



CLINIGENE CURRENT GENE THERAPY WEEKLY

From June 21st to June 28th 2010

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PMID:
20577574

Expert Rev Hematol. 2009 Dec;2(6):673-683.

Advancements in gene transfer-based therapy for hemophilia A.

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Gene therapy has promised clinical benefit to those suffering with hemophilia A, but this benefit has not yet been realized. However, during the past two decades, basic and applied gene therapy research has progressed and the goal of gene therapy for hemophilia A is once again in our sights. The hemophilia A patient population suffers from a disease that requires invasive, lifelong management, is exorbitantly expensive to treat, has geographically limited treatment access and can become untreatable due to immune reactions to the treatment product. Subsequent to the cloning of the factor VIII gene and cDNA in the early 1980s, academic and commercial research laboratories began to pursue gene transfer-based therapies to supplement or supplant the available protein replacement therapy. However, to date, clinical trials for gene therapy of hemophilia A have been unsuccessful. Three trials have been conducted with each having tested a different gene-transfer strategy and each demonstrating that there is a considerable barrier to achieving sustained expression of therapeutic amounts of factor VIII. Recent progress has been made in gene-transfer technology and, relevant to hemophilia A, towards increasing the biosynthetic efficiency of factor VIII. These advances are now being combined to develop novel strategies to treat and possibly cure hemophilia A.

PMID:
20577035

Physiol Meas. 2010 Jul;31(7):995-1009. Epub 2010 Jun 24.

Electrical impedance characterization of normal and cancerous human hepatic tissue.

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The four-electrode method was used to measure the ex vivo complex electrical impedance of tissues from 14 hepatic tumors and the surrounding normal liver from six patients. Measurements were done in the frequency range 1-400 kHz. It was found that the conductivity of the tumor tissue was much higher than that of the normal liver tissue in this frequency range (from 0.14 +/- 0.06 S m⁻¹ versus 0.03 +/- 0.01 S m⁻¹ at 1 kHz to 0.25 +/- 0.06 S m⁻¹ versus 0.15 +/- 0.03 S m⁻¹ at 400 kHz). The Cole-Cole models were estimated from the experimental data and the four parameters ($\rho(0)$, $\rho(\infty)$, α , $f(c)$) were obtained using a least-squares fit algorithm. The Cole-Cole parameters for the cancerous and normal liver are 9 +/- 4 Ω m⁻¹, 2.2 +/- 0.7 Ω m⁻¹, 0.5 +/- 0.2, 140 +/- 103 kHz and 50 +/- 28 Ω m⁻¹, 3.2 +/- 0.6 Ω m⁻¹, 0.64 +/- 0.04, 10 +/- 7 kHz, respectively. These data can contribute to developing bioelectric applications for tissue diagnostics and in tissue treatment planning with electrical fields such as radiofrequency tissue ablation, electrochemotherapy and gene therapy with reversible electroporation, nanoscale pulsing and irreversible electroporation.

PMID:
20574710

Mol Biol Rep. 2010 Jun 24. [Epub ahead of print]

Targeted killing effects of double CD and TK suicide genes controlled by survivin promoter on gastric cancer cell.

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Suicide genes such as cytosine deaminase (CD) and herpes simplex virus thymidine kinase (TK) encode products that convert nontoxic substances (prodrugs) into toxic metabolites. Studies in recent years indicated that survivin(sur) expression was associated with the biological behaviors of gastric carcinoma. In the present study, targeted killing effects of double CD and TK suicide genes controlled by survivin promoter on gastric cancer cell were investigated, the recombinant pSCT vector containing CD and TK genes driven by sur promoter was constructed and transfected into SGC-7901 cells. After adding the CCV and 5-FC, the effects of double suicide genes on cell growth, cell cycle and proliferation were determined by MTT assay and flow cytometry (FCM). The results showed that sur promoter could specifically drive the expression of double CD/TK gene in SGC-7901 cells, whereas not in the normal GES-1 cell. After using CCV and 5-FC, the growth of SGC-7901 cells was inhibited. G1 phase proportion was significantly higher in SGC-7901 cells transfected with double suicide genes than the untransfected cells. These results suggest that CD and TK double suicide genes driven by sur promoter could provide a new approach for enhancing selective suicide gene therapy of CD/5-FC for the treatment of advanced gastric carcinoma.

PMID:
20574453

Leukemia. 2010 Jun 24. [Epub ahead of print]

Correction of B-cell development in Btk-deficient mice using lentiviral vectors with codon-optimized human BTK.

