



CLINIGENE CURRENT GENE THERAPY WEEKLY

From February 1st to February 8th 2010

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PMID:
20135358

In Vitro Cell Dev Biol Anim. 2010 Feb 5. [Epub ahead of print]

Side populations of glioblastoma cells are less sensitive to HSV-TK/GCV suicide gene therapy system than the non-side population.

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Side populations of glioblastoma cells are resistant to chemotherapy basically due to ABCG2-mediated efflux of small-molecule drugs. The herpes simplex virus thymidine kinase/ganciclovir suicide gene therapy system is one of the best-characterized strategies for malignant tumors including glioblastoma. Since this system involves a small-molecule drug ganciclovir, we wonder if glioblastoma side population cells are able to "pump out" ganciclovir and thus resistant to this suicide gene therapy. By 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay, we found that side populations are more resistant to this system than non-side populations. By flow cytometry and competition assay, we found that ganciclovir is a substrate for ABCG2.

PMID:
20134468

Nat Med. 2010 Feb;16(2):163-5.

Gene therapy activates EVI1, destabilizes chromosomes.

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No abstract available

PMID:
20133969

In Vivo. 2010 Jan-Feb;24(1):1-8.

Effectiveness of Combined Modality Radiotherapy of Orthotopic Human Squamous Cell Carcinomas in Nu/Nu Mice Using Cetuximab, Tirapazamine and MnSOD-Plasmid Liposome Gene Therapy.

Epperly MW, Lai SY, Kanai AJ, Mason N, Lopresi B, Dixon T, Franicola D, Niu Y, Wilson WR, Greenberger JS.

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Hypoxic regions limit the radiocontrollability of head and neck carcinomas. Whether or not combinations of plasmid/liposome mediated overexpression of normal tissue protective manganese superoxide dismutase (MnSOD), cetuximab (C225), and the hypoxic cytotoxin tirapazamine (TPZ) enhanced radiotherapeutic effects was tested in a CAL-33 orthotopic mouse cheek tumor model. The tumor volume continued to increase in the control (untreated) mice, with a ninefold increase by 10 days when the tumors exceeded 2 cm³. The mice receiving 14 Gy only showed reduced tumor growth to 3.1±0.1 fold at day 10. The mice receiving MnSOD-PL, C225, TPZ plus 14 Gy had the best outcome with 0.7±0.1 fold increase in tumor volume by 10 days (p=0.015) compared to irradiation only. The addition of MnSOD-PL, TPZ, and C225 to irradiation optimized the therapeutic ratio for the local control of hypoxic region-containing CAL-33 orthotopic tumors.

PMID:
20133756

Proc Natl Acad Sci U S A. 2010 Jan 26. [Epub ahead of print]

Reengineering orthogonally selective riboswitches.

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The ability to independently control the expression of multiple genes by addition of distinct small-molecule modulators has many applications from synthetic biology, functional genomics, pharmaceutical target validation, through to gene therapy. Riboswitches are relatively simple, small-molecule-dependent, protein-free, mRNA genetic switches that are attractive targets for reengineering in this context. Using a combination of chemical genetics and genetic selection, we have developed riboswitches that are selective for synthetic "nonnatural" small molecules and no longer respond to the natural intracellular ligands. The orthogonal selectivity of the riboswitches is also demonstrated in vitro using isothermal titration calorimetry and x-ray crystallography. The riboswitches allow highly responsive, dose-dependent, orthogonally selective, and dynamic control of gene expression in vivo. It is possible that this approach may be further developed to reengineer other natural riboswitches for application as small-molecule responsive genetic switches in both prokaryotes and eukaryotes.

PMID:
20133638

Proc Natl Acad Sci U S A. 2010 Feb 1. [Epub ahead of print]

Lens epithelium-derived growth factor fusion proteins redirect HIV-1 DNA integration.

Ferris AL, Wu X, Hughes CM, Stewart C, Smith SJ, Milne TA, Wang GG, Shun MC, Allis CD, Engelman A, Hughes SH.

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Lens epithelium-derived growth factor (LEDGF) fusion proteins can direct HIV-1 DNA integration to novel sites in the host genome. The C terminus of LEDGF contains an integrase binding domain (IBD), and the N terminus binds chromatin. LEDGF normally directs integrations to the bodies of expressed genes. Replacing the N terminus of LEDGF with chromatin binding domains (CBDs) from other proteins changes the specificity of HIV-1 DNA integration. We chose two well-characterized CBDs: the plant homeodomain (PHD) finger from ING2 and the chromodomain from heterochromatin binding protein 1alpha (HP1alpha). The ING2 PHD finger binds H3K4me3, a histone mark that is associated with the transcriptional start sites of expressed genes. The HP1alpha chromodomain binds H3K9me2,3, histone marks that are widely distributed throughout the genome. A fusion protein in which the ING2 PHD finger was linked to the LEDGF IBD directed integrations near the start sites of expressed genes. A similar fusion protein in which the HP1alpha chromodomain was linked to the LEDGF IBD directed integrations to sites that differed from both the PHD finger fusion-directed and LEDGF-directed integration sites. The ability to redirect HIV-1 DNA integration may help solve the problems associated with the activation of oncogenes when retroviruses are used in gene therapy.

PMID:
20133233

Hepatobiliary Pancreat Dis Int. 2010 Feb;9(1):69-77.

Growth inhibition induced by short hairpin RNA to silence survivin gene in human pancreatic cancer cells.

