



CLINIGENE CURRENT GENE THERAPY WEEKLY

From February 8th to February 15th 2010

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PMID:
20151201

J Inherit Metab Dis. 2010 Feb 12. [Epub ahead of print]

Long-term correction of murine phenylketonuria by viral gene transfer: liver versus muscle.

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Current therapy for phenylketonuria (PKU) consists of life-long dietary restriction of phenylalanine (Phe), which presents problems of adherence for patients. Alternative therapies under investigation include, among others, the use of gene therapy to provide copies of wild-type, non-mutant, phenylalanine hydroxylase (PAH) enzyme. Expression of PAH in both liver (the usual metabolic source of this enzyme) and skeletal muscle is under investigation. Liver gene therapy, using a viral vector based on the adeno-associated viruses (AAVs), provided effective clearance of serum Phe that was sustained for 1 year in some mice. In order for PAH expression to be effective in skeletal muscle, the essential metabolic cofactor, tetrahydrobiopterin (BH(4)), must also be provided, either by supplementation or gene therapy. Both these approaches were effective. When transgenic PKU mice that constitutively expressed PAH in muscle were given intraperitoneal supplementation with BH(4), this produced (transient) effective clearance of Phe to normal levels. In addition, use of an AAV vector containing the genes for PAH, and for two key synthetic enzymes for BH(4), provided substantial and long-lasting correction (more than 1 year) of blood Phe levels when injected into skeletal muscle of PKU mice. These two strategies provide promising treatment alternatives for the management of PKU in patients.

PMID:
20150932

Cancer Gene Ther. 2010 Feb 12. [Epub ahead of print]

Enhanced delivery of mda-7/IL-24 using a serotype chimeric adenovirus (Ad.5/3) improves therapeutic efficacy in low CAR prostate cancer cells.

Dash R, Dmitriev I, Su ZZ, Bhutia SK, Azab B, Vozhilla N, Yacoub A, Dent P, Curiel DT, Sarkar D, Fisher PB.

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Gene therapy is being examined as a potential strategy for treating prostate cancer. Serotype 5 adenovirus (Ad.5) is routinely used as a vector for transgene delivery. However, the infectivity of Ad.5 is dependent on Coxsackie-adenovirus receptors (CARs); many tumor types show a reduction in this receptor in vivo, thereby limiting therapeutic gene transduction. Serotype chimerism is one approach to circumvent CAR deficiency; this strategy is used to generate an Ad.5/3-recombinant Ad that infects cancer cells through Ad.3 receptors in a CAR-independent manner. In this report, the enhanced transgene delivery and efficacy of Ad.5/3-recombinant virus was evaluated using an effective wide-spectrum anticancer therapeutic melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL-24). Our data show that in low CAR human prostate cancer cells (PC-3), a recombinant Ad.5/3 virus delivering mda-7/IL-24 (Ad.5/3-mda-7) is more efficacious than an Ad.5 virus encoding mda-7/IL-24 (Ad.5-mda-7) in infecting tumor cells, expressing MDA-7/IL-24 protein, inducing cancer-specific apoptosis, inhibiting in vivo tumor growth and exerting an antitumor 'bystander' effect in a nude mouse xenograft model. Considering the fact that Ad.5-mda-7 has shown significant objective responses in a phase I clinical trial for solid tumors, Ad.5/3-mda-7 is predicted to exert enhanced therapeutic benefit in patients with prostate cancer.

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20150931

Cancer Gene Ther. 2010 Feb 12. [Epub ahead of print]

Bleomycin/interleukin-12 electrochemogene therapy for treating naturally occurring spontaneous neoplasms in dogs.