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X-linked agammaglobulinemia (XLA) is the most common primary immunodeficiency (PID) in man and caused by mutations in the Bruton's tyrosine kinase (BTK) gene. XLA is characterized by a B-cell differentiation arrest in bone marrow, absence of mature B cells and immunoglobulins (Igs), and recurrent bacterial infections. We used self-inactivating lentiviral vectors expressing codon-optimized human BTK under the control of three different ubiquitous or B cell-specific promoters. Btk^{-/-} mice engrafted with transduced cells showed correction of both precursor B-cell and peripheral B-cell development. Lentiviral vectors containing the wildtype BTK sequence did not correct the phenotype. All treated mice with codon-optimized BTK exhibited the recovery of B1 cells in the peritoneal cavity, and of serum IgM and IgG3 levels. Calcium mobilization responses upon B-cell receptor stimulation as well as in vivo responses to T cell-independent antigens were restored. Viral promoters overexpressing BTK >100-fold above normal resulted in erythro-myeloid proliferations independent of insertional mutagenesis. However, transplantation into secondary Btk^{-/-} recipients using cellular promoters resulted in functional restoration of peripheral B cells and IgM levels, without any adverse effects. In conclusion, transduction of human BTK corrects B-cell development and antigen-specific antibody responses in Btk^{-/-} mice, thus indicating the feasibility of lentiviral gene therapy for XLA, provided that BTK expression does not vastly exceed normal levels.

PMID:
20574029

Invest Ophthalmol Vis Sci. 2010 Jun 23. [Epub ahead of print]

Progressive Loss of Cones in Achromatopsia. An Imaging Study using Spectral-Domain Optical Coherence Tomography.

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Purpose: Achromatopsia (ACHM) is a congenital autosomal recessive cone disorder with a presumed stationary nature and only a few causative genes. Animal studies suggest that ACHM may be a good candidate for corrective gene therapy. Future implementation of this therapy in humans requires the presence of viable cone cells in the retina. We investigated the presence of cone cells in ACHM as a function of age. Methods: We evaluated the appearance and thickness of all retinal layers in 40 ACHM patients (age range 4-70 years) with known mutations in the CNGB3, CNGA3 and PDE6C genes using spectralis domain optical coherence tomogram (SD-OCT; Heidelberg Spectralis). A comparison was made with 55 healthy age-matched controls. Results: The initial feature of cone cell decay was loss of inner- and outer segments with disruption of the ciliary layer on OCT, which was observed as early as age 8 years. Cone cell loss further progressed with age, and occurred in 8/19 (42%) patients below 30 years, and in 20/21 (95%) of those aged 30+ years. Retinal thickness was significantly thinner in the fovea of all patients (126 μ m in ACHM vs 225 μ m in controls, $P < 0.001$); this correlated with age ($\beta = 0.065$; $P = 0.011$). Fovea hypoplasia was present in 24/30 (80%) of patients, and in 1/55 controls. Conclusions: ACHM is not a stationary disease. The first signs of cone cell loss already occur in early childhood. If intervention becomes available in the future, our results imply that this should be applied in the first decades.

PMID:
20573836

J Virol. 2010 Jun 23. [Epub ahead of print]

V α 14iNKT cells Promote Liver Pathology during Adenovirus Infection by Inducing CCL5 Production: Implications for Gene Therapy.

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Replication-defective recombinant Adenoviruses are the most widely studied replication-defective vectors for the potential treatment of inherited human diseases. However, broad clinical application of replication-defective Adenoviruses in gene therapy is being hindered by the induction of vigorous innate and adaptive immune responses against the vector which causes deleterious effects in the liver. V α 14 invariant natural killer T (V α 14iNKT) cells are thymic-derived innate T cells at the interface between the two arms of the immune response and provide full engagement of host defense. The pathophysiological role of intrahepatic V α 14iNKT cells during replication-defective Adenovirus infection is not known, and is the main focus of our study. Our data showed that intrahepatic V α 14iNKT cells were activated in response to Adenovirus infection to induce significant levels of hepatic CCL5 and subsequent liver toxicity. Moreover, intrahepatic CCL5 production was selectively reduced by V α 14iNKT cell deficiency. In vivo studies utilizing CCL5 deficient mice or V α 14iNKT cell deficient mice demonstrated that CCL5 or V α 14iNKT cell deficiency were each associated with reduced liver pathology. Similar results were seen after blocking the biological effects of the CCL5 receptors. In conclusion, we have identified an important pro-inflammatory role for activated intrahepatic V α 14iNKT cells in positively influencing hepatic CCL5 production to promote acute liver inflammation and injury. Therefore, our findings highlight the blockade of CCL5 interaction with cognate receptor(s) as an important potential strategy to alleviate liver pathology associated with replication-defective Adenovirus infection.

