using an adenoviral vector system in a mouse colon cancer model. Adenovirus-mediated cancer gene therapy using MDR1 shRNA and hNIS would be a useful tool for the treatment of cancer cells expressing multi-drug resistant genes.

PMID:
20184930

J Control Release. 2010 Feb 22. [Epub ahead of print]

Optimized pulmonary gene transfection in mice by spray-freeze dried powder inhalation.

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Spray-freeze drying (SFD) is an attractive technique to prepare highly porous dry powders for inhalation. However, there have been few reports of its application to dry powder inhalers (DPIs). Therefore, in this study, we prepared dry plasmid DNA (pDNA) powders with different molecular ratios of chitosan to pDNA (N/P ratios) by SFD. All the pDNA powders were spherical and highly porous, with particles approximately 20-40µm in geometric diameter. The morphology changed little with the alteration of the N/P ratio. On electrophoresis, a band of linear pDNA was detected in the preparation without chitosan, suggesting the destabilization of pDNA through SFD. However, the addition of chitosan protected pDNA from destabilization. Moreover, the pDNA powders were evaluated for pulmonary gene transfection efficiency using an in vivo dual imaging technique for gene DPIs developed previously. Maximum gene expression was observed at 9-12h following pulmonary administration of the powders into mice. The powder with the N/P ratio of 10 had the highest gene transfection efficiency. A higher affinity of chitosan for pDNA and a smaller (approximately 100nm) pDNA/chitosan complex (N/Pf10) were found at pH 6.5 (in lung) than at pH 7.4 (in physiological conditions), suggesting that the effective compaction of pDNA by chitosan at the N/P ratio of 10 at pH 6.5 contributes to the gene transfection efficiency in the lung. These results suggest inhalable dry pDNA powders with chitosan prepared by SFD to be a suitable formulation for pulmonary gene therapy. Copyright © 2010 Elsevier B.V. All rights reserved.

PMID:
20183925

Childs Nerv Syst. 2010 Mar;26(3):323-31.

Neuroprotective effect of combined hypoxia-induced VEGF and bone marrow-derived mesenchymal stem cell treatment.

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PURPOSES: To avoid unwanted adverse effects of higher doses of single treatment of stem cells and gene therapy and increase the therapeutic efficacies, we hypothesized the combined therapy with stem cells and gene therapy. This study assessed the neuroprotective effects of combined gene therapy and stem cell treatment under ischemic hypoxia conditions using hypoxia-inducible vascular endothelial growth factor (VEGF) and bone marrow-derived mesenchymal stem cells (BMSC). **METHODS:** Experimental groups included the control which was N2A cells transfected with empty vectors, the transfection only group which was N2A cells treated with pEpo-SV-VEGF alone, the BMSC only group which was N2A cells transfected with empty vectors and cocultured with BMSCs, and the combined treatment group which was N2A cells treated with pEpo-SV-VEGF and cocultured with BMSCs. Each group was transfected for 4 h and cultured at 37 degrees C and 5% CO₂ for 24 h. Each group was then cultivated under hypoxic conditions (1% O₂) for 12 h. Neuroprotective effects were assessed by reverse transcription polymerase chain reaction, annexin V, and cytotoxicity assay. **RESULTS:** Neurons exposed to hypoxic conditions exhibited neuronal apoptosis. Compared to single treatments, the combined hypoxia-inducible VEGF and BMSC treatment demonstrated a significant increase in VEGF expression and decreased neuronal apoptosis. **CONCLUSIONS:** These results suggest that combined pEpo-SV-VEGF and BMSC treatment is effective in protecting neurons against hypoxic ischemic injury.

PMID:
20183612

Nucleosides Nucleotides Nucleic Acids. 2009 Aug;28(8):725-35.

Method to improve DNA condensation efficiency by alkali treatment.