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On the basis of superior outcomes from electrochemogene therapy (ECGT) compared with electrochemotherapy in mice, we determined the efficacy of ECGT applied to spontaneous canine neoplasms. Intralesional bleomycin and feline interleukin-12 DNA (fIL-12 DNA) injection combined with translesional electroporation resulted in complete cure of two recurrent World Health Organization stage T(2b)N(0)M(0) oral squamous cell carcinomas (SCCs) and one T(2)N(0)M(0) acanthomatous ameloblastoma. Three remaining dogs, which had no other treatment options, had partial responses to ECGT; one had mandibular T(3b)N(2b)M(1) melanoma with pulmonary and lymph node metastases; one had cubital T(3)N(0)M(1) histiocytic sarcoma with spleen metastases; and one had soft palate T(3)N(0)M(0) fibrosarcoma. The melanoma dog had decrease in size of the primary tumor before recrudescence and euthanasia. The histiocytic sarcoma dog had resolution of the primary tumor, but was euthanized because of metastases 4 months after the only treatment. The dog with T(3)N(0)M(0) fibrosarcoma had tumor regression with recrudescence. Treatment was associated with minimal side effects and was easy to perform. It was associated with repair of bone lysis in cured dogs, it improved quality of life of dogs with partial responses and extended overall survival time. ECGT seems to be a safe and resulted in complete responses in SCC and acanthomatous ameloblastoma.

PMID:
20150930

Cancer Gene Ther. 2010 Feb 12. [Epub ahead of print]

Cytokine-armed vaccinia virus infects the mesothelioma tumor microenvironment to overcome immune tolerance and mediate tumor resolution.

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Intratumoral (i.t.) administration of cytokine genes expressed by viral vectors represents a rational approach that induces cytokine secretion at the site they are needed, and i.t. vaccinia virus (VV) has shown promise in mesothelioma patients. However, we and others have shown that the mesothelioma tumor microenvironment includes macrophages, dendritic cells (DCs), and T cells. Therefore, we investigated which of these cell types are infected after exposure to VV or Fowlpox virus (FPV)-cytokine gene constructs. In vitro studies showed that mesothelioma tumor cells were resistant to FPV infection yet highly permissive to infection by VV vectors resulting in significant cytokine production and impaired proliferation. Macrophages secreted low levels of cytokine suggestive of resistance to overt infection. DCs transiently secreted virally derived cytokines, but did not mature during VV infection. VV inhibition of T cell proliferation was rescued by the interleukin (IL)-2 and IL-12 VV constructs. In vivo studies of murine mesotheliomas showed that i.t. injection of the parent VV could not hinder tumor progression. In contrast, the VV-cytokine constructs induced profound tumor regression. These data suggest that i.t. VV-cytokine gene constructs retard tumor growth by infecting mesothelioma cells and targeting the immune system through tumor-infiltrating DC and T cells.

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20149427

Mediating high levels of gene transfer without cytotoxicity via hydrolytic cationic ester polymers.

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Cationic polymers are widely studied as gene-delivery vehicles, but are limited by low transfection due to inhibited release of DNA, and high cytotoxicity from the requisite positive charges. Here, we introduce a hydrolytic cationic ester polymer containing both tertiary and quaternary amines, which packages DNA into nanoparticles and then releases DNA upon hydrolysis. Cells were transfected with these nanoparticles. Luciferase expression from a polymer with the tertiary/quaternary ratio of 1:1 was equal to that obtained using branched polyethylenimine (PEI), and expression from an acidified polymer with the ratio of 3:1 was 20 times higher than branched PEI. These ratios best balance proton sponging from tertiary amines and packaging ability from cations. Importantly, no hydrolysed polymer exhibited cytotoxicity; the zwitterionic nature of the hydrolysed polymer ensured that the quaternary amines in this work do not cause cell death. Hydrolysis is critical for effective and safe gene therapy. Copyright © 2010 Elsevier Ltd. All rights reserved.

PMID: Virol J. 2010 Feb 11;7(1):35. [Epub ahead of print]
20149250

Targeting lentiviral vector to specific cell types through surface displayed single chain antibody and fusogenic molecule.

Lei Y, Joo KI, Zarzar J, Wong C, Wang P.

