



CLINIGENE CURRENT GENE THERAPY WEEKLY

From September 14th to September 21st 2009

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19061425

Toll-like receptor-mediated signaling in human adipose-derived stem cells: implications for immunogenicity and immunosuppressive potential.

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Human adipose-derived stem cells (hASCs) are mesenchymal stem cells with reduced immunogenicity and the capability to modulate immune responses. These properties make hASCs of special interest as therapeutic agents in the settings of chronic inflammatory and autoimmune diseases. Exogenous and endogenous toll-like receptor (TLR) ligands have been linked with the perpetuation of inflammation in a number of chronic inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis because of the permanent exposure of the immune system to TLR-specific stimuli. Therefore, hASCs employed in therapy are potentially exposed to TLR ligands, which may result in the modulation of hASC activity and therapeutic potency. In this study, we demonstrate that hASCs possess active TLR2, TLR3, and TLR4, because activation with specific ligands resulted in induction of nuclear factor kappa B-dependent genes, such as manganese superoxide dismutase and the release of interleukin (IL)-6 and IL-8. TLR3 and TLR4 ligands increased osteogenic differentiation, but no effect on adipogenic differentiation or proliferation was observed. Moreover, we show that TLR activation does not impair the immunogenic and immunosuppressive properties of hASCs. These results may have important implications with respect to the safety and efficacy of hASC-based cell therapies.

PMID: Cancer Chemother Pharmacol. 2009 Nov;64(6):1149-55. Epub 2009 Mar 26.
19322566

Phase I trial of GTI-2040, oxaliplatin, and capecitabine in the treatment of advanced metastatic solid tumors: a California Cancer Consortium Study.

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BACKGROUND: GTI-2040 is a 20-mer antisense oligonucleotide targeting the mRNA of ribonucleotide reductase M2. It was combined with oxaliplatin and capecitabine in a phase I trial in patients with advanced solid tumors based on previous studies demonstrating potentiation of chemotherapy with ribonucleotide reductase inhibitors. **METHODS:** Patients at least 18 years of age with advanced incurable solid tumors and normal organ function as well as a Karnofsky performance status of $>$ or $=60\%$ were eligible. One prior chemotherapy regimen for advanced disease or relapse within 12 months of adjuvant chemotherapy was required. Patients could have received prior fluoropyrimidines, including capecitabine, but not oxaliplatin. Treatment cycles were 21 days. In each cycle, GTI-2040 was given as a continuous intravenous infusion over 14 days, oxaliplatin as a 2-h intravenous infusion on day 1, and capecitabine orally twice a day for 14 days. In cycle 1 only, oxaliplatin and capecitabine were started on day 2 to allow ribonucleotide reductase mRNA levels to be measured with and without oxaliplatin and capecitabine. Doses were escalated in cohorts of three patients using a standard 3 + 3 design until the maximum tolerated dose was established, defined as no more than one first-cycle dose-limiting toxicity among six patients treated at a given dose level. **RESULTS:** The maximum tolerated dose was estimated to be the combination of GTI-2040 3 mg/kg per day for 14 days, capecitabine 600 mg/m² twice daily for 14 days, and oxaliplatin 100 mg/m² every 21 days. Dose-limiting toxicities were hematologic. GTI-2040 pharmacokinetics, obtained at steady-state on days 7 and 14, showed the high inter-patient variability previously reported. Two of six patients had stable disease at the maximum tolerated dose and one patient, with heavily pre-treated non-small cell lung cancer, had a partial response at a higher dose level. In samples from a limited number of patients, there was no clear decrease in ribonucleotide reductase expression in peripheral blood mononuclear cells during treatment. **CONCLUSION:** A combination of GTI-2040, capecitabine and oxaliplatin is feasible in patients with advanced solid tumors.

PMID:
19419197

Mol Pharm. 2009 Jul-Aug;6(4):1100-9.

Development of a genetically engineered biomimetic vector for targeted gene transfer to breast cancer cells.

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A biomimetic vector was genetically engineered to contain at precise locations (a) an adenovirus mu peptide to condense pDNA into nanosize particles, (b) a synthetic cyclic peptide to target breast cancer cells and enhance internalization of nanoparticles, (c) a pH-responsive synthetic fusogenic peptide to disrupt endosome membranes and facilitate escape of the nanoparticles into the cytosol, and (d) a nuclear localization signal from human immunodeficiency virus for microtubule mediated transfer of genetic material to the nucleus. The vector was characterized using physicochemical and biological assays to demonstrate the functionality of each motif in the vector backbone. The results demonstrated that the vector is able to condense plasmid DNA into nanosize particles (<100 nm), protect pDNA from serum endonucleases, target ZR-75-1 breast cancer cells and internalize, efficiently disrupt endosome membranes, exploit microtubules to reach nucleus and mediate gene expression. The therapeutic potential of the vector was evaluated by complexing with plasmid DNA encoding TRAIL (pTRAIL) and transfecting ZR-75-1 cells. The results demonstrated that up to 62% of the ZR-75-1 breast cancer cells can be killed after administration of pTRAIL in complex with the vector.

PMID:
19525455

J Nucl Med. 2009 Jul;50(7):1088-94. Epub 2009 Jun 12.

Combined reporter gene PET and iron oxide MRI for monitoring survival and localization of transplanted cells in the rat heart.

Higuchi T, Anton M, Dumler K, Seidl S, Pelisek J, Saraste A, Welling A, Hofmann F, Oostendorp RA, Gansbacher B, Nekolla SG, Bengel FM, Botnar RM, Schwaiger M.

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There is a need for in vivo monitoring of cell engraftment and survival after cardiac cell transplantation therapy. This study assessed the feasibility and usefulness of combined PET and MRI for monitoring cell engraftment and survival after cell transplantation. METHODS: Human endothelial progenitor cells (HEPCs), derived from CD34+ mononuclear cells of umbilical cord blood, were retrovirally transduced with the sodium iodide symporter (NIS) gene for reporter gene imaging by (124)I-PET and labeled with iron oxides for visualization by MRI. Imaging and histologic analysis were performed on 3 groups of nude rats on days 1, 3, and 7 after intramyocardial injection of 4 million HEPCs. RESULTS: In vitro studies demonstrated stable expression of functional NIS protein and normal viability of HEPCs after transduction. On day 1, after intramyocardial transplantation, iron- and NIS-labeled HEPCs were visualized successfully on MRI as a regional signal void in the healthy myocardium and on PET as (124)I accumulation. The (124)I uptake decreased on day 3 and was undetectable on day 7, and the MRI signal remained unchanged throughout the follow-up period. Histologic analysis with CD31 and CD68 antibodies confirmed the presence of either labeled or nonlabeled control transplanted HEPCs at the site of injection on day 1 but not on day 7, when only iron-loaded macrophages were seen. Furthermore, deoxyuride-5'-triphosphate biotin nick end labeling showed extensive apoptosis at the site of transplantation. CONCLUSION: The combination of MRI and PET allows imaging of localization and survival of transplanted HEPCs together with morphologic information about the heart. Although iron labeling rapidly loses specificity for cell viability because of phagocytosis of iron particles released from dead cells, reporter gene expression provided specific information on the number of surviving cells. This multimodality approach allows complementary analysis of cell localization and viability.

PMID:
19585550

Hepatology. 2009 Sep;50(3):815-24.

Long-term reduction of jaundice in Gunn rats by nonviral liver-targeted delivery of Sleeping Beauty transposon.