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The improvement of DNA's bioactivities by altering their structure is meaningful for their biological applications, ranging from DNA condensation study to gene therapeutic research. In this study, we treated the plasmid DNA with alkali and investigated the structure and the condensation efficiency of the alkali-treated DNA. We noticed that the alkali treatment could significantly increase the DNA condensation efficiency with spermidine and polyethylenimine (PEI). In addition, due to the improved interactions between the alkali-treated DNA and PEI, gene transfection experiments could be performed in the presence of less PEI. This research can contribute to the creation of a universal method to enhance the interaction between DNA and gene delivery vectors by alkali treatment, and should have significant potential in the field of gene therapy.

PMID:
20183607

Nucleosides Nucleotides Nucleic Acids. 2009 May;28(5):642-56.

Regioselective metalation of 6-methylpurines: synthesis of fluoromethyl purines and related nucleosides for suicide gene therapy of cancer.

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Metalation of 6-methyl-9-(tetrahydro-2H-pyran-2-yl)purine (10) with lithiating agents of varying basicities such as n-BuLi and LiHMDS in THF at -78 degrees C resulted in metalation at both of the 6-CH(3) moiety and the 8-CH position, irrespective of the molar equivalence of the base. On the other hand, a regioselective metalation at the 6-CH(3) moiety of 10 was observed with NaHMDS or KHMDS, under similar conditions. Treatment of the potassium salts of 10 and of the protected riboside derivative 6-methyl-9-(beta-D-2,3,5-tri-O-tert-butylidimethylsilylribofuranosyl)purine (22) with N-fluorobenzenesulfonamide (NFSI) at -78 degrees C gave the corresponding 6-fluoromethylpurine derivatives 11 and 23, respectively, in good yields. Deprotection of 11 and 23 under standard conditions gave 6-fluoromethylpurine (6-FMeP, 3) and 6-fluoromethyl-9-(beta-D-ribofuranosyl)purine (6-FMePR, 4), respectively, in high yield. Both 3 and 4 demonstrated cytotoxic activity against CCRF-CEM cells in culture. 6-FMePR is a good substrate for E. coli purine nucleoside phosphorylase (E. coli PNP) with a comparable substrate activity to that of the parent nucleoside, 6-methyl-9-(beta-D-ribofuranosyl)purine (6-MePR, 21). The cytotoxic activity of 6-FMeP along with the substrate activity of 6-FMePR with E. coli PNP meet the fundamental requirements for using 6-FMeP as a potential toxin in PNP/prodrug based cancer gene therapy.

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20182520

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Insulin expressed from endogenously active glucose-responsive EGR1 promoter in bone marrow mesenchymal stromal cells as diabetes therapy.

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Advances in islet transplantation have encouraged efforts to create alternative insulin-secreting cells that overcome limitations associated with current therapies. We have recently demonstrated durable correction of murine and porcine diabetes by syngeneic and autologous implantation, respectively, of primary hepatocytes non-virally modified with a glucose-responsive promoter-regulated insulin transgene. As surgical procurement of hepatocytes may be clinically unappealing, we here describe primary bone marrow-derived mesenchymal stromal cells (BMMSC) as alternative insulin-secreting bioimplants. BMMSC are abundant and less invasively procured for clinical autologous transplantation. Electroporation achieved high transgene transfection efficiencies in human BMMSC (HBMMSC) and porcine BMMSC (PBMMSC). We transcriptomically identified an HBMMSC glucose-responsive promoter, EGR1. This endogenously active promoter drove rapid glucose-induced transgene secretions in BMMSC with near-physiological characteristics during static and kinetic induction assays simulating normal human islets. Preparatory to preclinical transplantation, PBMMSC transfected with the circular insulin transgene vector or stably integrated with the linearized vector were evaluated by intrahepatic or intraperitoneal xenotransplantation in streptozotocin-diabetic and non-diabetic NOD-SCID mice. Hyperglycemia, glucose tolerance and body weight were corrected in a dose-responsive manner. Hypoglycemia was not observed even in identically implanted non-diabetic mice. These results establish human EGR1 promoter-insulin construct-modified BMMSC as safe and efficient insulin-secreting bioimplants for diabetes treatment.

