



CLINIGENE CURRENT GENE THERAPY WEEKLY

From March 01st to March 08th 2010

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Blocking effects of siRNA on VEGF expression in human colorectal cancer cells.

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AIM: To investigate the expression of vascular endothelial cell growth factor (VEGF) and its receptors Fms-like tyrosine kinase 1 (FLT-1) and fetal liver kinase 1 (FLK-1) in colorectal carcinoma (CRC), and the blocking effects of small interfering RNAs (siRNAs) on VEGF expression in human colorectal cancer HCT116 cells. METHODS: Immunohistochemical staining for VEGF, FLT-1 and FLK-1 proteins was performed in 82 cases of CRC and 14 normal colorectal mucosae. A siRNA targeting VEGF was synthesized and transfected into HCT116 cells using lipofectamine 2000. Immunocytochemical staining and Western blotting analyses were performed to detect the expression of VEGF protein. The suppressive effect of the siRNA on cell proliferation was detected using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertrazolium bromide (MTT) assay. Cellular apoptosis was detected using flow cytometry (FCM). RESULTS: The expression of VEGF, FLT-1 and FLK-1 in tumor tissues was significantly higher than that in normal tissues ($P = 0.008$, $P = 0.000$, $P = 0.000$). The expression of VEGF was positively correlated with both lymph node metastasis and clinical stage ($P = 0.009$ and $P = 0.025$, respectively). Immunocytochemistry showed that the expression of VEGF was weakly positive and Western blotting indicated a significant reduction in VEGF-siRNA cell protein levels. VEGF-siRNA cell growth inhibition was assessed by the MTT assay, and the tumor cell proliferation rate was significantly different at 24, 48, and 72 h after transfection. FCM results showed that the VEGF-siRNA group had an apparent aneuploid peak. CONCLUSION: VEGF, FLT-1 and FLK-1 are associated with colorectal carcinogenesis. siRNA silencing of the VEGF gene suppresses proliferation, and induces apoptosis in HCT116 cells. The results suggest that VEGF may be a new gene therapy target for colorectal cancer.

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Site-specific gene therapy for cardiovascular disease.

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Gene therapy holds considerable promise for the treatment of cardiovascular disease and may provide novel therapeutic solutions for both genetic disorders and acquired pathophysiologies such as arteriosclerosis, heart failure and arrhythmias. Recombinant DNA technology and the sequencing of the human genome have made a plethora of candidate therapeutic genes available for cardiovascular diseases. However, progress in the field of gene therapy for cardiovascular disease has been modest; one of the key reasons for this limited progress is the lack of gene delivery systems for localizing gene therapy to specific sites to optimize transgene expression and efficacy. This review summarizes progress made toward the site-specific delivery of cardiovascular gene therapy and highlights selected promising novel approaches.

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20205051

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HSV-1-derived helper-independent defective vectors, replicating vectors and amplicon vectors, for the treatment of brain diseases.

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HSV-1 is a neurotropic virus that displays several important adaptations to the nervous system of the host organism, each of which can be rationally exploited in the design of gene therapy vectors for neurological applications. Replication-incompetent (replication-defective) helper-independent recombinant vectors are nontoxic tools for gene transfer that preserve most of the neurotropic features of HSV-1, particularly the ability to express genes after establishing latent infections, and are thus proficient candidates for therapeutic gene transfer in neurons. A clinical trial with the use of a replication-incompetent vector, NP-2 (Diamyd Inc), for the treatment of pain has been initiated. Attenuated replication-competent (oncolytic) vectors are becoming suitable and powerful tools to eradicate brain tumors, such as malignant gliomas, as a result of the ability to replicate and spread only within the tumor mass. Some attenuated replication-competent vectors, such as G-207 and HSV-1716 (Crusade Laboratories Ltd), have been used in clinical trials for the treatment of cancers including recurrent malignant glioma. Helper-dependent amplicon vector technology takes advantage of the capacity of the virus particle to accommodate ≤ 150 Kbp of foreign DNA, enabling these vectors to deliver complete genomic loci to the nucleus of mammalian cells, making amplicons particularly useful agents in protocols that require stable and physiological transgene expression. However, difficulties in obtaining large stocks of helper-free amplicons continue to limit the use of these vectors in the clinic.

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20203519

Eur Surg Res. 2010 Mar 5;44(3-4):133-141. [Epub ahead of print]

Induction of Lymphocyte Apoptosis in Rat Liver Allograft by Adenoviral Gene Transfection of Human Interleukin-10.

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Background/Aims: Gene therapy can provide a possible avenue in organ transplantation to treat acute allograft rejection. This study was designed to investigate the effect of adenovirus-mediated human IL-10 (hIL-10) gene transfer on the apoptosis of infiltrating lymphocytes and examine the efficacy of hIL-10 gene transfer in combination with subtherapeutic doses of cyclosporine A (CsA) in a rat liver transplantation model. Methods: Inbred male DA and LEW rats were used for liver donors and recipients, respectively. The rats were divided into saline, Ad-lacZ, CsA, Ad-hIL-10 and Ad-hIL-10 + CsA groups. Graft survival, histopathological, enzyme-linked immunosorbent assay, reverse transcriptase-polymerase chain reaction and flow cytometry were performed in liver specimens obtained from different time points after transplantation in the 5 groups. Results: Ad-hIL-10 pretreatment inhibited allograft rejection, prolonged the survival of hepatic allografts, and downregulated the expression of IFN-gamma and IL-2 mRNA, with simultaneous upregulation of IL-4 mRNA. In addition, Ad-hIL-10 pretreatment upregulated the expression of Fas mRNA in the isolated graft-infiltrating lymphocytes and induced graft-infiltrating lymphocyte apoptosis. A single subtherapeutic dose of CsA acted synergistically with it. Conclusion: hIL-10 gene therapy induced alloreactive lymphocyte apoptosis via Fas/FasL pathway. hIL-10 gene transfection in combination with subtherapeutic doses of CsA facilitates the long-term survival of liver grafts. Copyright © 2010 S. Karger AG, Basel.

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20203170

Hum Mol Genet. 2010 Mar 4. [Epub ahead of print]

WIDESPREAD ENZYMATIC CORRECTION OF CNS TISSUES BY A SINGLE INTRACEREBRAL INJECTION OF THERAPEUTIC LENTIVIRAL VECTOR IN LEUKODYSTROPHY MOUSE MODELS.