PMID:
20573316

J Biomater Sci Polym Ed. 2010 Jun 22. [Epub ahead of print]

A Comparative Evaluation of Disulfide-Linked and Hydrophobically-Modified PEI for Plasmid Delivery.

C RB, Uludağ H.

Non-viral gene therapy has become an important approach for treatment of hereditary and acquired diseases as a result of better understanding of molecular mechanisms involved in disease development. To design more effective gene carriers, plasmid DNA (pDNA) delivery to 293T cells was investigated by using two types of polymeric carriers; polymer constructed with disulfide (-S-S-) linkages and polymers modified with hydrophobic moieties. The base polymer used for this study was 2-kDa poly(ethylene imine) (PEI2), a relatively cell-compatible but ineffective gene carrier. The -S-S- linking was achieved via Michael addition reaction using cystamine bisacrylamide (CBA), whereas hydrophobic modification by N-acylation of PEI2 amines with palmitoyl chloride (PA). The cytotoxicity of the polymers was found to be lower than that of the 25-kDa branched PEI, but both types of modifications increased the toxicity of PEI2 to some extent. The polymers were able to form polyplexes with pDNA with variable hydrodynamic sizes (130-600 nm) and zeta-potential (3.6-20.9 mV). Based on the expression of the reporter gene Enhanced Green Fluorescent Protein (EGFP), disulfide linking significantly increased the efficiency of native PEI2, which was not effective on its own. The PA-modified PEI2 was also effective for gene delivery, but disulfide linkage of this polymer did not increase its efficiency any further. Our results showed that hydrophobic modification of 2-kDa PEI significantly improved its transfection efficiency but improvements in transfection efficiency as a result of disulfide linking was dependent on the nature of the polymeric building blocks.

PMID:
20573280

J Biomed Sci. 2010 Jun 24;17(1):51. [Epub ahead of print]

In vitro and in vivo targeted delivery of IL-10 interfering RNA by JC virus-like particles.

Chou MI, Hsieh YF, Wang M, Chang JT, Chang D, Zouali M, Tsay GJ.

ABSTRACT: **BACKGROUND:** RNA interference (RNAi) is a powerful tool to silence gene expression post-transcriptionally. Delivering sequences of RNAi in vivo remains a problem. The aim of this study was to use JC virus (JCV) virus-like particles (VLPs) as a vector for delivering RNAi in silencing the cytokine gene of IL-10. **METHODS:** JCV VLPs were generated by recombinant JCV VP1 protein in yeast expression system. DNA fragment containing IL-10 shRNA was packaged into VLPs by osmotic shock. **RESULTS:** In RAW 264.7 cells, IL-10 shRNA was found to reduce IL-10 expression by 85 to 89%, as compared with VLPs alone. IL-10 shRNA did not cross-react with TNF-alpha mRNA or influence the expression of TNF-alpha. In BALB/c mice IL-10 shRNA could reduce 95% of IL-10 secretion. Surprisingly, it also down regulated TNF-alpha expression. **CONCLUSIONS:** We show for the first time that JCV VLPs empty capsids are competent vectors to deliver RNAi and are nontoxic to cells, suggesting that JCV VLPs is an efficient agent to deliver RNAi in both murine macrophage cells and BALB/c mice. This system provides an efficient means for delivering the RNAi for gene therapy purposes.

PMID:
20572772

Annu Rev Neurosci. 2010;33:441-72.

The genomic, biochemical, and cellular responses of the retina in inherited photoreceptor degenerations and prospects for the treatment of these disorders.

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The association of more than 140 genes with human photoreceptor degenerations, together with studies of animal models of these monogenic diseases, has provided great insight into their pathogenesis. Here we review the responses of the retina to photoreceptor mutations, including mechanisms of photoreceptor death. We discuss the roles of oxidative metabolism, mitochondrial reactive oxygen species, metabolic stress, protein misfolding, and defects in ciliary proteins, as well as the responses of Müller glia, microglia, and the retinal vasculature. Finally, we report on potential pharmacologic and biologic therapies, the critical role of histopathology as a prerequisite to treatment, and the exciting promise of gene therapy in animal models and in phase 1 trials in humans.

PMID:
20572313

World J Gastroenterol. 2010 Jun 28;16(24):3078-82.

Construction and characterization of calreticulin-HBsAg fusion gene recombinant adenovirus expression vector.