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BACKGROUND: Survivin is known to be overexpressed in various human malignancies, including pancreatic cancer, and mediates cancer cell proliferation and tumor growth, so the regulation of this molecule could be a new strategy for treating pancreatic cancer. In this study, short hairpin RNAs (shRNAs) specific to survivin were introduced into human pancreatic cancer Patu8988 cells to investigate the inhibitory effects on survivin expression and cell proliferation in vitro and in vivo. **METHODS:** Three kinds of shRNA specific to the survivin gene were designed and cloned into eukaryotic expression plasmid pGenesil-1 vector. Subsequently the recombinant plasmids were transfected into human pancreatic cancer Patu8988 cells with lipfectamineTM 2000 reagent. The mRNA and protein expressions of survivin in the transiently transfected Patu8988 cells were determined by RT-PCR, flow cytometry, and Western blotting analysis. The proliferation inhibition rates of stably transfected Patu8988 cells were determined by MTT assay. The antitumor activities of the three kinds of survivin-shRNA plasmids were evaluated in BALB/c nude mice inoculated with Patu8988 cells and bearing human pancreatic cancer. **RESULTS:** The three survivin-shRNA plasmids named pGenesil-1-survivin-1, pGenesil-1-survivin-2 and pGenesil-1-survivin-1+2 (with double interfering RNA sites) were successfully constructed, and were confirmed by restriction enzyme cutting and sequencing. At 48 hours after transfection, the expression of survivin mRNA and protein was inhibited in Patu8988 cells transfected with pGenesil-1-survivin-1, pGenesil-1-survivin-2, and pGenesil-1-survivin-1+2 when compared with that of either pGenesil-1-NC (with scrambled small interfering RNA) transfected cells or control cells ($P < 0.05$). The MTT results showed that the proliferation rates of Patu8988 cells stably transfected with survivin-shRNA plasmids were reduced when compared with that of either pGenesil-1-NC transfected cells or control cells ($P < 0.01$). Furthermore, when Patu8988 cells stably transfected with survivin-shRNA were injected into BALB/c nude mice, tumor growth was dramatically lower and the tumor was smaller than that of either pGenesil-1-NC transfected cells or control cells ($P < 0.01$). The inhibitory effect of pGenesil-1-survivin-1 was the best among the three kinds of survivin-shRNA plasmids, but no combination of inhibitory effects was found in pGenesil-1-survivin-1+2. **CONCLUSIONS:** shRNAs specific to survivin have gene silencing effects and inhibit pancreatic cancer cell proliferation. shRNA activity against survivin could be of potential value in gene therapy for pancreatic cancer. However, shRNAs with double combining sites did not significantly enhance the interference compared with single site shRNAs, therefore further studies on this are needed.

PMID:
20133039

Ultrasound Med Biol. 2010 Feb 2. [Epub ahead of print]

Sonoporation Mediated Immunogene Therapy of Solid Tumors.

Casey G, Cashman JP, Morrissey D, Whelan MC, Larkin JO, Soden DM, Tangney M, O'Sullivan GC.

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Development of gene-based therapies for the treatment of inherited and acquired diseases, including cancer, has seen renewed interest in the use of nonviral vectors coupled to physical delivery modalities. Low-frequency ultrasound (US), with a well-established record in a clinical setting, has the potential to deliver DNA efficiently, accurately and safely. Optimal in vivo parameters for US-mediated delivery of naked plasmid DNA were established using the firefly luciferase reporter gene construct. Optimized parameters were used to administer a therapeutic gene construct, coding for granulocyte-macrophage colony-stimulating factor (GM-CSF) and B7-1 costimulatory molecule, to growing murine fibrosarcoma tumors. Tumor progression and animal survival was monitored throughout the study and the efficacy of the US-mediated gene therapy determined and compared with an electroporation-based approach. Optimal parameters for US-mediated delivery of plasmid DNA to tumors were deduced to be 1.0 W/cm² at 20% duty cycle for 5 min (60 J/cm²). In vivo US-mediated gene therapy resulted in a 55% cure rate in tumor-bearing animals. The immunological response invoked was cell mediated, conferring resistance against re-challenge and resistance to tumor challenge after transfer of splenocytes to naïve animals. US treatment was noninjurious to treated tissue, whereas therapeutic efficacy was comparable to an electroporation-based approach. US-mediated delivery of an immune-gene construct to growing tumors was therapeutically effective. Sonoporation has the potential to be a major factor in the development of nonviral gene delivery approaches. (E-mail: gerald@iol.ie). Copyright © 2010 World Federation for Ultrasound in Medicine & Biology. Published by Elsevier Inc. All rights reserved.

PMID:
20132845

J Biotechnol. 2010 Feb 1. [Epub ahead of print]

A Bicistronic Lentiviral Vector Based on the 1D/2A Sequence of Foot-and-Mouth Disease Virus Expresses Proteins Stoichiometrically.

Torres V, Barra L, Garcés F, Ordenes K, Leal-Ortiz S, Garner CC, Fernandez F, Zamorano P. Department of Medical Technology.

Classic IRES sequences are notorious for exerting biased expression in favor of upstream coding regions when placed into polycistronic vectors. Here, we report the development of a bicistronic lentiviral system based on the 1D/2A sequence from the foot-and-mouth disease virus that is able to maintain tightly balanced control of upstream and downstream protein expression for several days at a stoichiometry very closely approaching 1.0. Our results suggest that the 1D/2A sequence can be optimized in an FUGW lentiviral setting to coordinate expression of multiple polypeptides, presenting a potentially valuable tool to signaling network researchers and to the gene therapy community.

PMID:
20132187

APMIS. 2010 Mar;118(3):210-21.

Evaluation of coxsackievirus and adenovirus receptor expression in human benign and malignant thyroid lesions.

Giaginis C, Zarros A, Alexandrou P, Klijanienko J, Delladetsima I, Theocharis S.
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Evaluation of coxsackievirus and adenovirus receptor expression in human benign and malignant thyroid lesions. APMIS 2010; 118: 210-21. Coxsackievirus and adenovirus receptor (CAR) expression on tumor cells is associated with sensitivity to adenoviral infection, being considered as a surrogate marker for monitoring and/or predicting adenovirus-mediated gene therapy. The aim of this study was to evaluate the clinical significance of CAR expression in human benign and malignant thyroid lesions. CAR protein expression was assessed immunohistochemically on paraffin-embedded thyroid tissues from 107 patients with benign and malignant lesions and was statistically analyzed in relation to histopathologic type; tumor size; lymph node metastasis; capsular, lymphatic and vessel invasion; as well as follicular cells' proliferative capacity. CAR immunoreactivity was characterized as negative/weak in 53 (49.53%), moderate in 31 (28.97%) and strong in 23 (21.50%) of 107 thyroid cases. CAR immunoreactivity was significantly increased in malignant compared with that in benign thyroid lesions ($p = 0.00002$). Both malignant and benign thyroid lesions with enhanced follicular cells' proliferative capacity showed significantly increased CAR immunoreactivity ($p = 0.00027$). In malignant thyroid lesions, enhanced CAR immunoreactivity was significantly associated with larger tumor size ($p = 0.0067$). The current data revealed that CAR immunoreactivity could be considered of diagnostic utility in thyroid neoplasia. Further research effort is warranted to delineate whether CAR could be considered clinically important for both diagnosis and future (gene) therapeutic applications in thyroid neoplasia.