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Leukodystrophies are rare diseases caused by defects in the genes coding for lysosomal enzymes that degrade several glycosphingolipids. Gene therapy for leukodystrophies requires efficient distribution of the missing enzymes in CNS tissues to prevent demyelination and neurodegeneration. In this work we targeted the external capsule (EC), a white matter region enriched in neuronal projections, with the aim of obtaining maximal protein distribution from a single injection site. We used bidirectional (bd) lentiviral vectors (LV)(bdLV) to ensure coordinate expression of a therapeutic gene (beta-galactocerebrosidase, GALC; Arylsulphatase A, ARSA) and of a reporter gene, thus monitoring simultaneously transgene distribution and enzyme reconstitution. A single EC injection of bdLV.GALC in early symptomatic Twitcher mice (a murine model of Globoid Cell Leukodystrophy, GLD) resulted in rapid and robust expression of a functional GALC protein in the telencephalon, cerebellum, brainstem and spinal cord. This led to global rescue of enzymatic activity, significant reduction of tissue storage and decrease of activated astroglia and microglia. Widespread protein distribution and complete metabolic correction was also observed after EC injection of bdLV.ARSA in a mouse model of Metachromatic Leukodystrophy (MLD). Our data indicated axonal transport, distribution through cerebrospinal fluid flow and cross-correction as the mechanisms contributing to widespread bioavailability of GALC and ARSA proteins in CNS tissues. LV-mediated gene delivery of lysosomal enzymes by targeting highly interconnected CNS regions is a potentially effective strategy that, combined with a treatments able to target the PNS and peripheral organs, may provide significant therapeutic benefit to patients affected by leukodystrophies.

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20201883

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Cell and gene therapy strategies for the treatment of postmyocardial infarction ventricular arrhythmias.

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Ventricular arrhythmias in the setting of a healed myocardial infarction represent a major cause of morbidity and mortality. The underlying mechanism is the presence of slow conduction tissue within the infarct border zone. In the current review we describe experimental gene and cell therapy approaches targeting the electrophysiologic substrate of the border zone, with the aim of preventing postinfarction ventricular arrhythmias. These include strategies that aim to prevent reentry by improving conduction velocity or by prolonging refractoriness. Attempts to augment conduction velocity include cardiomyocyte transplantation to regenerate the infarct, overexpression of unique sodium channels (to improve excitability), and methods to improve cell-to-cell coupling. Strategies to prolong refractoriness include gene therapy to prolong action potential duration or cell therapy using engineered cell grafts transfected ex vivo to express unique potassium channels. Finally, we will also discuss the potential advantages and drawbacks of these strategies as well as a road map for future clinical use.

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20201784

Curr Cancer Drug Targets. 2010 Mar 4. [Epub ahead of print]

The Biology of the Sodium Iodide Symporter and its Potential for Targeted Gene Delivery.

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The sodium iodide symporter (NIS) is responsible for thyroidal, salivary, gastric, intestinal and mammary iodide uptake. It was first cloned from the rat in 1996 and shortly thereafter from human and mouse tissue. In the intervening years, we have learned a great deal about the biology of NIS. Detailed knowledge of its genomic structure, transcriptional and post-transcriptional regulation and pharmacological modulation has underpinned the selection of NIS as an exciting approach for targeted gene delivery. A number of in vitro and in vivo studies have demonstrated the potential of using NIS gene therapy as a means of delivering highly conformal radiation doses selectively to tumours. This strategy is particularly attractive because it can be used with both diagnostic ((99m)Tc, (125)I,(124I)) and therapeutic ((131)I,(186)Re, (188)Re,(211)At) radioisotopes and it lends itself to incorporation with standard treatment modalities, such as radiotherapy or chemoradiotherapy. In this article, we review the biology of NIS and discuss its development for gene therapy.

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20201737

Expert Opin Drug Deliv. 2010 Mar;7(3):331-9.

Endosomal disruptors in non-viral gene delivery.

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Importance of the field: Non-viral gene delivery for the treatment of genetic and non-genetic diseases has been under investigation for several decades, but there has been very little application in patients because of poor gene expression and toxicity. Areas covered in this review: As gene delivery almost invariably involves endocytosis, many of its limitations are related to compartmentalisation of the transgene within the endosomes. Gene expression enhancers have become an essential part of manipulating endosomal release, as well as protecting transgene from intracellular degradation. However, disruption of the endosomes can also release proteases that have been shown to activate apoptotic pathways. What the reader will gain: An understanding of the role that endosomal release plays in the toxicity of gene delivery vehicles will help identify new approaches to minimise adverse effects while enhancing non-viral gene expression. Take home message: The future of non-viral gene therapy needs to identify new approaches that limit endosome-induced toxicity while enhancing expression so that a pharmacological response can be reliably observed in vivo.

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20201712

Expert Opin Drug Deliv. 2010 Mar 4. [Epub ahead of print]

Electrostatic surface modifications to improve gene delivery.

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Importance of the field: Gene therapy has the potential to treat a wide variety of diseases, including genetic diseases and cancer. Areas covered in this review: This review introduces biomaterials used for gene delivery and then focuses on the use of electrostatic surface modifications to improve gene delivery materials. These modifications have been used to stabilize therapeutics in vivo, add cell-specific targeting ligands, and promote controlled release. Coatings of nanoparticles and microparticles as well as non-particulate surface coatings are covered in this review. Electrostatic principles are crucial for the development of multilayer delivery structures fabricated by the layer-by-layer method. What the reader will gain: The reader will gain knowledge about the composition of biomaterials used for surface modifications and how these coatings and multilayers can be utilized to improve spatial control and efficiency of delivery. Examples are shown for the delivery of nucleic acids, including DNA and siRNA, to in vitro and in vivo systems. Take home message: The versatile and powerful approach of electrostatic coatings and multilayers will lead to the development of enhanced gene therapies.

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20201709

Expert Opin Biol Ther. 2010 Mar 4. [Epub ahead of print]

Enhanced T cell receptor gene therapy for cancer.