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Asialoglycoprotein receptor (ASGPR)-mediated endocytosis has been used to target genes to hepatocytes in vivo. However, the level and duration of transgene expression have been low because of lysosomal translocation and degradation of the DNA and lack of its integration into the host genome. In this study we packaged the DNA of interest in proteoliposomes containing the fusogenic galactose-terminated F-glycoprotein of the Sendai virus (FPL) for targeted delivery to hepatocytes. After the FPL binds to ASGPR on the hepatocyte surface, fusogenic activity of the F-protein delivers the DNA into the cytosol, bypassing the endosomal pathway. For transgene integration we designed plasmids containing one transcription unit expressing the Sleeping Beauty transposase (SB) and another expressing human uridinediphosphoglucuronate glucuronosyltransferase-1A1 (pSB-hUGT1A1). The latter was flanked by inverted/direct repeats that are substrates of SB. In cell culture, FPL-mediated delivery of the *E. coli* beta-galactosidase gene (LacZ) resulted in transduction of ASGPR-positive cells (rat hepatocytes or Hepa1 cell line), but not of ASGPR-negative 293 cells. Intravenous injection of the FPL-entrapped pSB-hUGT1A1 (4-8 microg/day, 1-4 doses) into UGT1A1-deficient hyperbilirubinemic Gunn rats (model of Crigler-Najjar syndrome type 1) resulted in hUGT1A1 expression in 5%-10% of hepatocytes, but not in other cell types. Serum bilirubin levels declined by 30% +/- 4% in 2 weeks and remained at that level throughout the 7-month study duration. With histidine containing FPL, serum bilirubin was reduced by 40% +/- 5%, and bilirubin glucuronides were excreted into bile. No antibodies were detectable in the recipient rats against the F-protein or human UGT1A1. Conclusion: FPL is an efficient hepatocyte-targeted gene delivery platform in vivo that warrants further exploration toward clinical application.

PMID:
19641890

Arch Pharm Res. 2009 Jul;32(7):1077-86. Epub 2009 Jul 31.

Suppression of hepatitis B virus-derived human hepatocellular carcinoma by NF-kappaB-inducing kinase-specific siRNA using liver-targeting liposomes.

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Hepatitis B virus triggers an increase of NF-kappaB inducing kinase (NIK)-dependent NF-kappaB activation, followed by the promotion of hepatocellular carcinoma (HCC). Here, we examined the inhibitory effect of NIK-specific siRNA on NF-kappaB signaling and HCC. The results of this study indicated that these siRNAs suppressed HBV-derived HCC by regulating NIK activation. To exert a protective effect from degradation enzyme, cationic liposomes were contrived and modified to contain beta-sitosterol glucoside to target the asialoglycoprotein receptors in liver cancer cells. The cationic dimyristoyl diacyltrimethylammonium propane liposomes were prepared by a reverse-phase evaporation method with slight modification. beta-Sitosterol glucoside was added to the lipid mixture at the beginning of the liposome preparation for the purpose of liver targeting. These liposomes assisted the delivery of the siRNA to specific cells and protected it from various lyases, followed by the ultimate suppression of HCC.

PMID:
19654315

Cancer Res. 2009 Aug 15;69(16):6531-8. Epub 2009 Aug 4.

A nanoparticle system specifically designed to deliver short interfering RNA inhibits tumor growth in vivo.

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Use of short interfering RNA (siRNA) is a promising new approach thought to have a strong potential to lead to rapid development of gene-oriented therapies. Here, we describe a newly developed, systemically injectable siRNA vehicle, the "wrapsome" (WS), which contains siRNA and a cationic lipofection complex in a core that is fully enveloped by a neutral lipid bilayer and hydrophilic polymers. WS protected siRNA from enzymatic digestion, providing a long half-life in the systemic circulation. Moreover, siRNA/WS leaked from blood vessels within tumors into the tumor tissue, where it accumulated and was subsequently transfected into the tumor cells. Because the transcription factor KLF5 is known to play a role in tumor angiogenesis, we designed KLF5-siRNA to test the antitumor activity of siRNA/WS. KLF5-siRNA/WS exhibited significant antitumor activity, although neither WS containing control scrambled-siRNA nor saline containing KLF5-siRNA affected tumor growth. KLF5-siRNA/WS inhibited Klf5 expression within tumors at both mRNA and protein levels, significantly reducing angiogenesis, and we detected no significant acute or long-term toxicity. Our findings support the idea that siRNA/WS can be used to knock down specific genes within tumors and thereby exert therapeutic effects against cancers.

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19747066

Hum Gene Ther. 2009 Sep 11. [Epub ahead of print]

The Current State of Head and Neck Cancer Gene Therapy.

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The incidence of head and neck cancer continues to increase worldwide with tobacco exposure and HPV16 infections being the major etiological factors. Current therapeutic options are ineffective in approximately half of the individuals afflicted with this malignancy. Recent developments in the identification of molecules that sustain head and neck for squamous cell carcinoma (HNSCC) growth and survival have made molecular targeting using gene therapy approaches a feasible therapeutic strategy. Although gene therapy was originally designed to correct single gene defects, it has now evolved to encompass all forms of therapeutic interventions involving engineered cells and nucleic acids that modify the overall pattern of gene expression within target tissues. Several preclinical studies and clinical trials have tested the efficacy of targeting specific molecules in HNSCC patients using genetic therapy approaches. This review will discuss promising preclinical and clinical approaches and new directions for HNSCC gene therapy.

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19747118

Curr Mol Med. 2009 Sep 13. [Epub ahead of print]

Stem Cell and Gene Therapeutic Strategies for the Treatment of Multiple Sclerosis.

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Multiple sclerosis is a disease of the central nervous system that predominantly affects young adults. The pathogenic mechanisms are complex, however numerous studies indicate that the disease is initiated by an autoimmune attack on protein targets present in the central nervous system. Given that a dysfunctional immune system perpetuates the pathophysiological mechanisms that characterize this inflammatory disorder, several therapeutic approaches that target immune cells or their secreted mediators have been generated and are currently used clinically. Although these strategies have been partially beneficial to a proportion of patients, current therapies are not particularly effective at preventing disease progression. As such there is a large and unmet need for the development of more effective treatments. Owing to a number of promising results obtained in mouse models of multiple sclerosis, cell therapies implementing hematopoietic, mesenchymal and neural stem cells may provide practical vehicles for in situ immunomodulation, neuroprotection and regeneration. In concert with these approaches, gene therapy strategies are being investigated to restore antigen-specific tolerance or to deliver anti-inflammatory molecules. Furthermore targeted delivery of glial or neurotropic factors, which counteract the activity of inhibitory molecules within the neurodegenerative component of the lesion, is also being pursued. It is conceivable that these experimental approaches alone, or in combination with emerging and current treatments, may establish a rational protocol for the treatment of multiple sclerosis and potentially other autoimmune disorders.

PMID:
19747468

Brain Res. 2009 Sep 9. [Epub ahead of print]

Inhibition of cerebral ischemia/reperfusion-induced injury by adenovirus expressed C-terminal amino acids of GluR6.

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GluR6 kainate receptor subunit is largely expressed in hippocampus of brain regions and plays an important role in brain ischemia/reperfusion-mediated neuronal cell death. Our previous researches have shown that cerebral ischemia/reperfusion could facilitate the assembly of GluR6 and postsynaptic density protein 95(PSD95) as well as mixed lineage kinase 3(MLK3) and further induce the activation of c-Jun NH2-terminal kinase 3(JNK3), leading to neuronal death of hippocampal CA1. Here, we show that over-expression of C-terminal amino acids of GluR6 can interrupt the combination of GluR6 with PSD95, inhibit the assembly of GluR6.PSD-95.MLK3 signaling module, suppress the activation of JNK3 and the downstream signaling pathway. Thus, our results imply that over-expression of C-terminal amino acids of GluR6 induce neuroprotection against ischaemic brain injury in rat hippocampal CA1 region via suppressing proapoptosis signaling pathways, which can be an experimental foundation for gene therapy of stroke.