ABSTRACT: **BACKGROUND:** Viral delivery remains one of the most commonly used techniques today in the field of gene therapy. However, one of the remaining hurdles is the off-targeting effect of viral delivery. To overcome this obstacle, we recently developed a method to incorporate an antibody and a fusogenic molecule (FM) as two distinct molecules into the lentiviral surface. In this report, we expand this strategy to utilize a single chain antibody (SCAb) for targeted transduction. **RESULTS:** Two versions of the SCAb were generated to pair with our various engineered FMs by linking the heavy chain and the light chain variable domains of the anti-CD20 antibody (alphaCD20) via a GS linker and fusing them to the hinge-CH2-CH3 region of human IgG. The resulting protein was fused to either a HLA-A2 transmembrane domain or a VSVG transmembrane domain for anchoring purpose. Lentiviral vectors generated with either version of the SCAb and a selected FM were then characterized for binding and fusion activities in CD20-expressing cells. **CONCLUSION:** Certain combinations of the SCAb with various FMs could result in an increase in viral transduction. This two-molecule lentiviral vector system design allows for parallel optimization of the SCAb and FMs to improve targeted gene delivery.

PMID:
20148637

Nanomedicine (Lond). 2010 Feb;5(2):259-68.

Protein nanodisk assembling and intracellular trafficking powered by an arginine-rich (R9) peptide.

Vazquez E, Roldán M, Diez-Gil C, Unzueta U, Domingo-Espín J, Cedano J, Conchillo O, Ratera I, Veciana J, Daura X, Ferrer-Mirallès N, Villaverde A.

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Aims: Arginine(R)-rich cationic peptides are powerful tools in drug delivery since, alone or when associated with polyplexes, proteins or chemicals, they confer DNA condensation, membrane translocation and blood-brain barrier crossing abilities. The unusual stability and high in vivo performance of their associated drugs suggest a particulate organization or R(n) complexes, which this study aimed to explore. **Materials & methods:** We have analyzed the particulate organization and biological performance in DNA delivery of a model, R9-containing green fluorescent protein by dynamic light scattering, transmission electron microscopy, atomic force microscopy, single cell confocal microscopy and flow cytometry. **Results:** A deep nanoscale examination of R9-powered constructs reveals a novel and promising feature of R9, that when fused to a scaffold green fluorescent protein, promote its efficient self-assembling as highly stable, regular disk-shaped nanoparticles of 20 x 3 nm. These constructs are efficiently internalized in mammalian cells and rapidly migrate through the cytoplasm towards the nucleus in a fully bioactive form. Besides, such particulate platforms accommodate, condense and deliver plasmid DNA to the nucleus and promote plasmid-driven transgene expression. **Conclusion:** The architectonic properties of arginine-rich peptides at the nanoscale reveal a new category of protein nanoparticles, namely nanodisks, and provide novel strategic concepts and architectonic tools for the tailored construction of new-generation artificial viruses for gene therapy and drug delivery.

PMID:
20147982

Gene Ther. 2010 Feb 11. [Epub ahead of print]

Immunobiology of gene therapy for neurodegenerative disease: prospects and risks.

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Gene therapy for neurological, and in particular neurodegenerative, disease is now a reality. A number of early phase clinical trials have been completed and several are currently in progress. In view of this, it is critically important to evaluate the immunological risk associated with neurological gene therapy, which has clear implications for trial safety and efficacy. Moreover, it is imperative in particular to identify factors indicating potential high risk. In the light of recent advances in understanding immune regulation in the central nervous system (CNS) and with the continued development of new gene delivery vectors, this review critically assesses the current knowledge of immunobiology within the CNS in terms of likely immunological risk pertaining to viral vectors and gene therapy applications for neurodegenerative disease.

PMID:
20147411

J Virol. 2010 Feb 10. [Epub ahead of print]

Provirus Selected for High and Stable Expression of Transduced Genes Accumulate in Broadly Transcribed Genome Areas.