PMID:
20182519

Gene Ther. 2010 Feb 25. [Epub ahead of print]

Studies on protective effects of human paraoxonases 1 and 3 on atherosclerosis in apolipoprotein E knockout mice.

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Paraoxonase (PON) possesses antiatherogenic potentials, but the distinct functions of PON members in alleviating atherosclerosis are not yet clear. This study aimed to evaluate the protective effects of hPON1 and hPON3 against atherosclerosis, and thereby exploring their synergistic mechanism in atherosclerosis development. We generated the recombinant adenovirus AdPON1 and AdPON3, which were capable of expressing hPON1 and hPON3. After AdPON1 and AdPON3 were injected intravenously into 5-week-old apolipoprotein E knockout mice, abundant hPON1 and hPON3 mRNA expression levels were detected. However, increase in serum lactonase activity was detected only in AdPON1-treated mice. Serum antioxidation and anti-inflammation capabilities in AdPON1-treated mice, reflected by malondialdehyde, total antioxidant capability and tumor necrosis factor-alpha levels, were greatly enhanced, whereas those in AdPON3-treated mice were not significantly affected. Nevertheless, histological analysis revealed that adenovirus-mediated expression of hPON1, hPON3 or both of them reduced atherosclerotic plaque area to a similar extent. Although no synergistic mechanism was detected in reducing arterial lesion size, hPON1 and hPON3 showed synergistic effects on promoting macrophage cholesterol efflux. In conclusion, hPON1 and hPON3 exhibited similar potentials in reducing arterial lesion size, but they exerted antiatherogenic effects in distinct ways.

PMID:
20182518

Gene Ther. 2010 Feb 25. [Epub ahead of print]

Anti-inflammatory effect by lentiviral-mediated overexpression of IL-10 or IL-1 receptor antagonist in rat glial cells and macrophages.

van Strien ME, Mercier D, Drukarch B, Brevé JJ, Poole S, Binnekade R, Bol JG, Blits B, Verhaagen J, van Dam AM.

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Neuroinflammation, as defined by activation of local glial cells and production of various inflammatory mediators, is an important feature of many neurological disorders. Expression of pro-inflammatory mediators produced by glial cells in the central nervous system (CNS) is considered to contribute to the neuropathology observed in those diseases. To diminish the production or action of pro-inflammatory mediators, we have used lentiviral (LV) vector-mediated encoding rat interleukin-10 (rIL-10) or rat interleukin-1 receptor antagonist (rIL-1ra) to direct the local, long-term expression of these anti-inflammatory cytokines in the CNS. We have shown that cultured macrophages or astroglia transduced with LV-rIL-10 or LV-rIL-1ra produced far less tumor necrosis factor (TNF)alpha or IL-6, respectively in response to pro-inflammatory stimuli. Moreover, intracerebroventricular (i.c.v.) administration of LV-rIL-10 or LV-rIL-1ra resulted in transduction of glial cells and macrophages and, subsequently reduced TNFalpha, IL-6 and inducible nitric oxide synthase (iNOS) expression in various brain regions induced by inflammatory stimuli, whereas peripheral expression of these mediators remained unaffected. In addition, expression levels of the anti-inflammatory cytokines IL-4 and transforming growth factor-beta were not altered in either brain or pituitary gland. Furthermore, i.c.v. administration of LV-rIL-10 or LV-rIL-1ra given during the remission phase of chronic-relapsing experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, improved the clinical outcome of the relapse phase. Thus, local application of LV vectors expressing anti-inflammatory cytokines could be of therapeutic interest to counteract pro-inflammatory processes in the brain without interfering with the peripheral production of inflammatory mediators.

PMID:
20182517

Gene Ther. 2010 Feb 25. [Epub ahead of print]

TRAIL gene-armed oncolytic poxvirus and oxaliplatin can work synergistically against colorectal cancer.