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Importance of the field: Adoptive therapy with T cell receptor- (TCR-) redirected T cells has shown efficacy in mouse tumor models and first responses in cancer patients. One prerequisite to elicit effective anti-tumor reactivity is the transfer of high-avidity T cells. Their generation, however, faces several technical difficulties. Target antigens are often expressed at low levels and their recognition requires the use of high-affine receptors. Yet, mainly low-affine TCRs have been isolated from tumor-infiltrating lymphocytes. Furthermore, upon transfer into a T cell the introduced receptor has to compete with the endogenous TCR. Areas covered in this review: This review discusses how the functional avidity of TCR-modified T cells can be enhanced by i) increasing the amount of introduced TCR heterodimers on the cell surface; and ii) generating receptors with high affinity. Risks of TCR gene therapy and possible safety mechanisms are discussed. What the reader will gain: The reader will gain an overview of the technical developments in TCR and T cell engineering. Take home message: Despite technical obstacles many advances have been made in the generation of high-avidity T cells expressing enhanced TCRs. Mouse studies and clinical trials will evaluate the effect of these improvements.

20201626 Hum Gene Ther. 2010 Mar 4. [Epub ahead of print]

A novel, codon-optimised HSVtk(A168H) mutant [TK.007] for suicide gene therapy.

Preuß E, Treschow A, Newrzela S, Brücher D, Weber K, Felldin U, Alici E, Gahrton G, von Laer D, Dilber MS, Fehse B.

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Conditional elimination of infused gene-modified allo-reactive T cells using suicide gene activation has been shown to be an efficient strategy to abrogate severe graft-versus-host disease in the context of adoptive immunotherapy. To overcome shortcomings of the most widely used suicide gene, wild-type (splice-corrected) Herpes simplex virus thymidine kinase ("schHSVtk"), we generated two new variants - the codon-optimised "coHSVtk" and, by introducing an additional mutation (A168H), the novel "TK.007". We transduced human haematopoietic cell lines and primary T cells with retroviral "sort-suicide vectors" encoding combinations of selection markers (tCD34, OuaSelect) with one out of three HSVtk variants. In vitro we observed higher expression levels and sustained long-term expression of TK.007 indicating lower non-specific toxicity. Also, we noted significantly improved kinetics of ganciclovir-mediated killing for TK.007-transduced cells. In an experimental (murine) allogeneic transplantation model, TK.007-transduced T cells mediated severe GvHD which was readily abrogated by application of GCV (10 mg/kg). We finally established a modified allo-transplantation model which allowed comparing the in vivo activities for TK.007 vs. schHSVtk quantitatively. We found that TK.007 mediates both significantly faster and higher absolute killing at low GCV concentrations (10 and 25 mg/kg.). In summary, we demonstrate that the novel TK.007 suicide gene combines better killing performance with reduced unspecific toxicity (as compared to the frequently used splice-corrected wild-type schHSVtk gene) thus representing a promising alternative for suicide gene therapy.

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Progress and prospects: nuclear import of nonviral vectors.

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The nuclear envelope represents a key barrier to successful nonviral transfection and gene therapy both in vitro and in vivo. Although the main purpose of the nuclear envelope is to partition the cell to maintain cytoplasmic components in the cytoplasm and nuclear components, most notably genomic DNA, in the nucleus, this function poses a problem for transfections in which exogenous DNA is delivered into the cytoplasm. After delivery to the cytoplasm, nucleic acids rapidly become complexed with cellular proteins that mediate interactions with the cellular machinery for trafficking. Thus, it is these proteins that, in essence, control the nuclear import of DNA, and we must also understand their activities in cells. In this review, we will discuss the principles of nuclear import of proteins and DNA-protein complexes, as well as the various approaches that investigators have used to improve nuclear targeting of plasmids. These approaches include complexation of plasmids with peptides, native and engineered proteins, ligands and polymers, as well as the inclusion of transcription factor-binding sites for general and cell-specific delivery. Keywords: nonviral gene transfer, plasmid, nuclear pore complex, importin, nuclear localization signal, karyopherin. Gene Therapy advance online publication, 4 March 2010; doi:10.1038/gt.2010.31.

PMID: 20200565 Gene Ther. 2010 Mar 4. [Epub ahead of print]

Adeno-associated virus-mediated delivery of kringle 5 of human plasminogen inhibits orthotopic growth of ovarian cancer.

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Kringle 5 (K5) of human plasminogen is a potent angiogenesis inhibitor. In this study, we investigated the effects of recombinant adeno-associated virus (AAV)-mediated delivery of K5 in mouse models of human ovarian cancer. A single intramuscular injection of AAV-K5 resulted in sustained expression of K5 reaching a maximum serum level of 800 ng ml⁻¹. Gene therapy inhibited both vascular endothelial growth factor (VEGF)-induced and tumor cell-induced angiogenesis in matrigel plug assays. Furthermore, a single injection of AAV-K5 significantly inhibited both subcutaneous and intraperitoneal growth of human ovarian cancer cells. Immunofluorescence studies of residual tumors surgically resected from the treated animals showed reduced tumor burden, which correlated with the inhibition of tumor neovascularization. In addition, AAV-K5 gene therapy differentially affected the nascent vessels more than mature vasculature and induced apoptotic death of tumor cells. These data show that AAV-K5 can be effectively used to inhibit ovarian cancer. Gene Therapy advance online publication, 4 March 2010; doi:10.1038/gt.2010.15.

PMID: 20200564 Gene Ther. 2010 Mar 4. [Epub ahead of print]

A dual promoter lentiviral vector for the in vivo evaluation of gene therapeutic approaches to axon regeneration after spinal cord injury.

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The identification of axon growth-promoting genes, and overexpression of these genes in central nervous system (CNS) neurons projecting to the spinal cord, has emerged as one potential approach to enhancing CNS regeneration. Assessment of the regenerative potential of candidate genes usually requires axonal tracing of spinal projections, ideally limited to neurons that express the candidate gene. Alternatively, coexpression of a reporter gene such as enhanced green fluorescent protein (GFP) from an internal ribosomal entry site can be used to identify neurons expressing the candidate gene, but this strategy does not label corticospinal axons in the spinal cord. We therefore developed a dual promoter lentiviral vector in which a potentially therapeutic transgene is expressed from the cytomegalovirus-enhanced chicken beta-actin promoter and the fluorescent protein copGFP is expressed from the elongation factor-1alpha promoter. The vector was constructed to be compatible with the Gateway recombination system for efficient introduction of transgenes through entry shuttle vectors. We show both simultaneous expression of a candidate and reporter gene in corticospinal and red nucleus neurons, and efficient labeling of their axons after lesions in the cervical spinal cord. This expression system is therefore an accurate and efficient means of screening candidate genes in vivo for enhancement of axonal growth. Gene Therapy advance online publication, 4 March 2010; doi:10.1038/gt.2010.14.