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Retroviruses and retrovirus-derived vectors integrate non-randomly into the genomes of host cells with specific preferences for transcribed genes, gene-rich regions, and CpG islands. However, the genomic features that influence transcriptinal activity of integrated retroviruses or retroviral vectors are poorly understood. We report here the cloning and characterization of avian sarcoma virus integration sites from chicken tumors. Growing progressively in dependence on high and stable expression of the transduced v-src oncogene, these tumors represent clonal expansions of cells bearing transcriptionally active replication-defective proviruses. Therefore, integration sites in our study distinguish genomic loci favorable for the expression of integrated retroviruses and gene transfer vectors. Analysis of integration sites from avian sarcoma virus-induced tumors showed strikingly non-random distribution with proviruses found prevalently within or close to transcription units, particularly in genes broadly expressed in multiple tissues but not in tissue-specifically expressed genes. We infer that proviruses integrated in these genomic areas efficiently avoid transcriptional silencing and keep active for a long time during the growth of tumors. Defining the differences between unselected retroviral integration sites and sites selected for long terminal repeat-driven gene expression is relevant for retrovirus-mediated gene transfer and has ramifications for gene therapy.

PMID:
20147151

CSH Protoc. 2009 May 1;2009(5):pdb.prot5011.

Construction and characterization of adenovirus vectors.

Ross PJ, Parks RJ.

INTRODUCTION Genetically modified adenoviruses (Ads) make attractive vectors for the delivery of exogenous DNA to mammalian cells for basic science and gene therapy applications. Ad vector production consists of (1) cloning a transgene into an infectious plasmid by in vivo recombination in bacteria, (2) rescuing and propagating the vector in complementing cells, and (3) purifying the vector. All of this can be accomplished using commercially available reagents, plasmids, and cell lines. First-generation Ads have a large cloning capacity (5-14 kbp) and efficiently transduce a wide range of both quiescent and proliferating cell types. They are readily propagated to produce high-titer stocks (10¹¹-10¹³ vector particles from a 3-L culture). Furthermore, Ads rarely integrate into the host genome and are relatively safe. However, Ad vector production typically takes 4-6 wk, and promiscuous host-cell transduction can occur in vivo. Furthermore, immune responses against viral proteins encoded by the vector backbone can occur, which limits the duration of transgene expression in vivo. Regardless of these limitations, Ad remains one of the more versatile and efficient gene delivery systems. Here, we discuss methods for the generation, propagation, purification, and characterization of first-generation Ad vectors.

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20146634

Expert Opin Biol Ther. 2010 Feb 10. [Epub ahead of print]

T cell receptor (TCR) gene therapy to treat melanoma: lessons from clinical and preclinical studies.

Coccoris M, Straetemans T, Govers C, Lamers C, Sleijfer S, Debets R.

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Importance of the field: Adoptive T cell therapy (ACT) with tumour infiltrating lymphocytes is currently the best treatment option for metastatic melanoma. Despite its clinical successes, ACT has limitations in availability and generation of therapeutic T cells for a larger group of patients. Introduction of tumour-specific T cell receptors into T cells, termed TCR gene therapy, can provide an alternative for ACT that is more widely applicable and might be extended to other types of cancer. Areas covered in this review: The current status of TCR gene therapy studies including clinical challenges, such as on-target toxicity, compromised anti-tumour T cell responses, compromised T cell persistence and potential immunogenicity of receptor transgenes. Strategies to address these challenges are covered. What the reader will gain: A listing and discussion of strategies that aim at improving the efficacy and safety of TCR gene therapy. Such strategies address antigen choice, TCR mis-pairing, functional avidity and persistence of T cells, immune responses towards receptor transgenes, and combination of ACT with other therapies. Take home message: To ensure further clinical development of TCR gene therapy, it is necessary to choose safe T cell target antigens, and implement (combinations of) strategies that enhance the correct pairing of TCR transgenes and the functional avidity and persistence of T cells.

PMID:
20145605

Mol Ther. 2010 Feb 9. [Epub ahead of print]

TLR9 and IRF3 Cooperate to Induce a Systemic Inflammatory Response in Mice Injected With Liposome:DNA.