Ziauddin MF, Guo ZS, O'Malley ME, Austin F, Popovic PJ, Kavanagh MA, Li J, Sathaiah M, Thirunavukarasu P, Fang B, Lee YJ, Bartlett DL.

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We have explored a unique combination therapy for metastatic colorectal cancer. This strategy combines a potent and new oncolytic poxvirus expressing a membrane-bound tumor necrosis factor-related apoptosis-inducing ligand (TRAIL or TNFSF10) and oxaliplatin (Ox) chemotherapy. We hypothesized that TRAIL expression would increase the efficacy of the oncolytic poxvirus, and that the therapeutic efficacy would be further enhanced by combination with chemotherapy. The cytotoxicity to cancer cells by Ox, oncolytic vaccinia virus (VV) and trail gene-armed VV alone or in combination was tested in vitro. The trail gene armed oncolytic VV-expressed high levels of TRAIL in infected cancer cells and had greater potency as a cytotoxic agent compared with the parent VV. Ox alone exerted concentration-dependent cytotoxicity. In vitro, the combination of the two agents applied at suboptimal concentrations for individual therapy displayed synergy in inducing cancer cells into enhanced levels of apoptosis/necrosis. Western blot analyses were consistent with the notion that TRAIL induced cancer cell death mainly through apoptosis, whereas Ox and vJS6 induced cell death more through non-apoptotic death pathways. In two aggressive colorectal carcinomatosis models derived from human HCT116 and murine MC38 cells, the combination therapy displayed synergistic or additive antitumor activity and prolonged the survival of the tumor-bearing mice compared with either Ox chemotherapy or vvTRAIL-mediated oncolytic gene therapy alone. This combination strategy may provide a new avenue to treating peritoneal carcinomatosis and other types of metastases of colorectal cancer.

PMID:
20182516

Gene Ther. 2010 Feb 25. [Epub ahead of print]

Functional recovery of diabetic mouse hearts by glutaredoxin-1 gene therapy: role of Akt-FoxO-signaling network.

Lekli I, Mukherjee S, Ray D, Gurusamy N, Kim YH, Tosaki A, Engelman RM, Ho YS, Das DK. [1] Cardiovascular Research Center, University of Connecticut School of Medicine, Farmington, CT, USA [2] Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary.

Recent studies suggest that glutaredoxin-1 (Glx-1) may serve as therapeutic target for diabetic hearts. As the level of reactive oxygen species (ROS) is increased in the pathologic hearts including ischemia/reperfusion (I/R) and diabetes, we assumed that upregulation of Glrx-1 could reduce the cardiac risk factors associated with I/R and/or diabetes. Diabetes was induced in mice by i.p. injection of streptozotocin (150 mg kg⁻¹). Eight days after when the blood glucose was elevated to 400 mg per 100 ml, the animals were randomly assigned to one of the following three groups, which received either empty vector, or LacZ or Glrx-1 adenoviral construct. Four days later, isolated working hearts were subjected to 30 min ischemia followed by 2 h reperfusion. Glrx-1 gene therapy significantly enhanced the Glrx-1 level, which prevented I/R-mediated reduction of ventricular recovery, increased myocardial infarct size and cardiomyocyte apoptosis in diabetic myocardium. In concert, Glrx-1 prevented diabetes and ischemia-reperfusion induced reduction of cardioprotective proteins including Akt, FoxO-1, and hemoxygenase-1, and abolished the death signal triggered by Jnk, p38 mitogen-activated protein kinase, and c-Src. Glrx-1 gene therapy seems to prevent cardiac complications in diabetic heart due to the I/R by switching the death signal into survival signal by activating Akt-FoxO-signaling network. Gene Therapy advance online publication, 25 February 2010; doi:10.1038/gt.2010.9.

PMID: J Virol. 2010 Feb 24. [Epub ahead of print]
20181689

Effect of the internal promoter on insertional gene activation by lentiviral vectors with an intact HIV LTR.