PMID:
19747638

Biol Blood Marrow Transplant. 2009 Oct;15(10):1296-305. Epub 2009 Aug 8.

Interferon gamma 13-CA-repeat homozygous genotype and a low proportion of CD4(+) lymphocytes are independent risk factors for cytomegalovirus reactivation with a high number of copies in hematopoietic stem cell transplantation recipients.

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Cytomegalovirus (CMV) reactivation was analyzed in 92 recipients of allogeneic hematopoietic stem cell transplantation (HSCT) in relation to the proportion of CD4(+) lymphocytes in blood and a microsatellite polymorphism within the first intron of the interferon-gamma (IFNG) gene. CMV reactivation was found in 50% of the HSCT recipients; in 30% of these individuals, the level of CMV copies exceeded 100 per 10⁵ peripheral blood (PB) cells on at least one occasion during the 100-day post-HSCT observation period. This high CMV copy level was most frequently found between 31 and 60 days post-HSCT (P = .021). Patients with > or = 100 CMV copies/10⁵ cells were characterized by poorer overall survival (OS) compared with those lacking CMV copies or having < 100 CMV copies/10⁵ cells (P = .04), and they suffered from severe post-HSCT complications, including acute graft-versus-host disease (aGVHD) and relapse. Thus, patients with > or = 100 CMV copies/10⁵ cells were designated as having clinically significant CMV reactivation. Patients with < 10% CD4(+) lymphocytes had a higher number of CMV DNA copies than those with higher proportions of CD4(+) lymphocytes (0.62 vs 0.21, P = .001; mean +/- SEM, 4422 +/- 1667 vs 937 +/- 662 CMV copies/10⁵ cells, P < .001, for the proportion of cases with reactivation and numbers of copies, respectively). Similarly, patients carrying 2 IFNG 13-CA-repeat alleles (homozygotes) had more frequent CMV reactivation (0.50 vs 0.26; P = .039) and a higher CMV load (4111 +/- 1699 vs 950 +/- 591 CMV copies/10⁵ cells; P = .041) compared with those with other IFNG microsatellite allele constellations. Multivariate analysis demonstrated that the IFNG 13-CA-repeat homozygous genotype (odds ratio [OR] = 0.221; P = .044), a low proportion of CD4(+) lymphocytes (OR = 0.276; P = .050), and a lack of optimal (10/10 alleles) donor-recipient HLA match (OR = 15.19; P = .006) were independent risk factors for CMV reactivation with a high number of copies.

PMID:
19749258

Nucleic Acids Symp Ser (Oxf). 2009;(53):57-8.

Polyethylenimine derived nanoparticles for efficient gene delivery.

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Introduction of therapeutic genes into the cells of an organism in a safe and efficient way has become a challenging task in non-viral mediated gene therapy. Here, branched polyethylenimine (bPEI, 25 kDa) was converted into nanoparticles through electrostatic interactions with anionic polysaccharides (e.g. alginic acid, AI and hyaluronic acid, HA). A small library of PEI-AI and PEI-HA nanoparticles was synthesized by varying the amounts of anionic polysaccharides and evaluated in terms of their size, surface charge, cytotoxicity, transfection efficiency, etc. Both the series of nanoparticles exhibited higher cell viability and transfection efficiency as compared to native PEI and the standard transfection reagents. In vivo targeting efficacy of PEI-HA(4.6%) nanoparticles was examined in tumor induced mice.

PMID:
19749373

Nucleic Acids Symp Ser (Oxf). 2009;(53):287-8.

Inhibition of influenza virus by baculovirus-mediated shRNA.

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Influenza viruses A and B cause widespread infections of the human respiratory tract; however, existing vaccines and drug therapy are of limited value for their treatment. Here we show that bispecific short hairpin small-interfering RNA constructs containing an eight-nucleotide intervening spacer, targeted against influenza virus A or influenza virus B, can inhibit the production of both types of virus in infected cell lines. This multiple vector showed remarkable ability to cope with both influenza virus A or B. Furthermore, the *Autographa californica* multiple nuclear polyhedrosis virus can infect a range of mammalian cells, facilitating its use as a baculovirus vector for gene delivery into cells. In this study, baculovirus-mediated bispecific short-hairpin RNA expression markedly inhibited both influenza virus A and B production.

PMID:
19751100

Hum Gene Ther. 2009 Sep 14. [Epub ahead of print]

Enhanced pancreatic cancer gene therapy by combination of adenoviral vector expressing c-erb-B2(Her-2/neu) targeted immunotoxin with a replication competent adenovirus or etoposide.

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Pancreatic cancer is the 4th leading cause of cancer related death in the United States and even under optimal therapy these patients face a very poor prognosis. Here we report a novel gene therapy based strategy to battle this disease. We show that the majority of pancreatic tumors overexpress c-erb-B2 which therefore might serve as a good target for novel therapies. Based on these findings, we developed an adenoviral vector (Ad-e23(scFv)-PE40) encoding for a c-erb-B2(Her-2/neu) targeted immunotoxin. To improve viral gene delivery we co-infected the therapeutic adenovirus with a replication competent adenovirus (RCAd) at low doses which enhanced transduction efficiency of the former. In addition we show that target gene expression can be enhanced by adding etoposide (VP16) at non-therapeutic doses. To investigate the therapeutic efficiency of our approach we established a mouse model for advanced pancreatic cancer disease by i.p. injection of pancreatic cancer cell lines resulting in multifocal peritoneal xenograft tumors. Administration of Ad-e23(scFv)-PE40 in combination with RCAd and VP16 could significantly inhibit tumor growth in mice, with no apparent systemic toxicity. In this study we show that c-erb-B2 might be an effective molecular target in the treatment of pancreatic tumors and that co-administration of a therapeutic c-erb-B2 targeted non-replication competent adenovirus with a RCAd and VP16 could be a powerful approach to effectively deliver therapeutic genes to tumors. As we could demonstrate, this strategy can be employed to effectively treat pancreatic cancer in particular but it might as well be modified to treat other types of cancer.

PMID:
19751205

Curr Pharm Des. 2009 Sep 15. [Epub ahead of print]

RNAi Applications in Therapy Development for Neurodegenerative Disease.

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RNA-mediated interference (RNAi) is a powerful tool for experimental manipulation of gene expression and is widely used to investigate gene function both in vitro and in vivo. RNAi refers to an evolutionarily conserved cellular mechanism for sequence-specific post-transcriptional gene silencing, in which double-stranded RNAs promote selective degradation of homologous cellular mRNAs. Because RNAi-based techniques can be employed to reduce expression of specific genes, this approach holds great promise as a therapy for diverse diseases, including devastating neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases and amyotrophic lateral sclerosis (ALS). Importantly, in recent years RNAi has also emerged as a key tool in target identification and validation studies designed to complement traditional (i.e., small molecule-based) drug development strategies. These studies harness the power of RNAi-mediated reverse genetics to probe disease-associated pathways in both cell-based and animal models, and thus may provide critical data needed to focus drug development efforts around disease-relevant targets. This review highlights recent progress in the preclinical development of RNAi-based therapeutics for neurodegenerative disease and discusses the particular challenges that disorders of the central nervous system (CNS) pose for this approach. It further describes current applications of RNAi techniques for target identification and validation studies and underscores the importance of this methodology to developing treatments for neurological diseases.

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19752751

J Immunother. 2009 Sep 11. [Epub ahead of print]

Optimization of the PiggyBac Transposon System for the Sustained Genetic Modification of Human T Lymphocytes.