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Liposome:DNA is a promising gene therapy vector. However, this vector can elicit a systemic inflammatory response syndrome (SIRS). Prior reports indicate that liposome:DNA vectors activate Toll-like receptor (TLR)9. We hypothesized that liposome:DNA vectors also activate the cytosolic DNA-sensing pathway, which signals via interferon (IFN) regulatory factor (IRF)3. To test this, we treated dendritic cells (DCs) with liposome:DNA in vitro and found that IRF3 was phosphorylated independent of TLR9. To test the contribution of this pathway in vivo, we injected a liposome:DNA vector into wild-type (WT), TLR9-knockout (KO), IRF3-KO, and TLR9-IRF3-double-KO (DKO) mice. WT mice exhibited a systemic inflammatory response, evidenced by elevations in serum cytokines, serum enzyme changes indicating organ damage, hypothermia, and mortality. The cytokine response was reduced in TLR9-KO, IRF3-KO, and TLR9-IRF3-DKO mice and all three groups survived. We found that IFN-gamma-KO mice that receive liposome:DNA had a reduced cytokine response and 100% survival. CD11c(+) and NK1.1(+) cells produced IFN-gamma and depleting CD11c(+) cells reduced the cytokine response in mice injected with liposome:DNA. These findings may facilitate the development of immunologically inert gene therapy vectors and may provide general insight into the mechanisms of SIRS.

PMID:
20145150

Cancer Res. 2010 Feb 9. [Epub ahead of print]

A Placental Growth Factor Variant Unable to Recognize Vascular Endothelial Growth Factor (VEGF) Receptor-1 Inhibits VEGF-Dependent Tumor Angiogenesis via Heterodimerization.

Tarallo V, Vesci L, Capasso O, Esposito MT, Riccioni T, Pastore L, Orlandi A, Pisano C, De Falco S

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Angiogenesis is one of the crucial events for cancer development and growth. Two members of the vascular endothelial growth factor (VEGF) family, VEGF-A and placental growth factor (PIGF), which are able to heterodimerize if coexpressed in the same cell, are both required for pathologic angiogenesis. We have generated a PIGF1 variant, named PIGF1-DE in which the residues Asp(72) and Glu(73) were substituted with Ala, which is unable to bind and activate VEGF receptor-1 but is still able to heterodimerize with VEGF. Here, we show that overexpression in tumor cells by adenoviral delivery or stable transfection of PIGF1-DE variant significantly reduces the production of VEGF homodimer via heterodimerization, determining a strong inhibition of xenograft tumor growth and neoangiogenesis, as well as significant reduction of vessel lumen and stabilization, and monocyte-macrophage infiltration. Conversely, the overexpression of PIGF1wt, also reducing the VEGF homodimer production comparably with PIGF1-DE variant through the generation of VEGF/PIGF heterodimer, does not inhibit tumor growth and vessel density compared with controls but induces increase of vessel lumen, vessel stabilization, and monocyte-macrophage infiltration. The property of PIGF and VEGF-A to generate heterodimer represents a successful strategy to inhibit VEGF-dependent angiogenesis. The PIGF1-DE variant, and not PIGF1wt as previously reported, acts as a "dominant negative" of VEGF and is a new candidate for antiangiogenic gene therapy in cancer treatment. Cancer Res; 70(5); OF1-10.

PMID:
20144731

Int J Biochem Cell Biol. 2010 Feb 5. [Epub ahead of print]

MicroRNAs in Immune Regulation-Opportunities for Cancer Immunotherapy.

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Endogenously produced microRNAs are predicted to regulate the translation of over two-thirds all human gene transcripts. Certain microRNAs regulate expression of genes that are critically involved in both innate and adaptive immune responses. Immune cells represent a highly attractive target for microRNA gene therapy approaches, as these cells can be isolated, treated and then reintroduced into the patient. In this short review, we discuss how recent discoveries on the roles of microRNAs in immune-regulation will advance the field of cancer immunology and immunotherapy. Targets identified already in T cells include microRNAs, miR-17-92 family, miR-155, and miR-181a. In macrophages, miR-125b, miR-146, and miR-155 act as Pathogen Associated Molecular Pattern Molecule-associated microRNAs and miR-34C and miR-214 as Damage Associated Molecular Pattern Molecules-associated miRs. We have also demonstrated that the ability of tumors to serve as targets for cytolytic effectors is regulated by miR-222 and miR-339. Copyright © 2010. Published by Elsevier Ltd.