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Insertional mutagenesis by viral vectors is a problem in gene therapy. We recently reported that lentiviral vectors with an intact HIV LTR caused insertional gene activation, by transcripts from the 5'LTR splicing to an adjacent gene. Here we demonstrate that the level of transcription from the 5'LTR, and also insertional gene activation, is dependent on the internal promoter in the vector. We also show that there are more transcripts originating from the 5'LTR than from, or reading through, the 3'LTR. This study will allow the design of safer lentiviral vectors for applications in which an intact HIV LTR is required.

PMID: Cancer Genomics Proteomics. 2010 Jan-Feb;7(1):31-49.
20181629

Genetic basis and gene therapy trials for thyroid cancer.

Al-Humadi H, Zarros A, Al-Saigh R, Liapi C.
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Gene therapy is regarded as one of the most promising novel therapeutic approaches for hopeless cases of thyroid cancer and those not responding to traditional treatment. In the last two decades, many studies have focused on the genetic factors behind the origin and the development of thyroid cancer, in order to investigate and shed more light on the molecular pathways implicated in different differentiated or undifferentiated types of thyroid tumors. We, herein, review the current data on the main genes that have been proven to (or thought to) be implicated in thyroid cancer etiology, and which are involved in several well-known signaling pathways (such as the mitogen-activated protein kinase and phosphatidylinositol-3-kinase/Akt pathways). Moreover, we review the results of the efforts made through multiple gene therapy trials, via several gene therapy approaches/strategies, on different thyroid carcinomas. Our review leads to the conclusion that future research efforts should seriously consider gene therapy for the treatment of thyroid cancer, and, thus, should: (a) shed more light on the molecular basis of thyroid cancer tumorigenesis, (b) focus on the development of novel gene therapy approaches that can achieve the required antitumoral efficacy with minimum normal tissue toxicity, as well as (c) perform more gene therapy clinical trials, in order to acquire more data on the efficacy of the examined approaches and to record the provoked adverse effects.

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20179682

Mol Ther. 2010 Feb 23. [Epub ahead of print]

Comparison of AAV Serotypes for Gene Delivery to Dorsal Root Ganglion Neurons.

Mason MR, Ehler EM, Eggers R, Pool CW, Hermening S, Huseinovic A, Timmermans E, Blits B, Verhaagen J.

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For many experiments in the study of the peripheral nervous system, it would be useful to genetically manipulate primary sensory neurons. We have compared vectors based on adeno-associated virus (AAV) serotypes 1, 2, 3, 4, 5, 6, and 8, and lentivirus (LV), all expressing green fluorescent protein (GFP), for efficiency of transduction of sensory neurons, expression level, cellular tropism, and persistence of transgene expression following direct injection into the dorsal root ganglia (DRG), using histological quantification and qPCR. Two weeks after injection, AAV1, AAV5, and AAV6 had transduced the most neurons. The time course of GFP expression from these three vectors was studied from 1 to 12 weeks after injection. AAV5 was the most effective serotype overall, followed by AAV1. Both these serotypes showed increasing neuronal transduction rates at later time points, with some injections of AAV5 yielding over 90% of DRG neurons GFP(+) at 12 weeks. AAV6 performed well initially, but transduction rates declined dramatically between 4 and 12 weeks. AAV1 and AAV5 both transduced large-diameter neurons, IB4(+) neurons, and CGRP(+) neurons. In conclusion, AAV5 is a highly effective gene therapy vector for primary sensory neurons following direct injection into the DRG.

PMID:
20179679

Mol Ther. 2010 Feb 23. [Epub ahead of print]

Therapeutic Efficacy of Bone Marrow Transplant, Intracranial AAV-mediated Gene Therapy, or Both in the Mouse Model of MPS IIIB.