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Optimal implementation of adoptive T-cell therapy for cancer will likely require multiple and maintained genetic modifications of the infused T cells and their progeny so that they home to tumor sites and recognize tumor cells, overcome tumor immune evasion strategies, and remain safe. Retroviral vectors readily transduce T cells and integrate into the host cell genome, but have a limited capacity for multigene insertion and cotransduction and are prohibitively expensive to produce at clinical grade. Genetic modification of T cells using transposons as integrating plasmids is an attractive alternative because of the increased simplicity and cost of production. Of available transposons, piggyBac has the higher transposase activity and larger cargo capacity, and we now evaluate piggyBac for potential adoptive therapies with primary T cells. PiggyBac transposons mediated stable gene expression in approximately 20% of primary T cells without selection. Treatment and maintenance of T cells with interleukin-15 increased stable transgene expression up to approximately 40% and expression was sustained through multiple logs of expansion for over 9 weeks in culture. We demonstrate simultaneous integration of 2 independent transposons in 20% of T cells, a frequency that could be increased to over 85% by selection of a transgenic surface marker (truncated CD19). PiggyBac could also deliver transposons of up to 13 kb with 10,000-fold expansion of transduced T cells in culture and finally we demonstrate delivery of a functional suicide gene (iCasp9). PiggyBac transposons may thus be used to express the multiple integrated transgenes that will likely be necessary for the broader success of T-cell therapy.

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J Immunother. 2009 Sep 11. [Epub ahead of print]

Local AdCD40L Gene Therapy is Effective for Disseminated Murine Experimental Cancer by Breaking T-cell Tolerance and Inducing Tumor Cell Growth Inhibition.

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CD40 ligand (CD40L) is one of the most potent stimulators of Th1-type immunity through its maturation of dendritic cells that, in turn, stimulate effector cells such as T cells and NK cells. Lately, CD40-mediated cell growth inhibition and apoptosis have been in focus for the development of novel cancer treatment regimens, including recombinant soluble CD40L or CD40-stimulating antibodies. In this study, intravesical CD40L gene transfer through adenoviral vectors (AdCD40L) was used to treat an aggressive model of disseminated bladder cancer (MB49/C57BL/6). Three weekly AdCD40L vector instillations increased overall survival of tumor-bearing mice (mean 18.5 d, control mice 13 d). Furthermore, bladder tumors were eradicated (2 of 10) simultaneously as lung metastases (6 of 10) were cleared. FoxP3 levels were similar in the tumors of AdCD40L-treated mice and control mice but the tumor-infiltrating effector T cells in AdCD40L-treated mice were cytotoxic (CD107a+) in contrast to those in control-treated tumors. Furthermore, AdCD40L gene therapy could induce cell growth inhibition and cell death in the MB49 tumor cells in vitro and in vivo. However, this effect was not potent enough to cure growing tumors in immunodeficient mice. In conclusion, AdCD40L gene therapy is potent for disseminated cancer both by activation of T cells and controlling tumor cell growth and viability.

PMID:
19754469

Bosn J Basic Med Sci. 2009 Aug;9(3):174-81.

Comparative analysis of gene transfer to human and rat retinal pigment epithelium cell line by a combinatorial use of recombinant adeno- associated virus and ultrasound or/and microbubbles.

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Ultrasound-targeted microbubble destruction has been utilized to deliver a drug/gene into cells in both in vitro and in vivo studies. This work was performed to investigate the feasibility of gene transfer to human retinal pigment epithelium cell line (ARPE-19) and rat retinal pigment epithelium cell line (RPE-J) by a combinatorial use of recombinant adeno-associated virus (rAAV) and ultrasound (US) or/and microbubbles (MBs) and compare the difference between them. Different doses of serotype 2 rAAV encoding an enhanced green fluorescent protein (rAAV2-EGFP) gene and MBs was administered to ARPE-19 and RPE-J cells under different US conditions. Transfection efficiency and cell viability were assessed by fluorescence microscopy, flow cytometry (FCM) analysis, trypan blue staining. The results indicated that US and MBs could respectively improve rAAV2-mediated gene transfer to RPE-J cells, but neither US nor MBs could do so in ARPE-19 cells. US plus MBs could significantly enhance rAAV2-mediated gene transfer to ARPE-19 cells, however, the same effects were not seen in RPE-J cells. These findings demonstrated it is not always coincident that US, MBs and US plus MBs exert the similar effects on gene transfer in vitro RPE cells. So, it is necessary to choose appropriate RPE cell line for the study of US or/and MBs-mediated rAAV gene transfer in retinal gene therapy.

PMID:
19754943

J Immune Based Ther Vaccines. 2009 Sep 15;7(1):4. [Epub ahead of print]

DNA vaccine containing the mycobacterial hsp65 gene prevented insulinitis in MLD-STZ diabetes.

Santos-Junior RR, Sartori A, Lima DS, Souza PR, Coelho-Castelo AA, Bonato VL, Silva CL.

ABSTRACT: BACKGROUND: Our group previously demonstrated that a DNA plasmid encoding the mycobacterial 65-kDa heat shock protein (DNA-HSP65) displayed prophylactic and therapeutic effect in a mice model for tuberculosis. This protection was attributed to induction of a strong cellular immunity against HSP65. As specific immunity to HSP60 family has been detected in arthritis, multiple sclerosis and diabetes, the vaccination procedure with DNA-HSP65 could induce a cross-reactive immune response that could trigger or worsen these autoimmune diseases. **METHODS:** In this investigation was evaluated the effect of a previous vaccination with DNA-HSP65 on diabetes development induced by Streptozotocin (STZ). C57BL/6 mice received three vaccine doses or the corresponding empty vector and were then injected with multiple low doses of STZ. **RESULTS:** DNA-HSP65 vaccination protected mice from STZ induced insulinitis and this was associated with higher production of IL-10 in spleen and also in the islets. This protective effect was also concomitant with the appearance of a regulatory cell population in the spleen and a decreased infiltration of the islets by T CD8+ lymphocytes. The vector (DNA_v) also determined immunomodulation but its protective effect against insulinitis was very discrete. **CONCLUSION:** The data presented in this study encourages a further investigation in the regulatory potential of the DNA-HSP65 construct. Our findings have important implications for the development of new immune therapy strategies to combat autoimmune diseases.

PMID: Cell Signal. 2009 Sep 13. [Epub ahead of print]
19755151

Coupling cellular mitogenesis to apoptosis by designed biomolecules.

Lin Q, Zhu F, Yang W.

Cellular signal transduction pathways transduce input signals to produce the corresponding output effects, ensuring the correct response to extracellular signals. Manipulation of the components in the signaling pathway will alter the correlation of the input signals to output effects. Here we report that by reconstructing the components in mitogenic and apoptotic signaling pathways, Ras, Raf, and caspase-3, we manipulated the cells to couple mitogenic signal input to apoptotic output. The reconstructed biomolecules that couple mitogenesis to apoptosis are designated as "mitogenesis coupled-apoptosis molecular device" (MCAMD). As mitogenesis in cancer cells is constitutively active, MCAMD may have potential applications for cancer gene therapy.

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19755962

Rescue of Pyruvate Kinase Deficiency in Mice by Gene Therapy Using the Human Isoenzyme.