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AIM: To generate recombinant adenoviral vector containing calreticulin (CRT)-hepatitis B surface antigen (HBsAg) fusion gene for developing a safe, effective and HBsAg-specific therapeutic vaccine. **METHODS:** CRT and HBsAg gene were fused using polymerase chain reaction (PCR), endonuclease digestion and ligation methods. The fusion gene was cloned into pENTR/D-TOPO transfer vector after the base pairs of DNA (CACC) sequence was added to the 5' end. Adenoviral expression vector containing CRT-HBsAg fusion gene was constructed by homologous recombination. The human embryo kidney (HEK) 293A cells were transfected with linearized DNA plasmid of the recombinant adenoviral vector to package and amplify recombinant adenovirus. The recombinant adenovirus titer was characterized using the end-dilution assay. The expression of the CRT/HBsAg fusion protein in Ad-CRT/HBsAg infected 293A cells was detected by Western blotting. **RESULTS:** The CRT-HBsAg fusion gene was characterized by PCR and sequencing and its length and sequence were confirmed to be accurate. The CRT-HBsAg fusion gene recombinant pENTR/D-TOPO transfer vector was constructed. The recombinant adenoviral vector, Ad-CRT/HBsAg, was generated successfully. The titer of Ad-CRT/HBsAg was characterized as 3.9×10^{11} pfu/mL. The CRT-HBsAg fusion protein was expressed by HEK 293A cells correctly. **CONCLUSION:** CRT/HBsAg fusion gene recombinant replication-defective adenovirus expression vector is constructed successfully and this study has provided an experimental basis for further studies of Hepatitis B virus gene therapy.

PMID:
20571977

Curr Opin Investig Drugs. 2010 Jul;11(7):813-22.

NLX-P101, an adeno-associated virus gene therapy encoding glutamic acid decarboxylase, for the potential treatment of Parkinson's disease.

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Parkinson's disease (PD) is a neurodegenerative disease affecting nigrostriatal dopaminergic neurons. Dopamine depletion in the striatum leads to functional changes in several deep brain nuclei, including the subthalamic nucleus (STN), which becomes disinhibited and perturbs the control of body movement. Although there is no cure for PD, some pharmacological and surgical treatments can significantly improve the functional ability of patients, particularly in the early stages of the disease. Among neurodegenerative diseases, PD is a particularly suitable target for gene therapy because the neuropathology is largely confined to a relatively small region of the brain. Neurologix Inc is developing NLX-P101 (AAV2-GAD), an adeno-associated viral vector encoding glutamic acid decarboxylase (GAD), for the potential therapy of PD. As GAD potentiates inhibitory neurotransmission from the STN, sustained expression of GAD in the STN by direct delivery of NLX-P101 decreases STN overactivation. This procedure was demonstrated to be a safe and efficient method of reducing motor deficits in animal models of PD. A phase I clinical trial has demonstrated that NLX-P101 was safe and indicated the efficacy of this approach in patients with PD. Results from an ongoing phase II clinical trial of NLX-P101 are awaited to establish the clinical efficacy of this gene therapy.

PMID:
20571866

J Inherit Metab Dis. 2010 Jun 23. [Epub ahead of print]

Mitochondrial medicine: to a new era of gene therapy for mitochondrial DNA mutations.

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Mitochondrial disorders can no longer be ignored in most medical disciplines. Such disorders include specific and widespread organ involvement, with tissue degeneration or tumor formation. Primary or secondary actors, mitochondrial dysfunctions also play a role in the aging process. Despite progresses made in identification of their molecular bases, nearly everything remains to be done as regards therapy. Research dealing with mitochondrial physiology and pathology has >20 years of history around the world. We are involved, as are many other laboratories, in the challenge of finding ways to fight these diseases. However, our main limitation is the scarcity of animal models required for both understanding the molecular mechanisms underlying the diseases and evaluating therapeutic strategies. This is especially true for diseases due to mutations in mitochondrial DNA (mtDNA), since an authentic genetic model of mtDNA mutations is technically a very difficult task due to both the inability of manipulating the mitochondrial genome of living mammalian cells and to its multicopy nature. This has led researchers in the field to consider the prospect of gene therapy approaches that can roughly be divided into three groups: (1) import of wild-type copies or relevant sections of DNA or RNA into mitochondria, (2) manipulation of mitochondrial genetic content, and (3) rescue of a defect by expression of an engineered gene product from the nucleus (allotopic or xenotropic expression). We briefly introduce these concepts and indicate where promising progress has been made in the last decade.