PMID:
20132060

Expert Opin Biol Ther. 2010 Mar;10(3):395-408.

AAV-directed muscular dystrophy gene therapy.

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Importance of the field: Muscle-directed gene therapy for genetic muscle diseases can be performed by the recombinant adeno-associated viral (rAAV) vector delivery system to achieve long-term therapeutic gene transfer in all affected muscles. Areas covered in this review: Recent progress in rAAV-vector-mediated muscle-directed gene transfer and associated techniques for the treatment of muscular dystrophies (MD). The review covers literature from the past 2 - 3 years. What the reader will gain: rAAV-directed muscular dystrophy gene therapy can be achieved by mini-dystrophin replacement and exon-skipping strategies. The additional strategies of enhancing muscle regeneration and reducing inflammation in the muscle micro-environment should be useful to optimize therapeutic efficacy. This review compares the merits and shortcomings of different administration methods, promoters and experimental animals that will guide the choice of the appropriate strategy for clinical trials. Take home message: Restoration of muscle histopathology and function has been performed using rAAV systemic gene delivery. In addition, the combination of gene replacement and adjuvant therapies in the future may be beneficial with regard to improving muscle regeneration and decreasing myofiber necrosis. The challenges faced by large animal model studies and in human trials arise from gene transfer efficiency and immune response, which may be overcome by optimizing the rAAV vectors utilized and the administration methods.

PMID: Expert Opin Biol Ther. 2010 Mar;10(3):381-94.
20132059

Viral vector-mediated gene transfer for CNS disease.

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Importance of the field: Gene therapy is a promising strategy for the treatment of many neurological disorders that currently lack effective treatment. Recent improvements in vectorology and vector engineering have improved overall safety and delivery of viral vectors. Areas covered in this review: This review discusses the current state of viral vector development and clinical use, as well as routes of delivery, and clinical trials for neurological disorders. What the reader will gain: Viral vectors may be delivered directly or remotely to the CNS, largely depending on the nature of the disease and the tropism of the vector. Nonetheless, delivery remains one of the major limitations of successful gene transfer to the CNS. Take home message: Although the majority of clinical trials have centered on gene replacement and neuroprotection approaches, the field is advancing in the direction of neuromodulation, gene silencing and other newer strategies.

PMID: Expert Opin Biol Ther. 2010 Mar;10(3):353-68.
20132057

Oncolytic (replication-competent) adenoviruses as anticancer agents.

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Importance of the field: Whilst therapies for neoplasies have advanced tremendously in the last few decades, there is still a need for new anti-cancer treatments. One option is genetically-engineered oncolytic adenovirus (Ad) 'vectors'. These kill cancer cells via the viral replication cycle, and amplify the anti-tumor effect by producing progeny virions able to infect neighboring tumor cells. Areas covered in this review: We provide a description of basic Ad biology and summarize the literature for oncolytic Ads from 1996 to the present. What the reader will gain: An overall view of oncolytic Ads, the merits and drawbacks of the various features of these vectors, and obstacles to further development and future directions for research. Take home message: Ads are attractive for gene therapy because they are relatively innocuous, easy to produce in large quantities, genetically stable, and easy to manipulate. A variety of have been constructed and tested, in pre-clinical and clinical experiments. Oncolytic Ads proved to be remarkably safe; no dose-limiting toxicity was observed in any clinical trial, and the maximum tolerated dose was not reached. At present, the major challenge for researchers is to increase the efficacy of the vectors, and to incorporate oncolytic virotherapy into existing treatment protocols.

PMID:
20132050

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

Addition of a single E2 binding site to the HPV16 E2 long control region enhances killing of HPV-positive cells via HPV E2 protein-regulated HSV-1 thymidine kinase-mediated suicide gene therapy.

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Human papillomavirus type 16 (HPV16) is associated with the development of anogenital cancers and their precursor lesions, intraepithelial neoplasia (IN). Treatment strategies against HPV-induced IN are not HPV-specific and mostly consist of physical removal or ablation of lesions. We had previously designed an HPV-specific approach to killing HPV-infected cells using the herpes simplex virus type I thymidine kinase (HSV-1 TK) gene driven by HPV E2 binding to E2 binding sites in the native HPV16 long control region (LCR). E2-induced TK transcription renders the cells sensitive to the prodrug ganciclovir (GCV). To optimize this therapeutic approach, we modified the native LCR by adding variable numbers of E2 binding sites adjacent to E2 binding site 4 (E2BS4) resulting in greatly increased cell death in HPV-positive cell lines with variable levels of E2 protein expression and no reduction in HPV specificity. Our results showed maximum increase in TK transcription and cell killing when one additional E2 binding site was added adjacent to E2BS4. Since HPV-infected patients also exhibit variable E2 expression across lesions and within a lesion, this approach may potentiate the clinical utility of the HSV-1 TK/GCV therapeutic approach.

PMID:
20130655

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

Vaccination with a potent DNA vaccine targeting B-cell epitopes of hGRP induces prophylactic and therapeutic antitumor activity in vivo.

Yong L, Huiyong Z, Jing H, Huaqian W, Xiangbing H, Yanjun M, Xiaoyu G, Li H, Yanan Y, Rongyue C, Hao F, Jingjing L, Jie W.

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Gastrin-releasing peptide (GRP), a bombesin-like peptide, is an autocrine or paracrine growth factor that can stimulate the growth of various cancer cells, making it an ideal target antigen to develop vaccines against cancer. In this study, we developed a novel DNA vaccine that encodes six tandem repeats of B-cell epitope GRP(18-27) (GRP6) flanked by HSP65 as carrier and four tandem repeats of mycobacterial HSP70(407-426) (M4) as helper T-cell epitopes for enhancement of immunogenicity. When intramuscularly immunized to mice, this anti-GRP DNA vaccine-induced GRP-specific antibody (Ab) responses that were at least 10-fold higher in magnitude compared with HSP65-GRP6 protein vaccine. Both prophylactic and therapeutic antitumor immunities induced by vaccination significantly suppressed the growth of GRP-dependent prostate carcinoma RM-1 in vivo and prolonged the survival of tumor-inoculated mice. Our results also showed that the immune sera with high titer of GRP-specific Abs effectively inhibited the growth of tumor in mice and dose dependently inhibited proliferation of cultured RM-1 cells in vitro, suggesting that the GRP neutralizing Ab is responsible for the protective and therapeutic antitumor activity of vaccination. These findings may be of great importance in the further exploration of the applications of growth factors identified in human in cancer therapy.