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Human erythrocyte R-type pyruvate kinase deficiency (PKD) is a disorder caused by mutations in the PKLR gene that produces chronic nonspherocytic hemolytic anemia. Besides periodic blood transfusion and splenectomy, severe cases require bone marrow (BM) transplant, which makes this disease a good candidate for gene therapy. Here, the normal human R-type pyruvate kinase (hRPK) complementary (cDNA) was expressed in hematopoietic stem cells (HSCs) derived from pklr deficient mice, using a retroviral vector system. These mice show a similar red blood cell phenotype to that observed in human PKD. Transduced HSCs were transplanted into myeloablated adult PKD mice or in utero injected into nonconditioned PKD fetuses. In the myeloablated recipients, the hematological manifestations of PKD were completely resolved and normal percentages of late erythroid progenitors, reticulocyte and erythrocyte counts, hemoglobin levels and erythrocyte biochemistry were restored. Corrected cells preserved their rescuing capacity after secondary and tertiary transplant. When corrected cells were in utero transplanted, partial correction of the erythrocyte disease was obtained, although a very low number of corrected cells became engrafted, suggesting a different efficiency of cell therapy applied in utero. Our data suggest that transduction of human RPK cDNA in PKLR mutated HSCs could be an effective strategy in severe cases of PKD.

PMID:
19757082

AAPS J. 2009 Sep 9. [Epub ahead of print]

Lipidic Systems for In Vivo siRNA Delivery.

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The ability of small-interfering RNA (siRNA) to silence specific target genes not only offers a tool to study gene function but also represents a novel approach for the treatment of various human diseases. Its clinical use, however, has been severely hampered by the lack of delivery of these molecules to target cell populations in vivo due to their instability, inefficient cell entry, and poor pharmacokinetic profile. Various delivery vectors including liposomes, polymers, and nanoparticles have thus been developed in order to circumvent these problems. This review presents a comprehensive overview of the barriers and recent progress for both local and systemic delivery of therapeutic siRNA using lipidic vectors. Different strategies for formulating these siRNA-loaded lipid particles as well as the general concern about their safe use in vivo will also be discussed. Finally, current advances in the targeted delivery of siRNA and their impacts on the field of RNA interference (RNAi)-based therapy will be presented.

PMID:
19757182

Mol Biol Rep. 2009 Sep 12. [Epub ahead of print]

RNA interference: a potent technology in studying and modulating of dendritic cells, and potential in clinical therapy.

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RNA interference (RNAi) is a natural process by which small interfering RNA duplex directs sequencespecific post-transcriptional silencing of homologous genes by binding to its complementary mRNA and triggering its elimination. The history of RNAi has only about two decades, however, further studies have revealed that it is a potent method of gene silencing that has developed rapidly over the past few years as a result of its extensive importance in the study of genetics, molecular biology and physiology. RNAi technology has also recently yielded significant insight into dendritic cells (DCs) by helping to elucidate numerous mechanisms that regulate the development, activation and function of cells that mediate immunity. In addition, because of its ability to suppress gene expression effectively, this technique may be used to regulate the immune response for clinical purposes. As the key antigen presenting cells, DCs play a crucial part in the start of an immune response and in the enhancement and regulation of cell mediated immune reactions. The current studies indicated that targeting DCs with RNAi is a novel and effective therapeutic method for the fundamental research, and displayed great potential in clinical treatment.

PMID:
19757377

Int J Dev Biol. 2009 Aug 28. [Epub ahead of print]

Pdx1-transfected adipose tissue-derived stem cells differentiate into insulin-producing cells in vivo and reduce hyperglycemia in diabetic mice.

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Insulin-dependent diabetes mellitus (IDDM) is characterized by the rapid development of potentially severe metabolic abnormalities resulting from insulin deficiency. The transplantation of insulin-producing cells is a promising approach for the treatment of IDDM. The transcription factor pancreatic duodenal homeobox 1 (Pdx1) plays an important role in the differentiation of pancreatic beta cells. In this study, the human Pdx1 gene was transduced and expressed in murine adipose tissue-derived stem cells (ASCs). To evaluate pancreatic repair, we used a mouse model of pancreatic damage resulting in hyperglycemia, which involves injection of mice with streptozotocin (STZ). STZ-treated mice transplanted with Pdx1-transduced ASCs (Pdx1-ASCs) showed significantly decreased blood glucose levels and increased survival, when compared with control mice. While stable expression of Pdx1 in ASCs did not induce the pancreatic phenotype in vitro in our experiment, the transplanted stem cells became engrafted in the pancreas, wherein they expressed insulin and C-peptide, which is a marker of insulin-producing cells. These results suggest that Pdx1-ASCs are stably engrafted in the pancreas, acquire a functional beta-cell phenotype, and partially restore pancreatic function in vivo. The ease and safety associated with extirpating high numbers of cells from adipose tissues support the applicability of this system to developing a new cell therapy for IDDM.

PMID:
19757454

J Gene Med. 2009 Sep 15. [Epub ahead of print]

Efficient adenovirus-mediated gene transfer to gastric tissue by oral administration.

Liu XF, Shi Y, Zhang JY, Zhuang Y, Jia KR, Mao XH, Guo Y, Liu T, Liu Z, Wu C, Zhang WJ, Zhou WY, Guo G, Zou QM.

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BACKGROUND: Recombinant adenoviruses (rAd) are well-characterized viral vectors and have been studied in many human diseases. However, there are no detailed methods for transferring genes to the stomach using rAd. **METHODS:** Gastric epithelial cells were infected with rAd encoding green fluorescence protein (AdGFP) for different times, or with AdGFP that had been incubated in artificial gastric juice at different pH values for 1 h. Gene expression was detected by fluorescence microscope and flow cytometry. Mice were infected via oral administration with rAd encoding red fluorescence protein and beta-galactosidase (AdRFP-lacZ) or rAd encoding mouse interleukin-17 (AdmIL-17), and tissues were collected at the indicated times after infection. LacZ expression in different tissues was detected by X-gal staining and IL-17 expression in the stomach was assessed by the real-time polymerase chain reaction and an enzyme-linked immunosorbent assay. Inflammation in the stomach was also assessed. **RESULTS:** rAd could infect the gastric epithelial cells and tolerate pH 5 for 1 h in vitro. Adenovirus-mediated genes were specifically expressed in the gastrointestinal tract and transgene expression persisted in gastric tissue for up to 7 days after oral administration of AdRFP-lacZ. Oral administration of AdmIL-17 induced mIL-17 expression in gastric tissue at the mRNA and protein levels and protein level peaked on day 5 post-infection. IL-6, a target protein of IL-17, and gastric inflammation also increased in AdmIL-17-infected mice. **CONCLUSIONS:** The present study has established a detailed method for transferring adenovirus-mediated gene to the stomach, which may provide a valuable approach for gene therapy or the study of the basic biology of gastric diseases. Copyright (c) 2009 John Wiley & Sons, Ltd.

PMID:
19759406

Phys Med Biol. 2009 Oct 7;54(19):5949-63. Epub 2009 Sep 17.

In vivo electrical conductivity measurements during and after tumor electroporation: conductivity changes reflect the treatment outcome.

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Electroporation is the phenomenon in which cell membrane permeability is increased by exposing the cell to short high-electric-field pulses. Reversible electroporation treatments are used in vivo for gene therapy and drug therapy while irreversible electroporation is used for tissue ablation. Tissue conductivity changes induced by electroporation could provide real-time feedback of the treatment outcome. Here we describe the results from a study in which fibrosarcomas (n = 39) inoculated in mice were treated according to different electroporation protocols, some of them known to cause irreversible damage. Conductivity was measured before, within the pulses, in between the pulses and for up to 30 min after treatment. Conductivity increased pulse after pulse. Depending on the applied electroporation protocol, the conductivity increase after treatment ranged from 10% to 180%. The most significant conclusion from this study is the fact that post-treatment conductivity seems to be correlated with treatment outcome in terms of reversibility.

PMID:
19759534

Nature. 2009 Sep 16. [Epub ahead of print]

Gene therapy for red-green colour blindness in adult primates.