PMID:
20571544

Mol Ther. 2010 Jun 22. [Epub ahead of print]

Neonatal Gene Therapy of Glycogen Storage Disease Type Ia Using a Feline Immunodeficiency Virus-based Vector.

Grinshpun A, Condiotti R, N Waddington S, Peer M, Zeig E, Peretz S, Simerzin A, Chou J, Pann CJ, Giladi H, Galun E.

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Glycogen storage disease type Ia (GSD-Ia), also known as von Gierke disease, is caused by a deficiency of glucose-6-phosphatase-alpha (G6Pase), a key enzyme in glucose homeostasis. From birth, affected individuals cannot maintain normal blood glucose levels and suffer from a variety of metabolic disorders, leading to life-threatening complications. Gene therapy has been proposed as a possible option for treatment of this illness. Vectors have been constructed from feline immunodeficiency virus (FIV), a nonprimate lentivirus, because the wild-type virus does not cause disease in humans. Previously, we have shown that these vectors are capable of integrating stably into hepatocyte cell lines and adult murine livers and lead to long-term transgene expression. In the current work, we have assessed the ability to attenuate disease symptoms in a murine model of GSD-Ia. Single administration of FIV vectors containing the human G6Pase gene to G6Pase-alpha(-/-) mice did not change the biochemical and pathological phenotype. However, a double neonatal administration protocol led to normalized blood glucose levels, significantly extended survival, improved body weight, and decreased accumulation of liver glycogen associated with the disease. This approach shows a promising paradigm for treating GSD-Ia patients early in life thereby avoiding long-term consequences.

PMID:
20571542

Mol Ther. 2010 Jun 22. [Epub ahead of print]

Ex Vivo Expansion of Retrovirally Transduced Primate CD34(+) Cells Results in Overrepresentation of Clones With MDS1/EVI1 Insertion Sites in the Myeloid Lineage After Transplantation.

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Activation of proto-oncogenes by retroviral insertion is an important issue delaying clinical development of gene therapy. We have reported the nonrandom persistence of hematopoietic clones with vector insertions within the MDS1/EVI1 locus following transplantation of rhesus macaques. We now ask whether prolonged culture of transduced CD34(+) cells before transplantation selects for clones with insertions in the MDS1/EVI1 or other proto-oncogene loci. CD34(+) cells were transduced with standard retroviral vectors for 4 days and then continued in culture for an additional 6 days before transplantation. A 15% of insertions identified in granulocytes 6 months post-transplant were in MDS1/EVI1, significantly increased compared to the frequency in animals transplanted with cells immediately following transduction. MDS1/EVI1 clones became more dominant over time post-transplantation in one animal that was followed long term, accompanied by an increased overall copy number of vector-containing granulocytes, with one MDS1/EVI1 clone eventually accounting for 100% of transduced granulocytes and marrow colony-forming unit (CFU). This vector insertion increased the expression of Evi1 mRNA. There was no overrepresentation of MDS1/EVI1 insertions contributing to lymphoid lineages. Strategies involving prolonged ex vivo expansion of transduced cells may increase the risk of genotoxicity.

PMID:
20569532

Expert Rev Mol Med. 2010 Jun 23;12:e18.

Gene therapy for bone healing.

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Clinical problems in bone healing include large segmental defects, spinal fusions, and the nonunion and delayed union of fractures. Gene-transfer technologies have the potential to aid healing by permitting the local delivery and sustained expression of osteogenic gene products within osseous lesions. Key questions for such an approach include the choice of transgene, vector and gene-transfer strategy. Most experimental data have been obtained using cDNAs encoding osteogenic growth factors such as bone morphogenetic protein-2 (BMP-2), BMP-4 and BMP-7, in conjunction with both nonviral and viral vectors using in vivo and ex vivo delivery strategies. Proof of principle has been convincingly demonstrated in small-animal models. Relatively few studies have used large animals, but the results so far are encouraging. Once a reliable method has been developed, it will be necessary to perform detailed pharmacological and toxicological studies, as well as satisfy other demands of the regulatory bodies, before human clinical trials can be initiated. Such studies are very expensive and often protracted. Thus, progress in developing a clinically useful gene therapy for bone healing is determined not only by scientific considerations, but also by financial constraints and the ambient regulatory environment.

PMID:
20569421

Respir Res. 2010 Jun 23;11(1):84. [Epub ahead of print]

Lysophosphatidylcholine as an adjuvant for lentiviral vector mediated gene transfer to airway epithelium: effect of acyl chain length.