PMID:
20130441

Ophthalmic Res. 2010 Feb 3;44(1):1-16. [Epub ahead of print]

Applications of Nanobiotechnology in Ophthalmology - Part I.

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Much progress has been achieved in the field of nanotechnology and its applications in ophthalmology. It is evident that drug delivery, gene therapy, implantable devices and regenerative medicine are some of the key areas of active research. To the best of our knowledge, there is limited review work on this subject area in the current literature. To assist the interested clinicians and scientists, this bipartite commentary will focus the discussion on emerging researches in nano-ophthalmology and other enabling technologies that soon may be available in the clinician's armamentarium to maintain and restore eye sight. This installment will focus on recent discoveries in drug delivery, gene therapy, imaging and visual prostheses; the second installment will discuss the impact of nanotechnology on artificial environment, cell-nanostructure interaction, other enabling nano-ophthalmic technologies, and safety and biocompatibility of nanostructures. We will take this opportunity to introduce some exciting nano-ophthalmic applications under investigation in our laboratory. The accomplishments by the scientific community are tremendous and the future prospects are wide open.

PMID:
20130180

J Neurosci. 2010 Feb 3;30(5):1712-20.

Anti-glucocorticoid gene therapy reverses the impairing effects of elevated corticosterone on spatial memory, hippocampal neuronal excitability, and synaptic plasticity.

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Moderate release of the major stress hormones, glucocorticoids (GCs), improves hippocampal function and memory. In contrast, excessive or prolonged elevations produce impairments. Enzymatic degradation and reformation of GCs help to maintain optimal levels within target tissues, including the brain. We hypothesized that expressing a GC-degrading enzyme in hippocampal neurons would attenuate the negative impact of an excessive elevation in GC levels on synaptic physiology and spatial memory. We tested this by expressing 11-beta-hydroxysteroid dehydrogenase (type II) in dentate gyrus granule cells during a 3 d GC treatment followed by examination of synaptic responses in hippocampal slices or spatial performance in the Morris water maze. In adrenalectomized rats with basal GC replacement, additional GC treatments for 3 d reduced synaptic strength and promoted the expression of long-term depression at medial perforant path synapses, increased granule cell and CA1 pyramidal cell excitability, and impaired spatial reference memory (without influencing learning). Expression of 11-beta-hydroxysteroid dehydrogenase (type II), mostly in mature dentate gyrus granule cells, reversed the effects of high GC levels on granule cell and pyramidal cell excitability, perforant path synaptic plasticity, and spatial memory. These data demonstrate the ability of neuroprotective gene expression limited to a specific cell population to both locally and trans-synaptically offset neurophysiological disruptions produced by prolonged increases in circulating stress hormones. This report supplies the first physiological explanation for previously demonstrated cognitive sparing by anti-stress gene therapy approaches and lends additional insight into the hippocampal processes that are important for memory.

PMID:
20129936

Brain. 2010 Feb 2. [Epub ahead of print]

Optimized adeno-associated viral vector-mediated striatal DOPA delivery restores sensorimotor function and prevents dyskinesias in a model of advanced Parkinson's disease.

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Viral vector-mediated gene transfer utilizing adeno-associated viral vectors has recently entered clinical testing as a novel tool for delivery of therapeutic agents to the brain. Clinical trials in Parkinson's disease using adeno-associated viral vector-based gene therapy have shown the safety of the approach. Further efforts in this area will show if gene-based approaches can rival the therapeutic efficacy achieved with the best pharmacological therapy or other, already established, surgical interventions. One of the strategies under development for clinical application is continuous 3,4-dihydroxyphenylalanine delivery. This approach has been shown to be efficient in restoring motor function and reducing established dyskinesias in rats with a partial lesion of the nigrostriatal dopamine projection. Here we utilized high purity recombinant adeno-associated viral vectors serotype 5 coding for tyrosine hydroxylase and its co-factor synthesizing enzyme guanosine-5'-triphosphate cyclohydrolase-1, delivered at an optimal ratio of 5 : 1, to show that the enhanced 3,4-dihydroxyphenylalanine production obtained with this optimized delivery system results in robust recovery of function in spontaneous motor tests after complete dopamine denervation. We found that the therapeutic efficacy was substantial and could be maintained for at least 6 months. The tyrosine hydroxylase plus guanosine-5'-triphosphate cyclohydrolase-1 treated animals were resistant to developing dyskinesias upon peripheral l-3,4-dihydroxyphenylalanine drug challenge, which is consistent with the interpretation that continuous dopamine stimulation resulted in a normalization of the post-synaptic response. Interestingly, recovery of forelimb use in the stepping test observed here was maintained even after a second lesion depleting the serotonin input to the forebrain, suggesting that the therapeutic efficacy was not solely dependent on dopamine synthesis and release from striatal serotonergic terminals. Taken together these results show that vector-mediated continuous 3,4-dihydroxyphenylalanine delivery has the potential to provide significant symptomatic relief even in advanced stages of Parkinson's disease.

PMID:
20129661

Biomaterials. 2010 Feb 1. [Epub ahead of print]

The effects of Runx2 immobilization on poly (varepsilon-caprolactone) on osteoblast differentiation of bone marrow stromal cells in vitro.

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In vivo regenerative gene therapy is a promising approach for bone regeneration and can help to address cell-source limitations through surgical implantation of osteoinductive materials and subsequent recruitment of host-derived cells. Localized viral delivery may reduce the risk of virus dispersion, enhance transduction efficiency, and reduce administration/injection dosing, which subsequently increases patient safety. In this manuscript, we present a custom-tailored strategy to immobilize adenovirus expressing runt-related transcription factor 2 (AdRunx2) by using reactive polymer coatings to enhance in vitro osteoblast differentiation of bone marrow stromal cells (BMSCs). A thin polymer film of poly[p-xylylene carboxylic acid pentafluorophenol ester-co-p-xylylene] equipped with amine-reactive active ester groups was deposited on the surface of poly (varepsilon-caprolactone) (PCL) using the chemical vapor deposition (CVD) polymerization technique and then anti-adenovirus antibody was conjugated on the material with an amide chemical bond. Following antibody conjugation, AdRunx2 was conjugated to the PCL surface through antibody-antigen interaction. Osteoblast differentiation of BMSCs was induced by incubation in osteogenic medium. Alkaline phosphatase (ALP) activity, calcium deposition, and matrix mineralization were confirmed as markers of osteoblast formation. Incubation of the BMSCs in the presence of AdRunx2 modified PCL resulted in a 6.5-fold increase in ALP activity and significant increases in matrix mineralization when compared to controls. These results demonstrate that adenovirus vectors driving the expression of transcription factors can be delivered directly from biomaterials to direct cell differentiation..