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Red-green colour blindness, which results from the absence of either the long- (L) or the middle- (M) wavelength-sensitive visual photopigments, is the most common single locus genetic disorder. Here we explore the possibility of curing colour blindness using gene therapy in experiments on adult monkeys that had been colour blind since birth. A third type of cone pigment was added to dichromatic retinas, providing the receptor basis for trichromatic colour vision. This opened a new avenue to explore the requirements for establishing the neural circuits for a new dimension of colour sensation. Classic visual deprivation experiments have led to the expectation that neural connections established during development would not appropriately process an input that was not present from birth. Therefore, it was believed that the treatment of congenital vision disorders would be ineffective unless administered to the very young. However, here we show that the addition of a third opsin in adult red-green colour-deficient primates was sufficient to produce trichromatic colour vision behaviour. Thus, trichromacy can arise from a single addition of a third cone class and it does not require an early developmental process. This provides a positive outlook for the potential of gene therapy to cure adult vision disorders.

PMID:
19759562

Gene Ther. 2009 Sep 17. [Epub ahead of print]

Dose-dependent restoration of dystrophin expression in cardiac muscle of dystrophic mice by systemically delivered morpholino.

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We have earlier shown that antisense morpholino oligomers are able to restore dystrophin expression by systemic delivery in body-wide skeletal muscles of dystrophic mdx mice. However, the levels of dystrophin expression vary considerably and, more importantly, no dystrophin expression has been achieved in cardiac muscle. In this study, we investigate the efficiency of morpholino-induced exon skipping in cardiomyoblasts and myocytes in vitro, and in cardiac muscle in vivo by dose escalation. We showed that morpholino induces targeted exon skipping equally effectively in both skeletal muscle myoblasts and cardiomyoblasts. Effective exon skipping was achieved in cardiomyocytes in culture. In the mdx mice, morpholino rescues dystrophin expression dose dependently in both skeletal and cardiac muscles. Therapeutic levels of dystrophin were achieved in cardiac muscle albeit at higher doses than in skeletal muscles. Up to 50 and 30% normal levels of dystrophin were induced by single systemic delivery of 3 g kg(-1) of morpholino in skeletal and cardiac muscles, respectively. High doses of morpholino treatment reduced the serum levels of creatine kinase without clear toxicity. These findings suggest that effective rescue of dystrophin in cardiac muscles can be achieved by morpholino for the treatment of Duchenne muscular dystrophy.

PMID:
19759563

Gene Ther. 2009 Sep 17. [Epub ahead of print]

A versatile nonviral vector system for tetracycline-dependent one-step conditional induction of transgene expression.

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In this study, we describe a novel self-contained, nonviral vector system for the rapid development of tetracycline (Tet)-inducible transgene expression systems in mammalian cell lines. To avoid multiple rounds of clonal selection for the establishment of stably transfected cell clones, as is necessary with conventional systems, we constructed a multicomplementary DNA(cDNA) expression vector that enables both one-step targeted genomic integration and conditional induction of transgene expression. This vector system consists of several modules including a Tet-inducible promoter directing the expression of a transgene and two Tet repressor expression units placed in tandem on a single vector. The cell clones, generated using a one-step phiC31 integrase-mediated chromosomal integration of the multi-cDNA expression construct, showed a stable and robust expression with high induction rates upon addition of doxycycline inducer in five different cell lines tested. By using this system, we show c-Src-induced cell transformation and anticancer cell therapy for this transformation in cultured fibroblast cells. The results show a rapid production and accumulation of target protein on addition of the inducer starting from extremely low background levels and reduction to background levels in a matter of days after the inducer was withdrawn from the culture medium.

PMID:
19759564

Gene Ther. 2009 Sep 17. [Epub ahead of print]

The impact of antigen expression in antigen-presenting cells on humoral immune responses against the transgene product.

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Treatment of genetic diseases by gene therapy is hampered by immune responses against the transgene product. Promoter choice has been shown to be an important parameter of the presence or absence of antibodies against the transgene product after gene transfer. Here, the generality of some of these observations was tested by comparing different murine strains and different transgene products. We show immunological unresponsiveness for human apolipoprotein (apo) A-I in six murine strains after transfer with E1E3E4-deleted adenoviral vectors containing hepatocyte-specific expression cassettes. However, differences in the induction of a humoral immune response against human apo A-I after gene transfer with vectors driven by the major histocompatibility complex class II Ebeta promoter and the ubiquitously active cytomegalovirus promoter were not consistent in these six murine strains. Furthermore, use of a potent hepatocyte-specific expression cassette did not prevent a humoral immune response against human plasminogen in C57BL/6 mice. In contrast, human microplasminogen transfer resulted in stable expression in the absence of an immune response against the transgene product. Taken together, the molecular design of strategies to abrogate or induce an immune response against the transgene product may be hampered by the multitude of parameters affecting the outcome, thus limiting the external validity of results.

PMID:
19759566

Gene Ther. 2009 Sep 17. [Epub ahead of print]

AAV5-mediated gene transfer to the parotid glands of non-human primates.

Voutetakis A, Zheng C, Cotrim AP, Mineshiba F, Afione S, Roescher N, Swaim WD, Metzger M, Eckhaus MA, Donahue RE, Dunbar CE, Chiorini JA, Baum BJ.
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Salivary glands are potentially useful target sites for multiple clinical applications of gene transfer. Previously, we have shown that serotype 2 adeno-associated viral (AAV2) vectors lead to stable gene transfer in the parotid glands of rhesus macaques. As AAV5 vectors result in considerably greater transgene expression in murine salivary glands than do AAV2 vectors, herein we have examined the use of AAV5 vectors in macaques at two different doses (n=3 per group; 10¹⁰ or 3 x 10¹¹ particles per gland). AAV5 vector delivery, as with AAV2 vectors, led to no untoward clinical, hematological or serum chemistry responses in macaques. The extent of AAV5-mediated expression of rhesus erythropoietin (RhEpo) was dose-dependent and similar to that seen with an AAV2 vector. However, unlike results with the AAV2 vector, AAV5 vector-mediated RhEpo expression was transient. Maximal expression peaked at day 56, was reduced by approximately 80% on day 84 and thereafter remained near background levels until day 182 (end of experiment). Quantitative PCR studies of high-dose vector biodistribution at this last time point showed much lower AAV5 copy numbers in the targeted parotid gland (approximately 1.7%) than found with the same AAV2 vector dose. Molecular analysis of the conformation of vector DNA indicated a markedly lower level of concatamerization for the AAV5 vector compared with that of a similar AAV2 vector. In addition, cellular immunological studies suggest that host response differences may occur with AAV2 and AAV5 vector delivery at this mucosal site. The aggregate data indicate that results with AAV5 vectors in murine salivary glands apparently do not extend to macaque glands.

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19759567

Partial protection against SIV challenge by vaccination of adenovirus and MVA vectors in rhesus monkeys.

Wang HB, Kondo A, Yoshida A, Yoshizaki S, Abe S, Bao LL, Mizuki N, Ichino M, Klinman D, Okuda K, Shimada M.

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This study explores the effect of priming rhesus monkeys with an Ad5/35 vector expressing simian immunodeficiency virus (SIV) gag and gp120, and then boosting the animals with an modified vaccinia virus Ankara (MVA) vector encoding the same antigens after a 2-month interval. The animals were intravenously challenged with 100 TCID₅₀ of highly pathogenic SIVmac239 virus 2 months after the booster vaccination. The priming vaccination induced robust SIV-specific cell-mediated and humoral immune responses, and boosting further enhanced the cellular immunity. Vaccination reduced peak and long-term viral loads by 1-2 logs for a period of >6 months, as reflected by a reduction in both the SIV RNA and DNA levels. Of considerable interest, the immunized monkeys did not suffer from loss of CD4 T cells, particularly central memory CD4 T cells. These results demonstrate that prophylactic vaccination with Ad5/35 followed by MVA reduces viral replication and prevents CD4 T-cell loss, and that these effects may decrease the likelihood of disease progression.