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20200563

Gene Ther. 2010 Mar 4. [Epub ahead of print]

Gene-modified T cells as immunotherapy for multiple myeloma and acute myeloid leukemia expressing the Lewis Y antigen.

Peinert S, Prince HM, Guru PM, Kershaw MH, Smyth MJ, Trapani JA, Gambell P, Harrison S, Scott AM, Smyth FE, Darcy PK, Tainton K, Neeson P, Ritchie DS, Hönemann D.

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We have evaluated the carbohydrate antigen Lewis(Y) (Le(Y)) as a potential target for T-cell immunotherapy of hematological neoplasias. Analysis of 81 primary bone marrow samples revealed moderate Le(Y) expression on plasma cells of myeloma patients and myeloblasts of patients with acute myeloid leukemia (AML) (52 and 46% of cases, respectively). We developed a retroviral vector construct encoding a chimeric T-cell receptor that recognizes the Le(Y) antigen in a major histocompatibility complex-independent manner and delivers co-stimulatory signals to achieve T-cell activation. We have shown efficient transduction of peripheral blood-derived T cells with this construct, resulting in antigen-restricted interferon-gamma secretion and cell lysis of Le(Y)-expressing tumor cells. In vivo activity of gene-modified T cells was demonstrated in the delayed growth of myeloma xenografts in NOD/SCID mice, which prolonged survival. Therefore, targeting Le(Y)-positive malignant cells with T cells expressing a chimeric receptor recognizing Le(Y) was effective both in vitro and in a myeloma mouse model. Consequently, we plan to use T cells manufactured under Good Manufacturing Practice conditions in a phase I immunotherapy study for patients with Le(Y)-positive myeloma or AML. Gene Therapy advance online publication, 4 March 2010; doi:10.1038/gt.2010.21.

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20200562

Gene Ther. 2010 Mar 4. [Epub ahead of print]

Cutaneous vaccination using microneedles coated with hepatitis C DNA vaccine.

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The skin is potentially an excellent organ for vaccine delivery because of accessibility and the presence of immune cells. However, no simple and inexpensive cutaneous vaccination method is available. Micron-scale needles coated with DNA were tested as a simple, inexpensive device for skin delivery. Vaccination with a plasmid encoding hepatitis C virus nonstructural 3/4A protein using microneedles effectively primed specific cytotoxic T lymphocytes (CTLs). Importantly, the minimally invasive microneedles were as efficient in priming CTLs as more complicated or invasive delivery techniques, such as gene gun and hypodermic needles. Thus, microneedles may offer a promising technology for DNA vaccination. Gene Therapy advance online publication, 4 March 2010; doi:10.1038/gt.2010.22.

Selective suicide gene therapy of colon cancer exploiting the urokinase plasminogen activator receptor promoter.

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Colon cancer is the third and fourth most prevalent cancer among Iranian men and women, respectively. Suicide gene therapy is one of the alternative therapeutic modalities for cancer. The application of specific promoters for therapeutic genes should decrease the adverse effects of this modality. The combined aims of this study were to design a specific suicide gene therapy construct for colon cancer and study its effect in distinct representatives of transformed and nontransformed cells. The KRAS oncogene signaling pathway is one of the most important signaling pathways activated in colon cancer; therefore, we inserted the urokinase plasminogen activator receptor (uPAR; PLAUR gene) promoter as one of the upregulated promoters by this pathway upstream of a suicide gene (thymidine kinase [TK]) and a reporter gene (beta-galactosidase, beta-gal [LacZ]). This promoter is a natural combination of different motifs responsive to the RAS signaling pathway, such as the transcription factors AP1 (FOS/JUN), SP1, SP3, and AP2alpha, and nuclear factor kappa B (NFkappaB). The reporter plasmid under the control of the uPAR promoter (PUCUPARLacZ) had the ability to express beta-gal in colon cancer cells (human colon adenocarcinoma [SW480] and human colorectal carcinoma [HCT116] cell lines), while it could not express beta-gal in nontransformed human umbilical vein endothelial cells (HUVEC) and normal colon cells. After confirming the ability of pUCUPARTK (suicide plasmid) to express TK in SW480 and HCT116 cells by real-time PCR, cytotoxicity assays showed that pUCUPARTK decreased the viability of these cells in the presence of ganciclovir 20 and 40 mug/mL (and higher), respectively. Although M30 CytoDEATH antibody could not detect a significant rate of apoptosis induced by ganciclovir in pUCUPARTK-transfected HCT116 cells, the percentage of stained cells was marked in comparison with untreated cells. While this antibody could detect apoptosis in HCT116 cell line transfected with positive control plasmid, it could not detect apoptosis in SW480 cells transfected with the same positive control. This discrepancy could be attributed to the different mechanisms of TK/ganciclovir-induced apoptosis in tumor protein p53 (TP53)-expressing (HCT116) and -deficient (SW480) cells. Annexin-propidium iodide staining could detect apoptosis in treated, pUCUPARTK-transfected SW480 and HCT116 cells. This study showed that the uPAR promoter can be considered as a suitable candidate for specific suicide gene therapy of colon cancer and probably other cancers in which the RAS signaling pathway is involved in their carcinogenesis process.

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20198695

J Biomed Mater Res A. 2010 Mar 2. [Epub ahead of print]

Cationic copolymers nanoparticles for nonviral gene vectors: Synthesis, characterization, and application in gene delivery.

d'Ayala GG, Calarco A, Malinconico M, Laurienzo P, Petillo O, Torpedine A, Peluso G.
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The major aim of nonviral delivery systems for gene therapy is to mediate high levels of gene expression with low toxicity. Nowadays, one of the most successful synthetic polycations used in gene delivery research is poly(ethylenimine) (PEI) in its high-molecular weight (HMW) branched form. However, PEI is not the ideal transfection agent in vivo because of its overwhelming cytotoxicity. To overcome its toxic effects with a minimal impact on transfection efficiency, PEI has been conjugated with several nonionic biocompatible polymers. Here, we describe the synthesis of nanosized particles consisting of HMW PEI (25 kDa) crosslinked with poly(epsilon-caprolactone) (PCL, 50-60 kDa), a biodegradable aliphatic polyester. PCL was modified by the insertion of glycidyl groups able to condense with the amines of PEI to chemically bind PEI onto PCL. The nanoparticles obtained have been characterized in relation to their physicochemical and biological properties, and the results are extremely promising in terms of low cell toxicity and high transfection efficiency. These biological effects might be related to the peculiar DNA binding to covalently connected polymeric nanoparticles, without the formation of entangled DNA/polymer-soluble aggregates. (c) 2010 Wiley Periodicals, Inc. J Biomed Mater Res Part A, 2010.