PMID:
20127864

Int J Cancer. 2010 Feb 2. [Epub ahead of print]

Investigation of a plasmid containing a novel immunotoxin VEGF165-PE38 gene for antiangiogenic therapy in a malignant glioma model.

Hu CC, Ji HM, Chen SL, Zhang HW, Wang BQ, Zhou LY, Zhang ZP, Sun XL, Chen ZZ, Cai YQ, Qin LS, Lu L, Jiang XD, Xu RX, Ke YQ.
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Inhibition of tumor neovascularization has profound effects on the growth of solid tumors. Our previous studies have shown the effect of VEGF165-PE38 recombinant immunotoxin on proliferation and apoptosis in human umbilical vein endothelial cells (HUVECs) in vitro. In this study, we explored the direct inhibition of angiogenesis in chick chorioallantoic membrane (CAM) and antiangiogenic therapy in a malignant glioma model. HEK293 cells were transfected with the pVEGF165PE38-IRES2-EGFP plasmid. ELISA was used to confirm the expression of VEGF165-PE38 in the transfected cells. These cells released 1396 ± 131.9 pg VEGF165-PE38/ 1×10^4 cells/48 h into the culture medium and the supernatant was capable of inhibiting the growth of capillary-like structures in CAM assay. In a murine malignant glioma model, plasmid was directly administered via multiple local intratumoral delivery. After day 16 the tumor volume in mice treated with pVEGF165PE38-IRES2-EGFP was significantly lower than that in mice in the control groups. Immunohistochemistry studies showed that the treated group had decreased expression of CD31. Quantitative analysis of microvessel density (MVD) in the treated group was $1.99 \pm 0.69/0.74$ mm², and was significantly lower than that in the control groups ($9.33 \pm 1.99/0.74$ mm², $8.09 \pm 1.39/0.74$ mm² and $8.49 \pm 1.69/0.74$ mm²). Immunohistochemistry analysis indicated that immunotoxin VEGF165-PE38 was distributed in the treated group in malignant glioma tissue. Our findings provide evidence that the in vivo production of VEGF165-PE38 through gene therapy using a eukaryotic expression plasmid had potential antiangiogenic activity in malignant glioma in vivo. (c) 2010 UICC.

PMID:
20127551

IDrugs. 2010 Feb;13(2):63-5.

European Society of Gene & Cell Therapy - 17th Annual Congress.

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The 17th Annual Congress of the European Society of Gene & Cell Therapy, held in Hanover, Germany, included topics covering new developments in the field of stem cell therapy. This conference report highlights selected presentations on stem cell gene therapy and the use of stem cells in regenerative medicine. Investigational therapies discussed include Lenti-D (Genetix Pharmaceuticals Inc/INSERM) and LentiGlobin (Genetix), a lentiviral vector-based gene transfer system containing either a human beta-globin gene or a hybrid A-gamma/beta-globin gene.

PMID:
20127400

Cell Biol Toxicol. 2010 Feb 3. [Epub ahead of print]

Multifunctional nanocomplexes for gene transfer and gene therapy.

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DNA formulated into aggregates with polycationic reagents are referred to by a variety of terms including non-viral vectors, synthetic vectors, lipoplexes, polyplexes and more recently nanoparticles. The capacity for delivery of multiple genes, genomic-sized constructs and siRNA delivery, with a diversity of possible formulations, as well as the possibilities of improved efficiency of in vivo gene deliveries, means that nanoparticles, or nanocomplexes to reflect self-assembling systems, will be investigated with increasing vigour in the coming years. This review briefly outlines the applications and challenges for nanoparticle technologies in the field of gene therapy then focuses on the development of a specific kind of formulation, receptor-targeted nanocomplex (RTN), that we have found to be particularly useful in our gene therapy research. An overriding guiding concept that has emerged in the development of synthetic nanodelivery systems is the idea to develop formulations and structures that mimic viruses, whilst retaining the safety elements of synthetic, non-viral systems. RTNs have been optimised and developed for airway epithelial transfection, leading towards gene therapy for cystic fibrosis and for vascular transfection in vein grafts used in bypass surgery. The modular design of the RTN platform further allows for the testing of specific hypotheses relating to the structure and functional role of components in the formation of stable particles and in the transfection pathway, leading to their ultimate disassembly in the nucleus.

PMID:
20127041

Int J Mol Med. 2010 Mar;25(3):369-76.

Combination gene therapy of lung cancer with conditionally replicating adenovirus and adenovirus-herpes simplex virus thymidine kinase.

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A major obstacle to the success of gene therapy strategies that directly target cancer cells is the low gene transfer rate. To address this problem, we had previously proposed a combination adenoviral gene therapy containing a conditionally replicating adenovirus (CRAD) expressing mutant E1 (Delta24RGD), and a replication-defective E1-deleted adenovirus to enhance the efficiency of gene transfer. Suicide/pro-drug gene therapy has an important additional benefit to the therapy of cancer. This relates to the transfer and expression of non-mammalian genes encoding enzymes that convert non-toxic pro-drugs into cellular toxins. We investigated the interaction between CRAD (Delta24RGD) and a replication-defective E1-deleted adenovirus (ad-HSTK) containing a suicide gene (HSTK: herpes simplex virus thymidine kinase gene) with respect to therapeutic gene production and tumor cell killing efficacy. Combined transduction of CRAD and ad-HSTK increased the transduction efficiency of HSTK and increased its sensitivity to ganciclovir (GCV) more efficiently than ad-HSTK alone. Transfer of medium of CRAD and ad-HSTK co-transduced cells induced the transfer of HSTK (media transferable bystander effect), and enhanced its sensitivity to GCV. In an animal tumor model, combined intratumoral injection of CRAD and ad-HSTK followed by GCV administration induced prolonged expression of HSTK and stronger growth suppression of established lung cancer xenografts than single injections. These data demonstrate that the selective replication of ad-HSTK due to the presence of mutant E1, produced by a Delta24RGD and HSTK/GCV suicide gene system, resulted in a striking improvement in anti-tumor effects in vitro and in vivo.