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Sanfilippo syndrome type B (MPS IIIB) is a lysosomal storage disease resulting from a deficiency of N-acetyl-glucosaminidase (NAGLU) activity. In an attempt to correct the disease in the murine model of MPS IIIB, neonatal mice were treated with intracranial AAV2/5-NAGLU (AAV), syngeneic bone marrow transplant (BMT), or both (AAV/BMT). All treatments resulted in some improvement in clinical phenotype. Adeno-associated viral (AAV) treatment resulted in improvements in lifespan, motor function, hearing, time to activity onset, and daytime activity level, but no reduction of lysosomal storage. BMT resulted in improved hearing by 9 months, and improved circadian measures, but had no effect on lifespan, motor function, or central nervous system (CNS) lysosomal storage. AAV/BMT treatment resulted in improvements in hearing, time to activity onset, motor function, and reduced CNS lysosomal storage, but had no effect on lifespan. Combination therapy compared to either therapy alone resulted in synergistic effects on hearing and CNS lysosomal inclusions but antagonistic effects on motor function and lifespan. AAV alone is more efficacious than BMT or AAV/BMT treatment for lifespan. BMT was the least efficacious treatment by all measures. CNS-directed AAV treatment alone appears to be the preferred treatment, combining the most efficacy with the least toxicity of the approaches assessed.

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Expression of Dog Microdystrophin in Mouse and Dog Muscles by Gene Therapy.

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Duchenne muscular dystrophy (DMD) is characterized by the absence of dystrophin. Several previous studies demonstrated the feasibility of delivering microdystrophin complementary DNA (cDNA) into mouse and normal nonhuman primate muscles by ex vivo gene therapy. However, these animal models do not reproduce completely the human DMD phenotype, while the dystrophic dog model does. To progress toward the use of the best animal model of DMD, a dog microdystrophin was transduced into human and dystrophic dog muscle precursor cells (MPCs) with a lentivirus before their transplantation into mouse muscles. One month following MPC transplantation, myofibers expressing the dog microdystrophin were observed. We also used another approach to introduce this transgene into myofibers, i.e., the electrotransfer of a plasmid coding for the dog microdystrophin. The plasmid was injected into mouse and dog muscles, and brief electric pulses were applied in the region of injection. Two weeks later, the transgene was detected in both animals. Therefore, ex vivo gene therapy and electrotransfer are two possible methods to introduce a truncated version of dystrophin into myofibers of animal models and eventually into myofibers of DMD patients.

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Anti-angiogenic Gene Therapy of Solid Tumor by Systemic Injection of Polyplex Micelles Loading Plasmid DNA Encoding Soluble Flt-1.

Oba M, Vachutinsky Y, Miyata K, Kano MR, Ikeda S, Nishiyama N, Itaka K, Miyazono K, Koyama H, Kataoka K.

In this study, a polyplex micelle was developed as a potential formulation for anti-angiogenic gene therapy of subcutaneous pancreatic tumor model. Poly(ethylene glycol)-poly(L-lysine) block copolymers (PEG-PLys) with thiol groups in the side chain of the PLys segment were synthesized and applied for preparation of disulfide crosslinked polyplex micelles through ion complexation with plasmid DNA (pDNA) encoding the soluble form of vascular endothelial growth factor (VEGF) receptor-1 (sFlt-1), which is a potent anti-angiogenic molecule. Anti-tumor activity and gene expression of polyplex micelles with various crosslinking rates were evaluated in mice bearing subcutaneously xenografted BxPC3 cell line, derived from human pancreatic adenocarcinoma, and polyplex micelles with optimal crosslinking rate achieved effective suppression of tumor growth. Significant gene expression of this micelle was detected selectively in tumor tissue, and its anti-angiogenic effect was confirmed by decreased vascular density inside the tumor. Therefore, the disulfide crosslinked polyplex micelle loading sFlt-1 pDNA has a great potential for anti-angiogenic therapy against subcutaneous pancreatic tumor model by systemic application.

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High Transgene Expression by Lentiviral Vectors Causes Maldevelopment of Purkinje Cells In Vivo.