PMID:
20198635

Hepatology. 2009 Oct 27;51(3):912-921. [Epub ahead of print]

Insulin-like growth factor I gene transfer to cirrhotic liver induces fibrolysis and reduces fibrogenesis leading to cirrhosis reversion in rats.

Sobrevals L, Rodriguez C, Romero-Trevejo JL, Gondi G, Monreal I, Pañeda A, Juanarena N, Arcelus S, Razquin N, Guembe L, González-Asequinolaza G, Prieto J, Fortes P.

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We investigated whether gene transfer of insulin-like growth factor I (IGF-I) to the hepatic tissue was able to improve liver histology and function in established liver cirrhosis. Rats with liver cirrhosis induced by carbon tetrachloride (CCl₄) given orally for 8 weeks were injected through the hepatic artery with saline or with Simian virus 40 vectors encoding IGF-I (SVIGF-I), or luciferase (SVLuc). Animals were sacrificed 8 weeks after vector injection. In cirrhotic rats we observed that, whereas IGF-I was synthesized by hepatocytes, IGF-I receptor was predominantly expressed by nonparenchymal cells, mainly in fibrous septa surrounding hepatic nodules. Rats treated with SVIGF-I showed increased hepatic levels of IGF-I, improved liver function tests, and reduced fibrosis in association with diminished alpha-smooth muscle actin expression, up-regulation of matrix metalloproteases (MMPs) and decreased expression of the tissue inhibitors of MMPs TIM-1 and TIM-2. SVIGF-I therapy induced down-regulation of the profibrogenic molecules transforming growth factor beta (TGFbeta), amphiregulin, platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), and vascular endothelium growth factor (VEGF) and induction of the antifibrogenic and cytoprotective hepatocyte growth factor (HGF). Furthermore, SVIGF-I-treated animals showed decreased expression of Wilms tumor-1 (WT-1; a nuclear factor involved in hepatocyte dedifferentiation) and up-regulation of hepatocyte nuclear factor 4 alpha (HNF4alpha) (which stimulates hepatocellular differentiation). The therapeutic potential of SVIGF-I was also tested in rats with thioacetamide-induced liver cirrhosis. Also in this model, SVIGF-I improved liver function and reduced liver fibrosis in association with up-regulation of HGF and MMPs and down-regulation of tissue inhibitor of metalloproteinase 1 (TIMP-1). Conclusion: IGF-I gene transfer to cirrhotic livers induces MMPs and hepatoprotective factors leading to reversion of fibrosis and improvement of liver function. IGF-I gene therapy may be a useful alternative therapy for patients with advanced cirrhosis without timely access to liver transplantation. (HEPATOLOGY 2010;51:912-921.).

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20198335

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A new system for regulated functional gene expression for gene therapy applications: Nuclear delivery of a p16INK4A-estrogen receptor carboxy terminal fusion protein only in the presence of estrogen.

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The clinical use of gene therapy requires tight regulation of the gene of interest and functional expression only when it is needed. Thus, it is necessary to develop ways of regulating functional gene expression with exogenous stimuli. Many regulatable systems are currently under development. For example, the tetracycline-dependent transcriptional switch has been successfully employed for in vivo preclinical applications. However, there are no examples of regulatable systems that have been employed in human clinical trials. In the present study, we established an adenovirus-delivered functional gene expression system that is regulated by estrogen. This system uses p16INK4A fused at its C-terminus to the ligand-binding domain of the estrogen receptor (DeltaERalpha). We were able to establish cell lines expressing this gene wherein the functional expression of p16INK4A is estrogen-dependent and causes the arrest of several ovarian cancer cell lines. This inducible and adenovirus-mediated gene transfer system may allow gene therapy using nuclear functioning genes in postmenopausal or ovariectomized women.

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20198323

Int J Oncol. 2010 Apr;36(4):809-16.

Radiation-inducible silencing of uPA and uPAR in vitro and in vivo in meningioma.

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Stereospecific radiation treatment offers a distinct opportunity for temporal and spatial regulation of gene expression at tumor sites by means of inducible promoters. To this end, a plasmid, pCARG-U2, was constructed by incorporating nine CARG elements (in tandem) of EGR1 gene upstream to uPA and uPAR siRNA oligonucleotides in a pCi-neo vector. Radiation-induced siRNA expression was detected in a meningioma cell line (IOMM-Lee). Immunoblotting and RT-PCR analyses confirmed downregulation of uPA and uPAR. A similar effect was observed in transfected cells followed by H₂O₂ treatment. Moreover, pre-treatment of transfected cells with N-acetyl L-cysteine blocked the silencing of uPA and uPAR, which further confirmed the oxidative damage-mediated downregulation. Cell proliferation assays and Western blot analysis for apoptotic molecules confirmed cell death in a radiation-inducible fashion. Migration and matrigel invasion assays also revealed a marked decrease in migration and invasion. Immunocytochemistry showed a marked decrease in uPA and uPAR levels in transfected and irradiated cells. H&E staining revealed a decrease in the pre-established tumor volume among the animals treated with pCARG-U2 and radiation. Immunohistochemistry of the brain sections established with intracranial tumors also revealed a marked decrease in uPA and uPAR in a radiation-inducible fashion. Taken together, our data suggest pCARG-U2 as a suitable candidate for radiation-inducible gene therapy.

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20197146

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Anti-tumor immune response correlates with neurological symptoms in a dog with spontaneous astrocytoma treated by gene and vaccine therapy.