Cmielewski P, Anson DS, Parsons DW.

ABSTRACT: BACKGROUND: Poor gene transfer efficiency has been a major problem in developing an effective gene therapy for cystic fibrosis (CF) airway disease. Lysophosphatidylcholine (LPC), a natural airway surfactant, can enhance viral gene transfer in animal models. We examined the electrophysiological and physical effect of airway pre-treatment with variants of LPC on lentiviral (LV) vector gene transfer efficiency in murine nasal airways in vivo. **METHODS:** Gene transfer was assessed after 1 week following nasal instillations of a VSV-G pseudotype LV vector pre-treated with a low and high dose of LPC variants. The electrophysiological effects of a range of LPC variants were assessed by nasal transepithelial potential difference measurements (TPD) to determine tight junction permeability. Any physical changes to the epithelium from administration of the LPC variants were noted by histological methods in airway tissue harvested after 1 hour. **RESULTS:** Gene transduction was significantly greater compared to control (PBS) for our standard LPC (palmitoyl/stearoyl mixture) treatment and for the majority of the other LPC variants with longer acyl chain lengths. The LPC variant heptadecanoyl also produced significantly greater LV gene transfer compared to our standard LPC mixture. LV gene transfer and the transepithelial depolarization produced by the 0.1% LPC variants at 1 hour were strongly correlated ($r^2=0.94$), but at the 1% concentration the correlation was less strong ($r^2=0.59$). LPC variants that displayed minor to moderate levels of disruption to the airway epithelium were clearly associated with higher LV gene transfer. **CONCLUSIONS:** These findings show the LPC variants effect on airway barrier function and their correlation to the effectiveness of gene expression. The enhanced expression produced by a number of LPC variants should provide new options for preclinical development of efficient airway gene transfer techniques.

PMID:
20566345

J Biotechnol. 2010 May 26. [Epub ahead of print]

Analysis of adsorption of a baculovirus bioreaction bulk on an ion-exchange surface by surface plasmon resonance.

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The binding and elution of the key components of a bioreaction bulk for the production of recombinant baculoviruses—a promising viral vector for gene therapy and vaccination—on a model ion-exchange surface have been successfully measured and interpreted by surface plasmon resonance (SPR) spectroscopy. The micro-scaled, ion-exchange surface was produced by immobilizing a typical ion-exchange ligand, diethylaminoethyl, onto commercially available planar gold sensor chip surfaces, which were pre-derivatized with a self-assembled monolayer of 11-mercaptopundecanoic acid. Each isolated analyte was injected into the SPR cell at defined operating conditions of salt and solute concentrations to determine the adsorption equilibrium plateau, and then eluted at the same salt concentration, upon which a well-defined, residual desorption equilibrium plateau was observed. From the analysis of the binding and elution curves and equilibrium plateaus for seven key biomolecules, it is possible to determine the adsorption isotherms over a broad range of equilibrium conditions for the three main cuts of the baculovirus bioreaction bulk: the product (the infective baculovirus), the main product-related impurities, and the main process-related impurities. A model that quantitatively explains the SPR-derived adsorption/desorption data was successfully developed for this complex biological system.

PMID:
20565749

J Exp Clin Cancer Res. 2010 Jun 17;29(1):74. [Epub ahead of print]

Comparison of the inhibitory effects of three transcriptional variants of CDKN2A in human lung cancer cell line A549.

Zhang W, Zhu J, Bai J, Jiang H, Liu F, Liu A, Liu P, Ji G, Guan R, Sun D, Ji W, Yu Y, Jin Y, Meng X, Fu S.

ABSTRACT: Background The tumor suppressor gene CDKN2A generates at least three different transcriptional variants, each of which is thought to encode a tumor suppressor. However, the inhibitory activities of these variants have not yet been compared in the same cells. Protein therapy is known to have several advantages over gene therapy. Thus, investigation of the exogenous protein molecule of the most effective suppressor may yield meaningful information regarding protein-based cancer therapy. Methods The inhibitory effects of p16INK4a, p14ARF and p12 were studied in the human lung cancer cell line A549 which lacks the CDKN2A locus. The eukaryotic expression plasmids of the three transcriptional variants were constructed and stably transfected into the cells. RNA and protein expression by the plasmids was confirmed using RT-PCR and fluorescence immunocytochemistry, respectively. Cell growth inhibition and cell-cycle redistribution after transfection were investigated based on growth curve and flow cytometry analyses. An exogenous His-tag fusion p16INK4a protein was obtained and purified by affinity chromatography. Cell growth inhibition and cell cycle arrest induced by the expression of p16INK4a protein were measured in A549 cells transduced with the exogenous protein. Results While all three variants suppressed cell growth, p16INK4a had the strongest effect. Marked G1-phase accumulation and S-phase inhibition were induced by p16INK4a and p14ARF but not by p12. Exogenous p16INK4a protein was successfully expressed and purified and transduction of the fusion protein into A549 cells inhibited cell growth by G1->S arrest. Conclusions Among the three transcript variants, p16INK4a has a greater inhibitory effect than p14ARF and p12; exogenous p16INK4a protein should be further investigated for use in cancer therapy as a protein agent.