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20143927

Tissue Eng Part B Rev. 2010 Feb;16(1):13-20.

Direct gene therapy for bone regeneration: gene delivery, animal models, and outcome measures.

Pelled G, Ben-Arav A, Hock C, Reynolds DG, Yazici C, Zilberman Y, Gazit Z, Awad H, Gazit D, Schwarz EM.

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While various problems with bone healing remain, the greatest clinical change is the absence of an effective approach to manage large segmental defects in limbs and craniofacial bones caused by trauma or cancer. Thus, nontraditional forms of medicine, such as gene therapy, have been investigated as a potential solution. The use of osteogenic genes has shown great potential in bone regeneration and fracture healing. Several methods for gene delivery to the fracture site have been described. The majority of them include a cellular component as the carrying vector, an approach known as cell-mediated gene therapy. Yet, the complexity involved with cell isolation and culture emphasizes the advantages of direct gene delivery as an alternative strategy. Here we review the various approaches of direct gene delivery for bone repair, the choice of animal models, and the various outcome measures required to evaluate the efficiency and safety of each technique. Special emphasis is given to noninvasive, quantitative, in vivo monitoring of gene expression and biodistribution in live animals. Research efforts should aim at inducing a transient, localized osteogenic gene expression within a fracture site to generate an effective therapeutic approach that would eventually lead to clinical use.

PMID:
20143184

Mol Biotechnol. 2010 Feb 9. [Epub ahead of print]

Recombinant Baculovirus as a Highly Potent Vector for Gene Therapy of Human Colorectal Carcinoma: Molecular Cloning, Expression, and In Vitro Characterization.

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Present therapeutic strategies for most cancers are restricted mainly to the primary tumors and are also not very effective in controlling metastatic states. Alternatively, gene therapy can be a potential option for treating such cancers. Currently mammalian viral-based cancer gene therapy is the most popular approach, but the efficacy has been shown to be quite low in clinical trials. In this study, for the first time, the insect cell-specific baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) has been evaluated as a vector for gene delivery to colorectal cancer cells. Experiments involving factorial design were employed to study the individual and combined effects of different parameters such as multiplicity of infection (MOI), viral incubation time and epigenetic factors on transduction efficiency. The results demonstrate that baculovirus gene delivery system holds immense potential for development of a new generation of highly effective virotherapy for colorectal, as well as other major carcinomas (breast, pancreas, and brain), and offers significant benefits to traditional animal virus-based vectors with respect to safety concerns.

PMID:
20143172

Arch Immunol Ther Exp (Warsz). 2010 Feb 9. [Epub ahead of print]

Lentiviral Vectors in Gene Therapy: Their Current Status and Future Potential.

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The concept of gene therapy originated in the mid twentieth century and was perceived as a revolutionary technology with the promise to cure almost any disease of which the molecular basis was understood. Since then, several gene vectors have been developed and the feasibility of gene therapy has been shown in many animal models of human disease. However, clinical efficacy could not be demonstrated until the beginning of the new century in a small-scale clinical trial curing an otherwise fatal immunodeficiency disorder in children. This first success, achieved after retroviral therapy, was later overshadowed by the occurrence of vector-related leukemia in a significant number of the treated children, demonstrating that the future success of gene therapy depends on our understanding of vector biology. This has led to the development of later-generation vectors with improved efficiency, specificity, and safety. Amongst these are HIV-1 lentivirus-based vectors (lentivectors), which are being increasingly used in basic and applied research. Human gene therapy clinical trials are currently underway using lentivectors in a wide range of human diseases. The intention of this review is to describe the main scientific steps leading to the engineering of HIV-1 lentiviral vectors and place them in the context of current human gene therapy.

PMID:
20140496

Cytotechnology. 2010 Feb 6. [Epub ahead of print]

Immobilization of 293 cells using porous support particles for adenovirus vector production.