PMID: Biomacromolecules. 2009 Sep 17. [Epub ahead of print]

19761207

Development of a Functionalized Polymer for Stent Coating in the Arterial Delivery of Small Interfering RNA.

San Juan A, Bala M, Hlawaty H, Portes P, Vranckx R, Feldman LJ, Letourneur D.

Inserm, U698, Bio-ingenierie Cardiovasculaire, Universite Paris 7, Paris, France, and Universite Paris 13, Villetaneuse, France, Laboratoire d'Ingenierie des Materiaux et des Hautes Pressions (CNRS UPR 1311), Universite Paris 13, Villetaneuse, France, and AP-HP, Hopital Bichat, Departement de Cardiologie, Paris, France.

In patients receiving drug eluting stents, there is a growing concern about both the long-term toxicity/degradability of the polymers used for the coating, and the nature of the therapeutic agents. We hypothesized that the use of a functionalized biocompatible polymer for a stent coating could be appropriate for local arterial therapy. A cationized pullulan hydrogel was thus prepared to cover bare metal stents that could be further loaded with small interfering RNA (siRNA) targeted at MMP2 for gene silencing in vascular cells. The efficient coverage of the stent struts by a smooth polymeric layer, which can withstand the crimping of the stent on a balloon-catheter and its deployment, was demonstrated by fluorescence microscopy, scanning electron microscopy, and atomic force microscopy. The release of siRNA from the stents was modulated by the presence of the cationic groups, as compared to noncationized pullulan hydrogel. In vivo implantation of coated stents was successful and cationized pullulan-based hydrogels loaded with siRNA in rabbit balloon-injured carotid arteries induced an uptake of siRNA into the arterial wall and a decrease of pro-MMP2 activity. These results suggest that cationized pullulan-based hydrogel could be used as a new biocompatible and biodegradable stent coating for local gene therapy in the arterial wall.

PMID:
19761417

Expert Opin Biol Ther. 2009 Sep 18. [Epub ahead of print]

Strategies for short hairpin RNA delivery in cancer gene therapy.

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RNA interference (RNAi) gene silencing can be achieved by delivering vectors that transcribe short hairpin RNA (shRNA), which stably express small interfering RNA in target cells. Therefore, shRNA is of potential therapeutic use for inhibiting cancer cells, in which aberrant expression of certain mRNA's causes problems. However, this technique has not yet been developed for cancer therapy. The major problem for clinical use is lack of effective methods of delivery. In this article, we review the current strategies for shRNA delivery for target validation and their therapeutic uses in cancer to help further the understanding of challenges confronting shRNA technology, such as different principles of RNAi technology, basic construction of shRNA-expressing vectors and delivery barriers, which exist for both local and systemic delivery strategies. Even if there are data showing that shRNA can be used in mice, we are still a long way from its application in human cancer therapy, because serious problems remain, including biodistribution and clearance of nanoparticles following systemic delivery of shRNA-expressing vectors.

PMID:
19761539

Traffic. 2009 Oct;10(10):1414-28.

Transportin mediates nuclear entry of DNA in vertebrate systems.

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Delivery of DNA to the cell nucleus is an essential step in many types of viral infection, transfection, gene transfer by the plant pathogen *Agrobacterium tumefaciens* and in strategies for gene therapy. Thus, the mechanism by which DNA crosses the nuclear pore complex (NPC) is of great interest. Using nuclei reconstituted in vitro in *Xenopus* egg extracts, we previously studied DNA passage through the nuclear pores using a single-molecule approach based on optical tweezers. Fluorescently labeled DNA molecules were also seen to accumulate within nuclei. Here we find that this import of DNA relies on a soluble protein receptor of the importin family. To identify this receptor, we used different pathway-specific cargoes in competition studies as well as pathway-specific dominant negative inhibitors derived from the nucleoporin Nup153. We found that inhibition of the receptor transportin suppresses DNA import. In contrast, inhibition of importin beta has little effect on the nuclear accumulation of DNA. The dependence on transportin was fully confirmed in assays using permeabilized HeLa cells and a mammalian cell extract. We conclude that the nuclear import of DNA observed in these different vertebrate systems is largely mediated by the receptor transportin. We further report that histones, a known cargo of transportin, can act as an adaptor for the binding of transportin to DNA.

PMID:
19761832

Vaccine. 2009 Sep 14. [Epub ahead of print]

Antibody responses against NY-ESO-1 and HER2 antigens in patients vaccinated with combinations of cholesteryl pullulan (CHP)-NY-ESO-1 and CHP-HER2 with OK-432.

Aoki M, Ueda S, Nishikawa H, Kitano S, Hirayama M, Ikeda H, Toyoda H, Tanaka K, Kanai M, Takabayashi A, Imai H, Shiraishi T, Sato E, Wada H, Nakayama E, Takei Y, Katayama N, Shiku H, Kageyama S.

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Combination vaccines of the NY-ESO-1 protein complexed with cholesteryl pullulan (CHP), CHP-NY-ESO-1, and the truncated 146HER2 protein with CHP, CHP-HER2, were subcutaneously administered with the immuno-adjuvant OK-432 to eight esophageal cancer patients. Vaccination was well-tolerated. NY-ESO-1- and HER2-specific antibody responses were analyzed using the patients' sera and samples from previous single CHP-NY-ESO-1 or CHP-HER2 vaccine trial. The responses to NY-ESO-1 in the combination vaccine study were comparable to the single vaccine. For responses to HER2, there were fewer antibody responses in the combination vaccines. Although there were marked individual variations in the antibody responses to the NY-ESO-1 and HER2 antigens, the reaction patterns to these antigens were comparable within each patient. Antibodies to OK-432 were not augmented. Protein cancer vaccines targeting multiple antigens are feasible.

PMID:
19761848

Neuroimage. 2009 Sep 14. [Epub ahead of print]

Optimal Region of the Putamen for Image-Guided Convection-Enhanced Delivery of Therapeutics in Human and Non-human Primates.

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Optimal results in the direct brain delivery of brain therapeutics such as growth factors or viral vector into primate brain depends on reproducible distribution throughout the target region. In the present study, we retrospectively analyzed MRI of 25 convection-enhanced delivery (CED) infusions with MRI contrast into the putamen of non-human primates (NHP). Infused volume (V_i) was compared to total volume of distribution (V_d), vs. V_d within the target putamen. Excellent distribution of contrast agent within the putamen was obtained in 8 cases that were used to define an optimal target volume, or "green" zone. Partial or poor distribution with leakage into adjacent anatomical structures was noted in 17 cases, defining "blue" and "red" zones respectively. Quantitative containment (99 +/- 1%) of infused Gadoteridol within the putamen was obtained when the cannula was placed in the green zone, 87 +/- 3% in the blue zone and 49 +/- 0.05% in the red zone. These results were used to determine a set of 3D stereotactic coordinates that define an optimal site for putaminal infusions in NHP and human putamen. We conclude that cannula placement and definition of optimal (green zone) stereotactic coordinates have important implications in ensuring effective delivery of therapeutics into the putamen utilizing routine stereotactic MRI localization procedures, and should be considered when local therapies such as gene transfer or protein administration are being translated into clinical therapy.

PMID:
19763501

Methods Mol Biol. 2009;590:131-42.

Adenoviral gene transfer into isolated pancreatic islets.