PMID:
20564205

J Cell Biochem. 2010 May 19. [Epub ahead of print]

VEGF receptor binding peptide-linked high mobility box group-1 box A as a targeting gene carrier for hypoxic endothelial cells.

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High mobility group box-1 (HMGB-1) is a nuclear protein that can bind to and condense plasmid DNA. In this study, we developed a recombinant VEGF receptor binding peptide (VRBP) linked to HMGB-1 box A (VRBP-HMGB1A) as a targeting gene carrier to hypoxic endothelial cells. Hypoxic endothelial cells in ischemic tissues of solid tumors are important targets for gene therapy. A recombinant VRBP-HMGB1A expression vector, pET21a-VRBP-HMGB1A was constructed. VRBP-HMGB1A was over-expressed in BL21 strain and purified by nickel-chelate affinity chromatography. Complex formation between VRBP-HMGB1A and pCMV-Luc was confirmed by gel retardation assay. pCMV-Luc was retarded completely at a 2/1 weight ratio (peptide/plasmid). For transfection assays, calf pulmonary artery endothelial (CPAE) cells were incubated under hypoxia for 24 h, prior to transfection to induce the VEGF receptors on the cells. VRBP-HMGB1A/pCMV-Luc complexes were transfected to hypoxic CPAE cells. The highest transfection efficiency was at a 30/1 weight ratio (peptide/plasmid). In addition, VRBP-HMGB1A had higher efficiency than poly-L-lysine (PLL) specifically in hypoxic CPAE cells, However, VRBP-HMGB1A had lower efficiency than PLL in 293, H9C2, and normoxic CPAE cells. In MTT assay, VRBP-HMGB1A was less toxic than PLL to cells. In conclusion, VRBP-HMGB1A is a potential gene carrier for targeting hypoxic endothelial cells and thus, may be useful for cancer gene therapy. J. Cell. Biochem.

PMID:
20563754

Mol Imaging Biol. 2010 Jun 19. [Epub ahead of print]

[(18)F]FLT PET for Non-Invasive Monitoring of Early Response to Gene Therapy in Experimental Gliomas.

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The purpose of this study was to investigate the potential of 3'-deoxy-3'-[(18)F]fluorothymidine [(18)F]FLT positron emission tomography (PET) to detect early treatment responses in gliomas. Human glioma cells were stably transduced with genes yielding therapeutic activity, sorted for different levels of exogenous gene expression, and implanted subcutaneously into nude mice. Multimodality imaging during prodrug therapy included (a) magnetic resonance imaging, (b) PET with 9-(4-[(18)F]fluoro-3-hydroxymethylbutyl)guanine assessing exogenous gene expression, and (c) repeat [(18)F]FLT PET assessing antiproliferative therapeutic response. All stably transduced gliomas responded to therapy with significant reduction in tumor volume and [(18)F]FLT accumulation within 3 days after initiation of therapy. The change in [(18)F]FLT uptake before and after treatment correlated to volumetrically calculated growth rates. Therapeutic efficacy as monitored by [(18)F]FLT PET correlated to levels of therapeutic gene expression measured in vivo. Thus, [(18)F]FLT PET assesses early antiproliferative effects, making it a promising radiotracer for the development of novel treatments for glioma.

PMID:
20563597

J Cancer Res Clin Oncol. 2010 Jun 19. [Epub ahead of print]

Triple expression of B7-1, B7-2 and 4-1BBL enhanced antitumor immune response against mouse H22 hepatocellular carcinoma.