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Gene therapy and vaccination have been tested in malignant glioma patients with modest, albeit encouraging results. The combination of these therapies has demonstrated synergistic efficacy in murine models but has not been reported in large animals. Gemistocytic astrocytoma (GemA) is a low-grade glioma that typically progresses to lethal malignancy despite conventional therapies. Until now there has been no useful animal model of GemA. Here we report the treatment of a dog with spontaneous GemA using the combination of surgery, intracavitary adenoviral interferon gamma (IFN γ) gene transfer, and vaccination with glioma cell lysates mixed with CpG oligodeoxynucleotides. Surgical tumor debulking and delivery of Ad-IFN γ into the resection cavity were performed. Autologous tumor cells grew slowly in culture, necessitating vaccination with allogeneic tumor lysate in four of the five vaccinations. Transient left-sided blindness and hemiparesis occurred following the fourth and fifth vaccinations. These neurological symptoms correlated with a peak in the levels of tumor-reactive IgG and CD8(+) T cells measured in the blood. All symptoms resolved and this dog remains tumor-free over 450 days following surgery. This case report preliminarily demonstrates the feasibility of treating dogs with spontaneous glioma using immune-based therapy and warrants further study using this therapeutic approach. Copyright © 2010. Published by Elsevier Ltd.

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20196736

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Gene Therapy to Improve High-Density Lipoprotein Metabolism and Function.

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Plasma levels of high-density lipoprotein (HDL) cholesterol and its major apolipoprotein (apo), apo A-I, are inversely correlated with the incidence of ischemic cardiovascular diseases. Till now, evaluation of the hypothesis that elevation of HDL cholesterol reduces atherosclerotic burden and/or decreases ischemic cardiovascular events in humans has been hampered by the lack of drugs that selectively increase HDL cholesterol. In contrast to the lack of clinical data, evidence for a direct causal role of HDL in modulating atherogenesis in experimental models has been provided by investigations in human apo A-I transgenic mice and rabbits. The development of gene transfer technologies with a sufficiently high therapeutic index may pave the road for a selective and effective HDL raising therapeutic intervention. The goal of a therapeutic strategy that modulates HDL metabolism is not an increase of HDL cholesterol as such, but an enhancement of HDL function. The value of HDL cholesterol as a surrogate endpoint to predict reduced atherosclerosis or a decrease in clinical events may be highly dependent on the mechanism leading to an increased level of HDL cholesterol. In the case of gene transfer, this implies that beneficial effects of increasing HDL cholesterol will be dependent on the transgene that is expressed. Here, we critically review HDL metabolism and HDL function in relation to the development of HDL raising gene transfer, advances and drawbacks of different gene transfer technologies, and experimental gene transfer studies evaluating the effect of raised HDL on histological and functional outcomes in animal models.

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Cytotherapy. 2010 Apr;12(2):226-37.

Gene therapy in hemiparkinsonian rhesus monkeys: long-term survival and behavioral recovery by transplantation of autologous human tyrosine hydroxylase-expressing neural stem cells.

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Abstract Background aims. Neural stem cells (NSC) derived from bone marrow stromal cells (BMSC) (BMSC-D-NSC) are remarkably versatile in response to environmental signals, which render them useful in the search for neurodegenerative disease treatments. **Methods.** We isolated NSC from rhesus monkey bone marrow (BM), transfected them with the human tyrosine hydroxylase (hTH) gene, and transplanted them into 1-methyl-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned hemiparkinsonian rhesus monkeys to determine changes in neural transmitter production and alterations in behavior. **Results.** hTH-expressing cells produced monoamine agents in vitro, such as noradrenalin and dopamine. After cell transplantation in the caudate nucleus and substantia nigra of the experimental monkeys, their disease symptoms and dysfunctional glucose metabolism and dopamine transport were ameliorated. **Conclusions.** hTH-expressing BMSC-D-NSC survived in transplantation sites and assumed normal dopaminergic neuronal properties, playing an instrumental role in functional restoration.

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Mol Ther. 2010 Mar;18(3):456-9.

Immunology and gene therapy: shoulder to shoulder into the fray.

Smerdou C, Ochoa C, Quetglas JI, Fontanellas A, Gonzalez-Aseguinolaza G, Vile RG, Melero I.

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No abstract available

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Circulation. 2010 Mar 1. [Epub ahead of print]

Thioredoxin-1 Gene Therapy Enhances Angiogenic Signaling and Reduces Ventricular Remodeling in Infarcted Myocardium of Diabetic Rats.

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BACKGROUND: -The present study evaluated the reversal of diabetes-mediated impairment of angiogenesis in a myocardial infarction model of type 1 diabetic rats by intramyocardial administration of an adenoviral vector encoding thioredoxin-1 (Ad.Trx1). Various studies have linked diabetes-mediated impairment of angiogenesis to dysfunctional antioxidant systems in which thioredoxin-1 plays a central role. **Methods and Results-**Ad.Trx1 was administered intramyocardially in nondiabetic and diabetic rats immediately after myocardial infarction. Ad.LacZ was similarly administered to the respective control groups. The hearts were excised for molecular and immunohistochemical analysis at predetermined time points. Myocardial function was measured by echocardiography 30 days after the intervention. The Ad.Trx1-administered group exhibited reduced fibrosis, oxidative stress, and cardiomyocyte and endothelial cell apoptosis compared with the diabetic myocardial infarction group, along with increased capillary and arteriolar density. Western blot and immunohistochemical analysis demonstrated myocardial overexpression of thioredoxin-1, heme oxygenase-1, vascular endothelial growth factor, and p38 mitogen-activated protein kinase-beta, as well as decreased phosphorylated JNK and p38 mitogen-activated protein kinase-alpha, in the Ad.Trx1-treated diabetic group. Conversely, we observed a significant reduction in the expression of vascular endothelial growth factor in nondiabetic and diabetic animals treated with tin protoporphyrin (SnPP, a heme oxygenase-1 enzyme inhibitor), even after Ad.Trx1 therapy. Echocardiographic analysis after 4 weeks of myocardial infarction revealed significant improvement in myocardial functional parameters such as ejection fraction, fractional shortening, and E/A ratio in the Ad.Trx1-administered group compared with the diabetic myocardial infarction group. **Conclusions-**This study demonstrates for the first time that impairment of angiogenesis and myocardial dysfunction can be regulated by Ad.Trx1 gene therapy in streptozotocin-induced diabetic rats subjected to infarction.

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20193746

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Carbon nanotubes in cancer diagnosis and therapy.