PMID:
20127013

Oncol Rep. 2010 Mar;23(3):733-8.

Preparation of a novel adenovirus formulation with artificial envelope of multilayer polymer-coatings: Therapeutic effect on metastatic ovarian cancer.

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Layer-by-layer deposition of the ionic polymers onto adenovirus particles afforded the multilayer-coated virus vectors. The infectivity of the virus in the presence of anti-adenovirus antibody increased as the layer number and the viruses with five or six polymer layers allowed relatively high efficiency of reporter gene expression in vitro. Therapeutic effect of the intraperitoneal injection of the oncolytic adenovirus with quintal polymer multilayers on the mice bearing intraperitoneal metastatic ovarian cancer was examined. All the control mice injected with PBS died within 21 days after the tumor inoculation. On the other hand, the mice injected with the multilayer-coated oncolytic virus lived much longer and seven eighths of them lived >60 days without apparent accumulation of ascites. These approaches would open a new way to create a novel, safe and efficient viral gene therapy.

PMID: Trends Mol Med. 2010 Jan 30. [Epub ahead of print]
20122868

T cell receptor gene therapy: strategies for optimizing transgenic TCR pairing.

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T cell receptor (TCR) gene therapy provides patients with autologous T cells that are genetically engineered with TCRalpha chains and constitutes a promising approach for the treatment of tumors and virus infections. Among the current challenges of TCR gene therapy is the optimization of TCRalpha and beta transgene pairing to enhance the functional avidity of therapeutic T cells. Recently, various genetically modified TCRs have been developed that enhance TCR pairing and minimize mispairing, i.e. pairing between transgenic and endogenous TCR chains. Here, we classify such receptors according to their CD3-dependence for surface expression and review their abilities to address functional T cell avidity. In addition, we discuss the anticipated clinical value of these and other strategies to generate high-avidity T cells. Copyright © 2010 Elsevier Ltd. All rights reserved.

PMID: Endocrinol Metab Clin North Am. 2010 Mar;39(1):201-215.
20122459

The Human Genome and Sport, Including Epigenetics, Gene Doping, and Athleticogenomics.

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Hugh Montgomery's discovery of the first of more than 239 fitness genes together with rapid advances in human gene therapy have created a prospect of using genes, genetic elements, and cells that have the capacity to enhance athletic performance (to paraphrase the World Anti-Doping Agency's definition of gene doping). This brief overview covers the main areas of interface between genetics and sport, attempts to provide a context against which gene doping may be viewed, and predicts a futuristic legitimate use of genomic (and possibly epigenetic) information in sport.

PMID: J Law Med Ethics. 2009 Dec;37(4):659-84.
20122108

Gene therapy oversight: lessons for nanobiotechnology.

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Oversight of human gene transfer research ("gene therapy") presents an important model with potential application to oversight of nanobiology research on human participants. Gene therapy oversight adds centralized federal review at the National Institutes of Health's Office of Biotechnology Activities and its Recombinant DNA Advisory Committee to standard oversight of human subjects research at the researcher's institution (by the Institutional Review Board and, for some research, the Institutional Biosafety Committee) and at the federal level by the Office for Human Research Protections. The Food and Drug Administration's Center for Biologics Evaluation and Research oversees human gene transfer research in parallel, including approval of protocols and regulation of products. This article traces the evolution of this dual oversight system; describes how the system is already addressing nanobiotechnology in gene transfer: evaluates gene therapy oversight based on public opinion, the literature, and preliminary expert elicitation; and offers lessons of the gene therapy oversight experience for oversight of nanobiotechnology.

PMID:
20121594

Hum Gene Ther. 2010 Feb 2. [Epub ahead of print]

Clinical impact of Suicide Gene Therapy in Allogeneic Hematopoietic Stem Cell Transplantation.

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Allogeneic hematopoietic stem cell transplantation (allo-SCT) from an HLA-matched related or unrelated donor is a curative option for patients with high-risk hematological diseases. In the absence of a matched donor, patients have been offered investigational transplant strategies such as umbilical cord blood SCT or family haploidentical SCT. Besides the activity of the conditioning regimen, most of the antileukemic potential of allo-SCT resides on alloreactivity, promoted by donor lymphocytes reacting against patient-specific antigens, such as minor and major histocompatibility antigens, ultimately translating into cancer immunotherapy. Unfortunately, alloreactivity is also responsible for the most serious and frequent complication of allo-SCT: the Graft-versus-Host-Disease (GvHD). The risk of GvHD increases with the level of HLA disparity between host and donor, and leads to impaired quality of life and reduced survival expectancy, particularly in patients transplanted from HLA-mismatched donors. Gene transfer technologies are promising tools to manipulate donor T cell immunity to enforce the graft-versus-tumor (GvT) effect, to promote a functional immune reconstitution (graft-versus-infection-GvI) and to prevent or control GvHD. To this purpose, several cell and gene transfer approaches have been investigated at pre-clinical level, and are being implemented in clinical trials. Suicide gene therapy is to date the most extensive clinical application of T-cell based gene therapy. In several phase I-II clinical studies conducted worldwide this approach proved highly feasible, safe and effective in promoting a dynamic and patient-specific modulation of alloreactivity. This review will focus on this approach.

PMID:
20119855

Cell Mol Biol Lett. 2010 Jan 28. [Epub ahead of print]

A microarray gene analysis of peripheral whole blood in normal adult male rats after long-term GH gene therapy.