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OBJECTIVES: Costimulatory signals are essential for T-cell activation and hence play a very important role in antitumor immunity. B7 and 4-1BBL which belongs to tumor necrosis factor (TNF) family provide costimulatory interaction for T-cell activation and function. This study investigated the role of B7 and 4-1BBL in the amplification of tumor immunity by transduction of the B7-1, B7-2 and 4-1BBL into mouse hepatocellular carcinoma cell line H22. **METHODS:** The tumorigenicity of H22 variants expressing either B7-1, B7-2 (H22/B7-1/B7-2) or 4-1BBL was compared with an H22 variant expressing B7-1, B7-2 and 4-1BBL (H22/B7-1/B7-2/4-1BBL). The study next investigated whether the combination of B7-1/B7-2 and 4-1BBL cell injection induced cytotoxic T lymphocyte (CTL) response and IL-2/IFN-gamma secretion. The immune mechanisms underlying this combination treatment were then analyzed. **RESULTS:** Syngeneic BALB/c mice injected with H22/B7-1/B7-2/4-1BBL cells that expressed elevated levels of B7-1, B7-2 and 4-1BBL showed a tumor development frequency of 50% compared with 100% in mice injected with the H22 parental line, H22/neo, H22/B7-1/B7-2 and H22/4-1BBL. Mice inoculated with H22 tumor cells expressing B7-1, B7-2 and 4-1BBL developed a strong cytotoxic T lymphocyte response and long-term immunity against wild-type tumor, suggesting a synergistic effect between the B7 and 4-1BBL costimulatory pathways. Results showed that H22/B7-1/B7-2/4-1BBL tumor vaccines probably protect the infiltrating lymphocytes from apoptosis and induce NF-kappaB activation to improve T-cell-mediated antitumor response. **CONCLUSIONS:** In this study, the antitumor consequences of using B7-1, B7-2 and 4-1BBL gene transfer have demonstrated the therapeutic potential of gene therapy approach for hepatocellular carcinoma.

PMID:
20561681

Biomaterials. 2010 Jun 17. [Epub ahead of print]

Liposome-polyethylenimine complexes for enhanced DNA and siRNA delivery.

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The efficient delivery of nucleic acids into cells is critical for successful gene therapy or gene knockdown. Polyethylenimines (PEIs) are positively charged polymers which complex and deliver DNA for gene transfection or small interfering RNAs (siRNAs) for the induction of RNA interference (RNAi), and mediate their endosomal release. Likewise, various liposomes act as transfection reagents, with some lipids showing increased endocytosis and influencing endosomal escape. This study combines the favourable properties of PEI and lipid systems for DNA and siRNA delivery. Various lipids and lipid combinations, which cover a broad range of physicochemical properties and form optimal liposomes, are assessed. By addition of the liposomes to pre-formed polyplexes, based on the low molecular weight PEI F25-LMW, we establish liposome-PEI complexes (lipopolyplexes) and characterise them in comparison to their 'parent' polyplexes and liposomes regarding size, shape and zeta-potential. Furthermore, while the lipidation of polyplexes generally decreases their toxicity, our studies on DNA transfection and siRNA-mediated knockdown also establish certain lipopolyplexes based on rigid, negatively charged lipids as particularly efficient vehicles for nucleic acid delivery, further improving DNA transfection. The analysis of their mechanism and kinetics of cellular uptake confirms that the biological properties of lipopolyplexes are mainly determined by the liposome shell. We conclude that certain lipopolyplexes show improved biological properties over PEI complexes, thus representing potentially attractive non-viral vectors for gene therapy and RNAi. Copyright © 2010 Elsevier Ltd. All rights reserved.

PMID:
20560798

Expert Opin Ther Targets. 2010 Jun 20. [Epub ahead of print]

Targeting the TLR9-MyD88 pathway in the regulation of adaptive immune responses.

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Importance of the field: Toll-like receptors (TLRs) are innate immune receptors critical in the innate immune defense against invading pathogens. Recent advances also reveal a crucial role for TLRs in shaping adaptive immune responses, conferring a potential therapeutic value to their modulation in the treatment of diseases. Areas covered in this review: The aim of this review is to discuss TLR9, the TLR9-MyD88 signaling pathway and its role in regulation of adaptive immune responses, as well as potential therapeutic implications by targeting this pathway. What the reader will gain: This review shows that the TLR9-MyD88 signaling pathway plays a critical role in promoting adaptive immune responses and that modulation of this pathway may have enormous therapeutic potential in enhancing vaccine potency, controlling autoimmunity, as well as improving the outcome of viral-vector-mediated gene therapy. Take home message: Although TLR9 agonists have been used as adjuvants for enhancing vaccine potency, further exploitation of the TLR9-MyD88 pathway and its dynamic interaction with the immune system in vivo is needed to provide more effective therapeutic inventions in the design of vaccines for infectious diseases, allergies and cancer, in the control of autoimmunity, as well as in the improvement of viral-vector-mediated gene therapy.