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Lentiviral vectors are promising as gene-transfer vehicles for gene therapy targeted to intractable brain diseases. Although lentiviral vectors are thought to exert little toxicity on infected cells, the adverse influence of viral infection on vulnerable developing neurons has not been well studied. Here, we examined whether lentiviral vector infection and subsequent transgene expression affected the morphological and functional maturation of vigorously developing cerebellar Purkinje cells in vivo. Lentiviral vectors expressing GFP under the control of the murine stem cell virus (MSCV) promoter were injected into the cerebellar cortex of neonatal rat pups. Three weeks after treatment, GFP-expressing Purkinje cells were compared with control Purkinje cells from phosphate-buffered saline-injected rats. Analysis of the dendritic tree showed that total dendrite length in GFP-expressing Purkinje cells was almost 80% that in control Purkinje cells. Electrophysiological examination showed that short-term synaptic plasticity at parallel fiber-Purkinje cell synapses and climbing fiber-Purkinje cell synapses was significantly altered in GFP-expressing Purkinje cells. In contrast, maldevelopment of infected Purkinje cells was substantially attenuated when lentiviral vectors with much weaker promoter activity were used. These results suggest that the maldevelopment of Purkinje cells was mainly caused by subsequent expression of a high amount of GFP driven by the strong MSCV promoter. Thus, the use of lentiviral vectors carrying a strong promoter may require particular precautions when applying them to neurological disorders of infants.

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Cardiac-Targeted Delivery of Regulatory RNA Molecules and Genes for the Treatment of Heart Failure.

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Ribonucleic acid (RNA) in its many facets of structure and function is more and more understood, and therefore it is possible to design and use RNAs as valuable tools in molecular biology and medicine. Understanding of the role of RNAs within the cell has changed dramatically during the past few years. Therapeutic strategies based on non-coding regulatory RNAs include RNA interference (RNAi) for the silencing of specific genes, and microRNA (miRNA) modulations to alter complex gene expression patterns. Recent progress has allowed to target therapeutic RNAi to the heart for the treatment of heart failure and we discuss current strategies in this field. Due to the peculiar biochemical properties of small RNA molecules, the actual therapeutic translation of findings in vitro or in cell cultures is more demanding than with small molecule drugs or proteins. The critical requirement for animal studies after pre-testing of RNAi tools in vitro likewise applies for miRNA modulations which also have complex consequences in the recipient dependent on stability and distribution of the RNA tools. Problems in the field that are not yet fully solved are the prediction of targets and specificity of the RNA tools as well as their tissue-specific and regulatable expression. We discuss analogies and differences between regulatory RNA therapy and classical gene therapy, since recent breakthroughs in vector technology are of importance for both. Recent years have witnessed partially parallel progress in the fields of gene-based and regulatory RNA-based therapies that are likely to significantly expand the cardiovascular therapeutic repertoire within the next decade.

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20175784

Viral shedding after p53 adenoviral gene therapy in 10 cases of esophageal cancer.

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We detected adenoviral DNA fragments in excretions of 10 esophageal cancer patients by DNA-PCR after tumor injection of Ad-CMV-vector. A total of 220 samples consisting of feces, gargling saliva, urine, and blood plasma were assessed. A total of 29.7% of feces samples and 13.2% of gargling saliva samples were positive for adenoviral DNA fragments, but 89.7% of the positive feces samples and all of the positive gargling saliva samples turned negative on day 12 after tumor injection. Although adenoviral DNA fragments may be pathogen-free, patients' feces and gargling saliva contain adenoviral DNA fragments for 12 days after injection.

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20175124

Endocytic uptake of fluorescence labelled DNA in yeast.

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After dispiriting results using viral vectors in gene therapy, by which a number of patients acquired cancer as a result of the use of retroviral vector constructs, the percentage of non-viral approaches has increased over recent years. To elucidate potential bottlenecks in the non-viral transfection process we here introduce a novel method to directly visualize endocytic non-viral DNA uptake in a transfection approach. This novel method allows for the first time to monitor the location of DNA which is taken up by endocytosis in yeast (*Saccharomyces cerevisiae*) wild type and mutant strains. More specifically it enables drawing conclusions about conditions favouring non-viral gene transfection.

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20173779

Stem cell and gene therapies for diabetes mellitus.