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During the past years, great progress has been made in the field of nanomaterials given their great potential in biomedical applications. Carbon nanotubes (CNTs), due to their unique physicochemical properties, have become a popular tool in cancer diagnosis and therapy. They are considered one of the most promising nanomaterials with the capability of both detecting the cancerous cells and delivering drugs or small therapeutic molecules to these cells. Over the last several years, CNTs have been explored in almost every single cancer treatment modality, including drug delivery, lymphatic targeted chemotherapy, thermal therapy, photodynamic therapy, and gene therapy. In this review, we will show how they have been introduced into the diagnosis and treatment of cancer. Novel SWNT-based tumor-targeted drug delivery systems (DDS) will be highlighted. Furthermore, the in vitro and in vivo toxicity of CNTs reported in recent years will be summarized. Copyright © 2010. Published by Elsevier B.V.

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Two decades of clinical gene therapy--success is finally mounting.

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Human gene therapy has made substantial progress since the initiation of the first clinical trials 20 years ago. Here, we summarized important applications of gene transfer protocols in the treatment of various human diseases using different viral vectors. Recent successful trials on the treatment of ocular diseases and inherited immune deficiencies are particularly encouraging and have raised hopes that human gene therapy as a standard treatment option will finally become a reality. While immune responses and insertional mutagenesis pose obstacles for this novel form of molecular medicine, continuous progress suggests that a wider range of diseases can be treated with gene therapy in the future.

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20190814

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Human mesenchymal stem cells overexpressing pigment epithelium-derived factor inhibit hepatocellular carcinoma in nude mice.

Gao Y, Yao A, Zhang W, Lu S, Yu Y, Deng L, Yin A, Xia Y, Sun B, Wang X.

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The poor outcome of cancer gene therapy in clinical trials relates in part to insufficient gene delivery to tumor sites. Mesenchymal stem cells (MSCs) represent a new tool for the delivery of therapeutic agents to tumor cells. This study used an orthotopic nude mice model of hepatocellular carcinoma (HCC) to evaluate the potential of genetically modified human MSCs (hMSCs), to function as an effective delivery vehicle for therapeutic genes. hMSCs derived from the bone marrow were efficiently engineered to express human pigment epithelium-derived factor (PEDF) by lentiviral transduction, then tested in vitro for high-level expression and bioactivity of the transgenic protein. The preferential homing of hMSCs toward HCC was confirmed by in vitro and in vivo migration assays. In vivo efficacy experiments showed that intravenous (i.v.) injection of PEDF-expressing hMSCs significantly suppressed both the growth of primary liver tumors and the development of pulmonary metastases. Moreover, hMSCs-based PEDF gene delivery moderately increased the systemic levels of human PEDF. Immunohistochemistry of primary liver tumors demonstrated lower microvessel density in mice treated with hMSCs-PEDF than in control mice. This is the first study to show the potential of hMSCs as an effective delivery vehicle for therapeutic genes in the treatment of HCC. Oncogene advance online publication, 1 March 2010; doi:10.1038/onc.2010.38.

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20190786

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Challenges with advanced therapy medicinal products and how to meet them.

Committee for Advanced Therapies (CAT); CAT Scientific Secretariat, Schneider CK, Salmikangas P, Jilma B, Flamion B, Todorova LR, Paphitou A, Haunerova I, Maimets T, Trouvin JH, Flory E, Tsiftoglou A, Sarkadi B, Gudmundsson K, O'Donovan M, Migliaccio G, Ancāns J, Maciulaitis R, Robert JL, Samuel A, Ovelgönne JH, Hystad M, Fal AM, Lima BS, Moraru AS, Turcáni P, Zorec R, Ruiz S, Akerblom L, Narayanan G, Kent A, Bignami F, Dickson JG, Niederwieser D, Figuerola-Santos MA, Reischl IG, Beuneu C, Georgiev R, Vassiliou M, Pychova A, Clausen M, Methuen T, Lucas S, Schüssler-Lenz M, Kokkas V, Buzás Z, MacAleenan N, Galli MC, Linē A, Gulbinovic J, Berchem G, Fraczek M, Menezes-Ferreira M, Vilceanu N, Hrubisko M, Marinko P, Timón M, Cheng W, Crosbie GA, Meade N, di Paola ML, VandenDriessche T, Ljungman P, D'Apote L, Oliver-Diaz O, Büttel I, Celis P.

Advanced therapy medicinal products (ATMPs), which include gene therapy medicinal products, somatic cell therapy medicinal products and tissue-engineered products, are at the cutting edge of innovation and offer a major hope for various diseases for which there are limited or no therapeutic options. They have therefore been subject to considerable interest and debate. Following the European regulation on ATMPs, a consolidated regulatory framework for these innovative medicines has recently been established. Central to this framework is the Committee for Advanced Therapies (CAT) at the European Medicines Agency (EMA), comprising a multidisciplinary scientific expert committee, representing all EU member states and European Free Trade Association countries, as well as patient and medical associations. In this article, the CAT discusses some of the typical issues raised by developers of ATMPs, and highlights the opportunities for such companies and research groups to approach the EMA and the CAT as a regulatory advisor during development.

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20190738

Nat Biotechnol. 2010 Mar;28(3):271-4. Epub 2010 Feb 28.

Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN.

Foust KD, Wang X, McGovern VL, Braun L, Bevan AK, Haidet AM, Le TT, Morales PR, Rich MM, Burghes AH, Kaspar BK.
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Spinal muscular atrophy (SMA), the most common autosomal recessive neurodegenerative disease affecting children, results in impaired motor neuron function. Despite knowledge of the pathogenic role of decreased survival motor neuron (SMN) protein levels, efforts to increase SMN have not resulted in a treatment for patients. We recently demonstrated that self-complementary adeno-associated virus 9 (scAAV9) can infect approximately 60% of motor neurons when injected intravenously into neonatal mice. Here we use scAAV9-mediated postnatal day 1 vascular gene delivery to replace SMN in SMA pups and rescue motor function, neuromuscular physiology and life span. Treatment on postnatal day 5 results in partial correction, whereas postnatal day 10 treatment has little effect, suggesting a developmental period in which scAAV9 therapy has maximal benefit. Notably, we also show extensive scAAV9-mediated motor neuron transduction after injection into a newborn cynomolgus macaque. This demonstration that scAAV9 traverses the blood-brain barrier in a nonhuman primate emphasizes the clinical potential of scAAV9 gene therapy for SMA.