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Adenovirus vector production by anchorage-independent 293 cells immobilized using porous biomass support particles (BSPs) was investigated in static and shake-flask cultures for efficient large-scale production of adenovirus vectors for gene therapy applications. The density of cells immobilized within BSPs was evaluated by measuring their WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) reduction activity. In shake-flask culture, 293-F cells, which were adapted to serum-free suspension culture, were not successfully retained within reticulated polyvinyl formal (PVF) resin BSPs (2 x 2 x 2 mm cubes) with matrices of relatively small pores (pore diameter 60 μm). When the BSPs were coated with a cationic polymer polyethyleneimine, a high cell density of more than 10⁷ cells cm⁻³-BSP was achieved in both static and shake-flask cultures with regular replacement of the culture medium. After infection with an adenovirus vector carrying the enhanced green fluorescent protein gene (Ad EGFP), the specific Ad EGFP productivity of the immobilized cells was comparable to the maximal productivity of non-immobilized 293-F cells by maintaining favorable conditions in the culture environment.

PMID:
20139924

Cancer Gene Ther. 2010 Feb 5. [Epub ahead of print]

Adenoviral targeting of gene expression to tumors.

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Using biochemical, imaging and histological methods, we employed transcriptional targeting to increase the specificity of tumor gene expression in vivo for intravenously administered recombinant adenovirus vectors. Surprisingly, the relative specificity of tumor expression in comparison with other tissues was increased for a constitutively expressing recombinant adenovirus, AdCMVLuc, by simply reducing the viral dose. Even at lower doses, however, the high frequency of viral infection and transgene expression in the liver using constitutive promoters still represents a substantial problem. To further augment tumor specificity, we constructed a series of adenoviruses expressing luciferase from several other promoters and tested their ability to selectively transcribe genes in tumor cells, both in vitro and in vivo. Constitutively active viral promoters (RSV, SRalpha) varied widely in their tumor selectivity, but hypoxia-responsive promoters (carbonic anhydrase 9, PAI-1, SOD2 and several chimeric constructs) showed the most tumor-selective expression. Our results show that tumor targeting to HT1080 fibrosarcomas was readily achieved using transcriptional targeting mechanisms. We attribute the relatively high level of gene transfer and expression in HT1080 tumors in vivo to increased viral access to the tumor, presumably due to discontinuities in tumor vasculature and augmented expression from stress-responsive promoters in the hypoxic and inflammatory tumor microenvironment.

PMID:
20139275

J Immunol. 2010 Feb 5. [Epub ahead of print]

IL-12 and IL-27 Sequential Gene Therapy via Intramuscular Electroporation Delivery for Eliminating Distal Aggressive Tumors.

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Eradication of residual malignancies and metastatic tumors via a systemic approach is the key for successfully treating cancer and increasing cancer patient survival. Systemic administration of IL-12 protein in an acute large dose is effective but toxic. Systemic administration of IL-12 gene by persistently expressing a low level of IL-12 protein may reduce the systemic toxicity but only eradicates IL-12-sensitive tumors. Here, we discovered that sequential administration of IL-12- and IL-27-encoding DNA, referred to as sequential IL-12-->IL-27 (IL-12 administration followed by IL-27 administration 10 d after) gene therapy, not only eradicated IL-12-sensitive CT26 tumors from 100% of mice but also eradicated the highly malignant 4T1 tumors from 33% of treated mice in multiple independent experiments. This IL-12-->IL-27 sequential gene therapy is not only superior to IL-12-encoding plasmid DNA given a total of two times at a 10-d interval sequential gene therapy for eliminating tumors but also for inducing CTL activity, increasing T cell infiltration into tumors, and yielding a large number of tumor-specific IFN-gamma-positive CD8 T cells. Notably, depletion of either T or NK cells during the IL-27 treatment phase reverses tumor eradication, suggesting an NK cell requirement for this sequential gene therapy-mediated tumor eradication. Both reversal of the administration sequence and coadministration of IL-12 and IL-27 impaired tumor eradication in 4T1 tumor-bearing mice. This IL-12-->IL-27 sequential gene therapy, via sequential administration of IL-12- and IL-27-encoding plasmid DNA into tumor-bearing mice through i.m. electroporation, provides a simple but effective approach for eliminating inaccessible residual tumors.