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In this Perspectives article, we comment on the progress in experimental stem cell and gene therapies that might one day become a clinical reality for the treatment of patients with diabetes mellitus. Research on the ability of human embryonic stem cells to differentiate into islet cells has defined the developmental stages and transcription factors involved in this process. However, the clinical applications of human embryonic stem cells are limited by ethical concerns, as well as the potential for teratoma formation. As a consequence, alternative forms of stem cell therapies, such as induced pluripotent stem cells and bone marrow-derived mesenchymal stem cells, have become an area of intense study. Finally, gene therapy shows some promise for the generation of insulin-producing cells. Here, we discuss two of the most frequently used approaches: in vitro gene delivery into cells which are then transplanted into the recipient and direct delivery of genes in vivo.

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Mutant Macaque Factor IX T262A: A Tool for Hemophilia B Gene Therapy Studies in Macaques.

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INTRODUCTION: Gene therapy is expected to be the next generation therapy for hemophilia, and a good animal model is required for hemophilia gene therapy preclinical studies. **METHODS:** Taking advantage of the human factor IX (FIX) specificity of monoclonal antibody 3A6, the epitope of which resides in the amino acid polypeptide segment including Ala 262 of human FIX, mutant macaque FIX with an amino acid substitution of Thr 262 to Ala (macaque FIX T262A) was generated and its reactivity to monoclonal antibody 3A6, biological activity and expression in vivo were studied. **RESULTS:** Enzyme-linked immunosorbent assays (ELISAs) and Western blot analyses showed that monoclonal antibody 3A6 bound to human FIX and macaque FIX T262A but not to wild-type macaque FIX. Recombinant macaque FIX T262A exhibited a comparable coagulation activity to wild-type macaque FIX and human FIX. High expression of macaque FIX T262A was achieved in mice by injection of AAV8 vectors carrying the macaque FIX T262A gene and reached levels of up to 31.5microg/mL (1050% of the normal human FIX concentration). Macaque FIX T262A expressed in the liver of mice was as biologically active as that expressed in vitro. In addition, the macaque FIX T262A concentrations determined by a 3A6-based ELISA were not influenced by the presence of normal macaque plasma. **CONCLUSIONS:** The results of the present study suggest that macaque FIX T262A may be processed appropriately in vivo and that the macaque FIX T262A concentration in the macaque circulation can be quantified precisely by a monoclonal antibody 3A6-based ELISA. Copyright © 2010. Published by Elsevier Ltd.

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miR-22 functions as a micro-oncogene in transformed human bronchial epithelial cells induced by anti-benzo[a]pyrene-7,8-diol-9,10-epoxide.

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MicroRNAs (miRNAs) are small non-coding RNA molecules that negatively control the expression of target genes post-transcriptionally. In this study, transformed human bronchial epithelial cells induced by anti-benzo[a]pyrene-7,8-diol-9,10-epoxide were characterized for miRNA involved in carcinogenesis. We found miR-22, which was highly expressed in transformed cells, concomitant with downregulation of the tumour suppressor gene PTEN protein. Using computer-generated and experimental analysis, PTEN was identified as one of the targets of miR-22. Over-expression and inhibition studies of miRNA showed decreased and increased PTEN protein, respectively, with no alteration of PTEN mRNA levels. These findings suggest that miR-22 regulates PTEN expression through translational repression. A dual-reporter assay confirmed these findings and provided evidence to suggest that miR-22 regulates PTEN expression by binding with a target site in the PTEN 3'-untranslated region. A mutated seed sequence in the PTEN binding site can abrogate the regulatory role of miR-22 on PTEN. Moreover, we found that anti-miR-22 promoted cell apoptosis, decreased colony formation and reduced the motility of malignant cells. Together, the results indicate that miR-22 functions as a micro-oncogene that can invert the functionality of PTEN. Furthermore, the binding site for miR-22 might provide insight into a potential target for gene therapy. Copyright © 2010 Elsevier Ltd. All rights reserved.