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20188764

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Optimization of shRNA inhibitors by variation of the terminal loop sequence.

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Gene silencing by RNA interference (RNAi) can be achieved by intracellular expression of a short hairpin RNA (shRNA) that is processed into the effective small interfering RNA (siRNA) inhibitor by the RNAi machinery. Previous studies indicate that shRNA molecules do not always reflect the activity of corresponding synthetic siRNAs that attack the same target sequence. One obvious difference between these two effector molecules is the hairpin loop of the shRNA. Most studies use the original shRNA design of the pSuper system, but no extensive study regarding optimization of the shRNA loop sequence has been performed. We tested the impact of different hairpin loop sequences, varying in size and structure, on the activity of a set of shRNAs targeting HIV-1. We were able to transform weak inhibitors into intermediate or even strong shRNA inhibitors by replacing the loop sequence. We demonstrate that the efficacy of these optimized shRNA inhibitors is improved significantly in different cell types due to increased siRNA production. These results indicate that the loop sequence is an essential part of the shRNA design. The optimized shRNA loop sequence is generally applicable for RNAi knockdown studies, and will allow us to develop a more potent gene therapy against HIV-1. Copyright © 2010. Published by Elsevier B.V.

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20188261

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Preparation of pharmaceutical-grade plasmid DNA using methacrylate monolithic columns.

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Plasmid DNA (pDNA) used in vaccination and gene therapy has to be highly pure and homogenous, which point out necessity to develop efficient, reproducible and scalable downstream process. Convective Interaction Media (CIM) monolithic chromatographic supports being designed for purification of large molecules and nanoparticles seem to be a matrix of choice for pDNA purification. In present work we describe a pDNA purification process designed on two different CIM monolithic columns, based on anion-exchange (AEX) chromatography and hydrophobic interaction chromatography (HIC) chemistry. HIC monolith enabled separation of supercoiled (sc) pDNA from open circular (oc) pDNA, genomic DNA (gDNA) and endotoxins regardless to flow rates in the range at least up to 380cm/h. Dynamic binding capacity of new HIC monolith is up to 4mg of pDNA per milliliter of support. Combination of both chromatographic steps using optimized CaCl₂ precipitation enabled production of pure pDNA, satisfying all regulatory requirements. Process was found to be reproducible, scalable, and exhibits high productivity. In addition, in-line monitoring of pDNA purification process is shown, using CIM DEAE disk monolithic columns. Copyright 2009 Elsevier Ltd. All rights reserved.

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20187803

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Selective suicide gene therapy of colon cancer cell lines exploiting fibroblast growth factor 18 promoter.

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Fibroblast growth factor 18 (FGF18) is one of the genes downstream of Wnt, one of the most important signaling pathways activated in colon cancer. An FGF18 promoter containing a single T-cell factor/lymphocyte enhancing factor 1 (TCF/LEF1) binding site was inserted upstream of a thymidine kinase (TK) suicide gene module, while a bacterial beta-Gal (LacZ) element served as the reporter gene. Following transient transfection with pUCFGF18LacZ, beta-Gal staining showed that 5% of SW480, 10% of HCT116, 0% of human umbilical vein endothelial cells (HUVECs) and 0% of normal colon cells (NCCs) had expressed LacZ. beta-Gal enzyme-linked immunosorbent assay revealed that the ratio of pUCFGF18LacZ activity to that of positive control was 0.09 and 0.25 in SW480 and HCT116, respectively (significantly higher than mock plasmid), while there were no significant changes in the beta-Gal expression in HUVEC and NCC cells transfected with pUCFGF18LacZ or mock plasmid. Following transfection with pUCFGF18TK and pUCCMVTk (positive control), cytotoxicity analysis of transfected cells showed that treatment with ganciclovir (GCV) significantly decreased SW480 and HCT116 cell survival at GCV concentrations above 20 microg/mL. An inverse correlation between GCV concentration and cell viability was evident in both colon cancer cell lines following transfection with these suicide plasmids. pUCFGF18TK and pUCCMVTk induced apoptosis after the administration of GCV in HCT116, but not in SW480, as demonstrated by M30 cytodeath antibody. This discrepancy may stem from differences in the mechanisms of TK/GCV-induced apoptosis in p53-proficient (HCT116) and -deficient (SW480) cells. The specific activity of the FGF18 promoter in HCT116 and SW480 may reflect the advantage of this promoter over artificial promoters containing artificial TCF/LEF binding sites.

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20187794

Cancer Biother Radiopharm. 2010 Feb;25(1):29-38.

The in vitro and in vivo antitumor activity of adenovirus-mediated interleukin-24 expression for laryngocarcinoma.

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Interleukin-24 (IL-24)/melanoma differentiation associated gene-7 (mda-7) as a novel tumor-suppressor gene has potent antitumor activities in a broad spectrum of human cancers through the activation of various signaling pathways. However, the suppressive effect of adenovirus-mediated IL-24 (Ad-IL-24) expression on human laryngeal cancers is still elusive. In this study, we explored the therapeutic effect of Ad-IL-24 on human laryngeal cancers in vitro and in vivo in an athymic nude mouse model, using a Hep-2 human laryngocarcinoma cell line, and a WI-38 human diploid cell line served as a normal cell control. We demonstrated that Ad-IL-24 induced significant growth inhibition and apoptosis, upregulated the expression of P21, P27, and Bax, downregulated Bcl-2 expression, and activated caspase-3 in Hep-2 laryngeal tumor cells, while it exerted no direct effect on the in vitro proliferation of WI-38 normal diploid cells. Moreover, intratumoral injections of Ad-IL-24 in nude mice bearing Hep-2 tumors significantly suppressed the laryngeal xenografted tumor growth and reduced microvessel density (MVD) and VEGF expression in tumors. This retarded tumor growth in vitro and in vivo elicited by Ad-IL-24 was closely associated with the upregulation of proliferation-related molecules P21 and P27, decrease in the ratio of anti- to proapoptotic molecules Bcl-2/Bax, followed by the activation of caspase-3, leading to apoptosis via intrinsic apoptotic pathways, and the reduced expression of proangiogenic factor VEGF involved in the inhibition of tumor angiogenesis. Thus, our results indicate that the potent, selective killing activity of Ad-IL-24 in laryngeal cancer cells, but not in normal cells, makes this vector a potential candidate for laryngeal cancer gene therapy.