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The beta cells within the pancreatic islets are responsible for production of insulin, a peptide hormone required for maintaining normoglycemia. The establishment of efficient gene transfer into pancreatic islets is very important for studies of insulin and glucagon production and secretion, as well as for gene therapy purposes for the treatment of diabetes. We describe here in detail a protocol for adenoviral gene transfer into isolated mouse islets of the pancreas. Effective gene transfer into pancreatic islets using recombinant adenoviruses can be achieved with a multiplicity of infection (MOI) of 10. However, if the islets are not dispersed, adenoviral gene transfer is limited only to the cells on the periphery of the islets, which represent the glucagon-producing alpha cells in rodents. Dispersion of pancreatic islets with EGTA increases the efficiency of gene transfer into the cells within the core of the islets, which consist of insulin-producing beta cells.

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Fusion enzymes containing HSV-1 thymidine kinase mutants and guanylate kinase enhance prodrug sensitivity in vitro and in vivo.

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Herpes simplex virus thymidine kinase (HSVTK) with ganciclovir (GCV) is currently the most widely used suicide gene/prodrug system in cancer gene therapy. A major limitation in this therapy is the inefficient activation of GCV by HSVTK to its active antimetabolites. We described earlier two strategies to overcome this limitation: (1) generation of HSVTK mutants with improved GCV activation potential and (2) construction of a fusion protein encoding HSVTK and mouse guanylate kinase (MGMK), the second enzyme in the GCV activation pathway. As a means to further enhance GCV activation, two MGMK/HSVTK constructs containing the HSVTK mutants, mutant 30 and SR39, were generated and evaluated for their tumor and bystander killing effects in vitro and in vivo. One fusion mutant, MGMK/30, shows significant reduction in IC(50) values of approximately 12 500-fold, 100-fold, and 125-fold compared with HSVTK, mutant 30 or MGMK/HSVTK, respectively. In vitro bystander analyses show that 5% of MGMK/30-expressing cells are sufficient to induce 75% of tumor cell killing. In an xenograft tumor model, MGMK/30 displays the greatest inhibition of tumor growth at a GCV concentration (1 mg kg(-1)) that has no effect on wild-type HSVTK-, MGMK/HSVTK-, or mutant 30-transfected cells. Another fusion construct, MGMK/SR39, sensitizes rat C6 glioma cells to GCV by 2500-fold or 25-fold compared with HSVTK or MGMK/HSVTK, respectively. In vitro analyses show similar IC(50) values between cells harboring SR39 and MGMK/SR39, although MGMK/SR39 seems to elicit stronger bystander killing effects in which 1% of MGMK/SR39-transfected cells result in 60% cell death. In a xenograft tumor model, despite observable tumor growth inhibition, no statistical significance in tumor volume was detected between mice harboring SR39- and MGMK/SR39-transfected cells when dosed with 1 mg kg(-1) GCV. However, at a lower dose of GCV (0.1 mg kg(-1)), MGMK/SR39 seems to have slightly greater tumor growth inhibition properties compared with SR39 ($P \leq 0.05$). In vivo studies indicate that both mutant fusion proteins display substantial improvements in bystander killing in the presence of 1 mg kg(-1) GCV, even when only 5% of the tumor cells are transfected. Such fusion mutants with exceptional prodrug converting properties will allow administration of lower and non-myelosuppressive doses of GCV concomitant with improved tumor killing and as such are promising candidates for translational gene therapy studies.

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Breast tumor-initiating cells isolated from patient core biopsies for study of hormone action.

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In recent years, evidence has emerged supporting the hypothesis that cancer is a stem cell disease. The cancer stem cell field was led by the discovery of leukemia stem cells (Tan, B.T., Park, C.Y., Ailles, L.E., and Weissman, I.L. (2006) The cancer stem cell hypothesis: a work in progress. *Laboratory Investigation*. 86, 1203-1207), and within the past few years cancer stem cells have been isolated from a number of solid tumor including those of breast and brain cancer among others (Al-Hajj M., Wicha M.S., Benito-Hernandez A., Morrison, S.J., and Clarke, M.F. (2003) Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. USA* 100, 3983-3988; Singh, S.K., Clarke, I.D., Terasaki, M., Bonn, V.E., Hawkins, C., Squire, J., and Dirks, P.B. (2003) Identification of a Cancer Stem Cell in Human Brain Tumors. *Cancer Research*. 63, 5821-5828). Cancer stem cells exhibit far different properties than established cells lines such as relative quiescence, multidrug resistance, and multipotency (Clarke, M.F., Dick, J.E., Dirks, P.B., Eaves, C.J., Jamieson, C.H.M., Jones, D.L., Visvader, J., Weissman, I.L., and Wahl, G.M. (2006) Cancer Stem Cells-Perspectives on Current Status and Future Directions: AACR Workshop on Cancer Stem Cells. *Cancer Research*. 66, 9339-9344). In addition, our laboratory has demonstrated that breast cancer stem cells exhibit a strong metastatic phenotype when passaged in mice. Since stem cells exhibit these somewhat unique properties, it will be important for endocrinologists to evaluate hormonal action in these precursor cells for a more thorough understanding of cancer biology and development of more effective treatment modalities. A relatively easy and low cost method was developed to isolate breast cancer stem cells from primary needle biopsies taken from patients diagnosed with primary invasive ductal carcinoma during the routine care of patients with consent and IRB approval. Fresh needle biopsies (2-3 biopsies at 2 cm in length) were enzymatically dissociated in a collagenase (300 U/ml)/hyaluronidase (100 U/ml) solution followed by sequential filtration. Single cell suspensions were cultured on ultra low attachment plastic flasks in defined medium and formed non-adherent tumorspheres. The tumorspheres exhibited surface marker expression of CD44(+)/CD24(low/-)/ESA(+), previously defined as a "breast cancer stem cell" phenotype by Al Hajj et al. (Al-Hajj M., Wicha M.S., Benito-Hernandez A., Morrison, S.J., and Clarke, M.F. (2003) Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. USA* 100, 3983-3988).

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New therapies for non-muscle-invasive bladder cancer.

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The treatment of non-muscle-invasive bladder cancer (NMIBC) remains a challenge owing to its increased tendency to recur and the possibility of progression to potentially dangerous muscle-invasive disease. Treatment outcomes by current therapeutic modalities are still not optimal. In recent years, there have been a number of substantive advances in the therapeutic options for the management of NMIBC. New chemotherapeutic drugs have been introduced, along with efforts made to improve the efficacy of existing agents and enhance delivery of agents to the bladder. There is also a growing trend toward combination of agents and multimodal therapy. Also of considerable interest are the investigation of newer approaches such as gene therapy, chemoenhancement and newer forms of immunotherapy. Here, we review the recent pre-clinical and clinical developments in the treatment of NMIBC, described in the broad categories of immunotherapy, chemotherapeutic agents, improved or device-assisted agent delivery and gene therapy.

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Severe neonatal spondylometaphyseal dysplasia in two siblings.

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We report on two siblings with a severe neonatal form of spondylometaphyseal dysplasia (SMD). Similar cases have been reported in four publications. Analysis of pedigree data from the original and present families suggests an autosomal recessive mode of inheritance, although parental gonadal mosaicism is also possible. The similarities in the phenotype between our patients and spondyloepimetaphyseal dysplasia congenita (SEMDC) and spondyloepimetaphyseal dysplasia Strudwick (SEMDS) type, indicated that these patients could have a defect in the COL2A1 gene. Molecular analysis of genomic DNA of these patients excluded this gene. Another potential candidate gene PTHR1, was also analyzed in the selected regions and no mutation was found. This gene is probably causative in the Jansen type of SMD, which shares some phenotypic features with the siblings whom we documented. Our results indicate that a new candidate gene for the reported form of SMD should be sought. (c) 2009 Wiley-Liss, Inc.