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The main aims of this study were to determine the effects of GH gene abuse/misuse in normal animals and to discover genes that could be used as candidate biomarkers for the detection of GH gene therapy abuse/misuse in humans. We determined the global gene expression profile of peripheral whole blood from normal adult male rats after long-term GH gene therapy using CapitalBio 27 K Rat Genome Oligo Arrays. Sixty one genes were found to be differentially expressed in GH gene-treated rats 24 weeks after receiving GH gene therapy, at a two-fold higher or lower level compared to the empty vector group ($p < 0.05$). These genes were mainly associated with angiogenesis, oncogenesis, apoptosis, immune networks, signaling pathways, general metabolism, type I diabetes mellitus, carbon fixation, cell adhesion molecules, and cytokine-cytokine receptor interaction. The results imply that exogenous GH gene expression in normal subjects is likely to induce cellular changes in the metabolism, signal pathways and immunity. A real-time qRT-PCR analysis of a selection of the genes confirmed the microarray data. Eight differently expressed genes were selected as candidate biomarkers from among these 61 genes. These 8 showed five-fold higher or lower expression levels after the GH gene transduction ($p < 0.05$). They were then validated in real-time PCR experiments using 15 single-treated blood samples and 10 control blood samples. In summary, we detected the gene expression profiles of rat peripheral whole blood after long-term GH gene therapy and screened eight genes as candidate biomarkers based on the microarray data. This will contribute to an increased mechanistic understanding of the effects of chronic GH gene therapy abuse/misuse in normal subjects.

PMID:
20119625

Acta Biochim Biophys Sin (Shanghai). 2010 Feb;42(2):137-44.

Therapeutic potential of siRNA-mediated combined knockdown of the IAP genes (Livin, XIAP, and Survivin) on human bladder cancer T24 cells.

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Livin, X-linked inhibitor of apoptosis (XIAP), and Survivin are three well-known inhibitors of apoptosis almost exclusively over-expressed in cancer cells and are considered potent targets for cancer treatment. In the present study, we found that Livin, XIAP, and Survivin were simultaneously expressed in bladder cancer cells. We speculated that Livin, XIAP, and Survivin might have synergistic effects on cell growth and apoptosis. Our results confirmed that combined knockdown of all these three genes can synergistically inhibit the proliferation and transformation ability of high-grade bladder cancer T24 cells and promote the cell apoptotic sensitivity to chemotherapy. Furthermore, combined knockdown of Livin, XIAP, and Survivin can markedly increase the abundance of active caspase-3, active caspase-7, active caspase-9, and cytosolic Smac. Our findings imply that combined silencing of Livin, XIAP, and Survivin may be a potent multitargeted gene therapy for bladder cancer.

PMID:
20118988

EMBO Rep. 2010 Feb;11(2):75.

Gene therapy: back on track?

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No Abstract available

PMID:
20118370

Appl Environ Microbiol. 2010 Jan 29. [Epub ahead of print]

A Novel Antibiotic-free Plasmid Selection System Based on Complementation of Host Auxotrophy in NAD De Novo Synthesis Pathway.

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The use of antibiotic resistance genes in plasmids causes potential biosafety and clinical hazards, such as the possibility of horizontal spread of resistance genes or the rapid emergence of multi-drug resistant pathogens. This paper introduced a novel auxotrophy complementation system that allowed plasmids and host cells to be effectively selected and maintained without the use of antibiotics. An Escherichia coli strain carrying a defect in NAD de novo biosynthesis was constructed by knocking out the chromosomal quinolinic acid phosphoribosyltransferase (QAPRTase) gene. The resistance gene in the plasmids was replaced by the QAPRTase gene of E.coli or mouse. As a result, only expression of QAPRTase from the plasmids can complement and rescue host E.coli cells in minimal medium. This is the first time that a vertebrate gene has been used to construct a non-antibiotic selection system and it can be widely applied in DNA vaccine and gene therapy. As QAPRTase is ubiquitous in species ranging from bacteria to mammals, the potential environmental biosafety problems caused by horizontal gene transfer can be eliminated.

PMID:
20118222

Am J Respir Cell Mol Biol. 2010 Jan 29. [Epub ahead of print]

Gene Delivery of a Cytochrome P450 Epoxygenase Ameliorates Monocrotaline-induced Pulmonary Artery Hypertension in Rats.

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Pulmonary arterial hypertension (PAH) is a life-threatening disease that leads to progressive pulmonary hypertension, right heart failure and death. Endothelial dysfunction and inflammation have been implicated in the pathogenesis of PAH. Epoxyeicosatrienoic acids (EETs), products of cytochrome P450 epoxygenase metabolism of arachidonic acid, are potent vasodilators that possess anti-inflammatory and other protective properties in endothelial cells. We investigated whether gene delivery with the human cytochrome P450 epoxygenase 2J2 (CYP2J2) ameliorates monocrotaline (MCT)-induced pulmonary hypertension in rats. Significant pulmonary hypertension developed 3 weeks after administration of MCT, but gene therapy with CYP2J2 significantly attenuated the development of pulmonary hypertension and pulmonary vascular remodeling without causing changes in systemic arterial pressure or heart rate. These effects were associated with increased pulmonary endothelial NO synthase (eNOS) expression and its activity, inhibition of inflammation in the lung and TGF beta/BMPRII-Smads signaling. Collectively, these data suggest that gene therapy with CYP2J2 may have potential as a novel therapeutic approach to this progressive and oftentimes lethal disorder.

PMID:
20117155

J Control Release. 2010 Jan 30. [Epub ahead of print]

Flow-through electroporation based on constant voltage for large-volume transfection of cells.

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Genetic modification of cells is a critical step involved in many cell therapy and gene therapy protocols. In these applications, cell samples of large volume (10⁸-10⁹ cells) are often processed for transfection. This poses new challenges for current transfection methods and practices. Here we present a novel flow-through electroporation method for delivery of genes into cells at high flow rates (up to approximately 20 mL/min) based on disposable microfluidic chips, a syringe pump, and a low-cost direct current (DC) power supply that provides a constant voltage. By eliminating pulse generators used in conventional electroporation, we dramatically lowered the cost of the apparatus and improved the stability and consistency of the electroporation field for long-time operation. We tested the delivery of pEFGP-C1 plasmids encoding enhanced green fluorescent protein into Chinese hamster ovary (CHO-K1) cells in the devices of various dimensions and geometries. Cells were mixed with plasmids and then flowed through a fluidic channel continuously while a constant voltage was established across the device. Together with the applied voltage, the geometry and dimensions of the fluidic channel determined the electrical parameters of the electroporation. With the optimal design, approximately 75% of the viable CHO cells were transfected after the procedure. We also generalize the guidelines for scaling up these flow-through electroporation devices. We envision that this technique will serve as a generic and low-cost tool for a variety of clinical applications requiring large volume of transfected cells.