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GITR engagement preferentially enhances proliferation of functionally competent CD4+CD25+FoxP3+ regulatory T cells.

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Naturally occurring regulatory T cells (Treg) express high levels of glucocorticoid-induced tumour necrosis factor receptor (GITR). However, studies of the role of GITR in Treg biology has been complicated by the observation that upon activation effector CD4(+) T (Teff) cells also express the receptor. Here, we dissect the contribution of GITR-induced signaling networks in the expansion and function of FoxP3(+) Treg. We demonstrate that a high-affinity soluble Fc-GITR-L dimer, in conjugation with alphaCD3, specifically enhances in vitro proliferation of Treg, which retain their phenotypic markers (CD25 and FoxP3) and their suppressor function, while minimally affecting Teff cells. Furthermore, Fc-GITR-L does not impair Teff susceptibility to suppression, as judged by cocultures employing GITR-deficient and GITR-sufficient CD4(+) T-cell subsets. Notably, this expansion of Treg could also be seen in vivo, by injecting FoxP3-IRES-GFP mice with Fc-GITR-L even in the absence of antigenic stimulation. In order to test the efficacy of these findings therapeutically, we made use of a C3H/HeJ hemophilia B-prone mouse model. The use of liver-targeted human coagulation factor IX (hF.IX) gene therapy in this model has been shown to induce liver toxicity and the subsequent failure of hF.IX expression. Interestingly, injection of Fc-GITR-L into the hemophilia-prone mice that were undergoing liver-targeted hF.IX gene therapy increased the expression of F.IX and reduced the anticoagulation factors. We conclude that GITR engagement enhances Treg proliferation both in vitro and in vivo and that Fc-GITR-L may be a useful tool for in vivo tolerance induction.

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Antiangiogenic gene therapy of experimental pancreatic tumor by sFlt-1 plasmid DNA carried by RGD-modified crosslinked polyplex micelles.

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Disulfide crosslinked polyplex micelles with RGD peptides were formed through ion complexation of thiolated c(RGDfK)-poly(ethylene glycol)-block-poly(L-lysine) (c(RGDfK)-PEG-P(Lys-SH)) and plasmid DNA encoding sFlt-1 and tested for their therapeutic effect in BxPC3 pancreatic adenocarcinoma tumor bearing mice. These micelles, systemically injected, demonstrated significant inhibition of tumor growth up to day 18, as a result of the antiangiogenic effect that was confirmed by vascular density measurements. Significant therapeutic activity of the 15% crosslinked micelle (c(RGDfK)-PEG-P(Lys-SH15)) was achieved by combined effect of increased tumor accumulation, interaction with endothelial cells and enhanced intracellular uptake through receptor-mediated endocytosis. These results suggest that RGD targeted crosslinked polyplex micelles can be effective plasmid DNA carriers for antiangiogenic gene therapy. Copyright © 2010. Published by Elsevier B.V.

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Hepatoma-targeted gene delivery using a tumor cell-specific gene regulation system combined with a human liver cell-specific bionanocapsule.

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Hepatoma (hepatocellular carcinoma) is the most common type of malignant tumor originating in the liver and has a relatively low 5-year survival rate. The development of hepatoma-targeted therapy is needed to increase treatment efficiency and to reduce the incidence of undesirable side effects. In this study, we developed a novel hepatoma-targeted gene delivery system. The gene delivery system was prepared by combining a human liver cell-specific bionanocapsule (BNC) and a tumor cell-specific gene regulation polymer, which responds to hyperactivated protein kinase C (PKC) α in hepatoma cells. The complex of the polymer/DNA with BNC was delivered into cells and tissues. The developed system showed increased transfection efficiency and resulted in cell-specific gene expression in hepatoma cells and tissues (HuH-7), but no gene expression in normal human hepatocytes or human epidermoid tumor cells (A431). The combination of a tumor cell-specific gene regulation system responding to PKC α and BNC showed novel potential for the selective treatment of hepatomas. The system could be a useful method with applications in hepatoma-specific gene therapy and molecular imaging.