PMID:
20117154

J Control Release. 2010 Jan 30. [Epub ahead of print]

A top-down approach for construction of hybrid polymer-virus gene delivery vectors.

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Safe and efficient delivery of therapeutic nucleic acids remains the primary hurdle for human gene therapy. While many researchers have attempted to re-engineer viruses to be suited for gene delivery, others have sought to develop non-viral alternatives. We have developed a complementary approach in which viral and synthetic components are combined to form hybrid nanoparticulate vectors. In particular, we complexed non-infectious retrovirus-like particles lacking a viral envelope protein, from Moloney murine leukemia virus (M-VLP) or human immunodeficiency virus (H-VLP), with poly-L-lysine (PLL) or polyethylenimine (PEI) over a range of polymer/VLP ratios. At appropriate stoichiometry (75-250microg polymer/10(6) VLP), the polymers replace the function of the viral envelope protein and interact with the target cell membrane, initiate cellular uptake and facilitate escape from endocytic vesicles. The viral particle, once in the cytosol, efficiently completes its normal infection process including integration of viral genes with the host genome as demonstrated by long-term (at least 5weeks) transgene expression. In addition, hybrid vectors comprising H-VLP were shown to be capable of infecting non-dividing cells.

PMID:
20117135

Pharmacol Ther. 2010 Feb 1. [Epub ahead of print]

Role of apoptosis in pulmonary hypertension: From experimental models to clinical trials.

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Pulmonary arterial hypertension (PAH) is a progressive and lethal disease that has a strong female predominance, often affecting the young. Current therapies are mostly vasodilator agents, and while mainly addressing the endothelial dysfunction that has been widely reported in this disease, they may be less effective in treating arterial remodeling. The lung pathology of PAH is characterized by medial hypertrophy and intimal hyperplasia of muscular arteries as well as plexiform lesions, that lead to a widespread narrowing or obliteration of the pulmonary arteriolar bed. However, the pathogenesis of the functional and structural abnormalities of the lung microcirculation in PAH is poorly understood. Perhaps the greatest advancement in the last decade has been the discovery of the "PAH gene," bone morphogenetic receptor 2 (Bmpr2), however how the loss-of-function mutations in Bmpr2 lead to PAH is unclear. The BMPR2 pathway has recently been shown to mediate survival signaling in endothelial cells (EC), and thus the reduced activity will favor endothelial apoptosis, likely increasing the susceptibility to endothelial injury in response to various environmental triggers. EC apoptosis has been implicated as an initiating event in experimental PAH, leading either directly to the degeneration of pre-capillary arterioles or to the selection of hyperproliferative, apoptosis-resistant ECs that may contribute to "angioproliferative" plexiform lesions. The idea that EC apoptosis may play a central role in the initiation and progression of PAH suggests that therapeutic strategies aimed at endothelial repair and regeneration of ECs may be uniquely effective in the treatment of this disease. Preclinical evaluation and validation on the use of endothelial progenitor cells (EPCs) for the prevention and reversal of experimental PAH is reviewed and the design of a "first in man" clinical trial to assess the safety and efficacy of a combined EPC and endothelial NO-synthase gene therapy for patients that are refractory to standard therapies is discussed.

PMID:
20113473

J Transl Med. 2010 Jan 29;8(1):10. [Epub ahead of print]

Anti-viral state segregates two molecular phenotypes of pancreatic adenocarcinoma: potential relevance for adenoviral gene therapy.

Monsurro V, Beghelli S, Wang R, Barbi S, Coin S, Di Pasquale G, Bersani S, Castellucci M, Sorio C, Eleuteri S, Worschech A, Chiorini JA, Pederzoli P, Alter H, Marincola FM, Scarpa A.

ABSTRACT: **BACKGROUND:** Pancreatic adenocarcinoma (PDAC) remains a leading cause of cancer mortality for which novel gene therapy approaches relying on tumor-tropic adenoviruses are being tested. **METHODS:** We obtained the global transcriptional profiling of primary PDAC using RNA from eight xenografted primary PDAC, three primary PDAC bulk tissues, three chronic pancreatitis and three normal pancreatic tissues. The Affymetrix GeneChip HG-U133A was used. The results of the expression profiles were validated applying immunohistochemical and western blot analysis on a set of 34 primary PDAC and 10 established PDAC cell lines. Permissivity to viral vectors used for gene therapy, Adenovirus 5 and Adeno-Associated Viruses 5 and 6, was assessed on PDAC cell lines. **RESULTS:** The analysis of the expression profiles allowed the identification of two clearly distinguishable phenotypes according to the expression of interferon-stimulated genes. The two phenotypes could be readily recognized by immunohistochemical detection of the Myxovirus-resistance A protein, whose expression reflects the activation of interferon dependent pathways. The two molecular phenotypes discovered in primary carcinomas were also observed among established pancreatic adenocarcinoma cell lines, suggesting that these phenotypes are an intrinsic characteristic of cancer cells independent of their interaction with the host's microenvironment. The two pancreatic cancer phenotypes are characterized by different permissivity to viral vectors used for gene therapy, as cell lines expressing interferon stimulated genes resisted to Adenovirus 5 mediated lysis in vitro. Similar results were observed when cells were transduced with Adeno-Associated Viruses 5 and 6. **CONCLUSION:** Our study identified two molecular phenotypes of pancreatic cancer, characterized by a differential expression of interferon-stimulated genes and easily recognized by the expression of the Myxovirus-resistance A protein. We suggest that the detection of these two phenotypes might help the selection of patients enrolled in virally-mediated gene therapy trials.

PMID:
20112968

ACS Nano. 2010 Jan 29. [Epub ahead of print]

Host-Guest Interaction Mediated Polymeric Assemblies: Multifunctional Nanoparticles for Drug and Gene Delivery.

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Novel core-shell structured nanoassemblies are assembled by a beta-cyclodextrin containing a positively charged host polymer and a hydrophobic guest polymer. The hydrophobic core of these types of assemblies serves as a nanocontainer to load and release the hydrophobic drugs, while the positively charged hydrophilic shell is able to condense the plasmid DNA and achieve its transfection/expression in osteoblast cells. These assemblies may be used as a new generation of multifunctional nanocarriers for simultaneous drug delivery and gene therapy.