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Interleukin-32 (IL-32) is a pro-inflammatory cytokine conditionally produced by T cells, natural killer (NK) cells, monocytes, epithelial cells and keratinocytes, which has an important role in host resistance against infectious disease. Interestingly, elevated levels of IL-32 transcripts in fine needle aspirates of tumor tissue have also been correlated with objective clinical responses in cancer patients receiving immunotherapy. To evaluate the antitumor impact of IL-32 gene therapy, we treated BALB/c mice bearing established subcutaneous CMS4 sarcomas with intratumoral (i.t.) injections of syngenic dendritic cells (DCs) engineered to express human IL-32β complementary DNA (that is, DC.IL32). Although ectopic expression of IL-32β by DC resulted in only modest phenotypic changes in these antigen-presenting cells, DC.IL32 produced higher levels of IL-12p70 than control DC. DC.IL32 were more potent activators of type-1 T-cell responses in vitro and in vivo, with i.t. administration of DC.IL32 leading to the CD8(+) T-cell-dependent (but CD4(+) T-cell- and NK cell-independent) suppression of tumor growth. Effective DC.IL32-based therapy promoted infiltration of tumors by type-1 (that is, CXCR3(+)VLA-4(+)GrB(+)) CD8(+) T cells and CD11b(+)CD11c(+) host myeloid DC, but led to reductions in the prevalence of CD11b(+)Gr1(+) myeloid-derived suppressor cells and CD31(+) blood vessels.

Fragile histidine triad (Fhit) protein encoded by tumour suppressor FHIT gene is a proapoptotic protein with diadenosine polyphosphate (Ap(n)A, n=2-6) hydrolase activity. It has been hypothesised that formation of Fhit-substrate complex results in an apoptosis initiation signal while subsequent hydrolysis of Ap(n)A terminates this action. A series of Ap(n)A analogues have been identified in vitro as strong Fhit ligands [Varnum, J. M.; Baraniak, J.; Kaczmarek, R.; Stec, W. J.; Brenner, C. BMC Chem. Biol.2001, 1, 3]. We assumed that in Fhit-positive cells these compounds might preferentially bind to Fhit and inhibit its hydrolytic activity what would prolong the lifetime of apoptosis initiation signalling complex. Therefore, several Fhit inhibitors were tested for their cytotoxicity and ability to induce apoptosis in Fhit-positive HEK293T cells. These experiments have shown that Ap(4)A analogue, containing a glycerol residue instead of the central pyrophosphate and two terminal phosphorothioates [A(PS)-CH(2)CH(OH)CH(2)-(PS)A (1)], is the most cytotoxic among test compounds (IC50=17.5±4.2µM) and triggers caspase-dependent cell apoptosis. The Fhit-negative HEK293T cells (in which Fhit was silenced by RNAi) were not sensitive to compound 1. These results indicate that the Ap(4)A analogue 1 induces Fhit-dependent apoptosis and therefore, it can be considered as a drug candidate for anticancer therapy in Fhit-positive cancer cells and in Fhit-negative cancer cells, in which re-expression of Fhit was accomplished by gene therapy.
Differential global gene expression in cystic fibrosis nasal and bronchial epithelium.


Medical Genetics Section, Molecular Medicine Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Edinburgh, UK; UK Cystic Fibrosis Gene Therapy Consortium, London, Edinburgh, Oxford, UK.

Respiratory epithelium is the target of therapies, such as gene therapy, for cystic fibrosis (CF) lung disease. To determine the usefulness of the nasal epithelium as a pre-screen for lung-directed therapies, we profiled gene expression in CF and non-CF nasal and bronchial epithelium samples using Illumina HumanRef-8 Expression BeadChips. 863 genes were differentially expressed between CF and non-CF bronchial epithelium but only 15 were differentially expressed between CF and non-CF nasal epithelium (≥1.5-fold, P≤0.05). The most enriched pathway in CF bronchial epithelium was inflammatory response, whereas in CF nasal epithelium it was amino acid metabolism. We also compared nasal and bronchial epithelium in each group and identified differential expression of cellular movement genes in CF patients and cellular growth genes in non-CF subjects. We conclude that CF and non-CF nasal and bronchial epithelium are transcriptionally distinct and CF nasal epithelium is not a good surrogate for the lung respiratory epithelium.

Bone marrow-derived mesenchymal stem cells expressing the bFGF transgene promote axon regeneration and functional recovery after spinal cord injury in rats.

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Department of Orthopedics, Affiliated Union Hospital of Fujian Medical University, Fuzhou, China.

OBJECTIVE:
To investigate neurological effects of transplanting bone marrow-derived mesenchymal stem cells (BMSCs) transfected with the basic fibroblast growth factor (bFGF) gene in spinal cord-injured rats.

METHODS:
Ninety-six male adult Sprague-Dawley rats were randomized into four groups: (1) pcDNA3·1-bFGF group; (2) pcDNA3·1 group; (3) BMSCs group; and (4) vehicle control (DMEM) group. After the rat model of acute spinal cord injury (SCI) was established, 1×10⁶ BMSCs or cells transfected with pcDNA3·1-bFGF or pcDNA3·1 were injected into rats of groups 1-3. At days 1, 7, 14, and 21 after injection, the Basso-Beattie-Bresnahan (BBB) locomotor rating scale was used to evaluate recovery of motor function. Expression changes of bFGF, myelin basic protein (MBP), and NF200 were examined by immunohistochemistry.

RESULTS:
The BBB score of DMEM group was significantly lower than those of groups 1-3 (P<0·05), but the score of pcDNA3·1-bFGF group was significantly higher than that of BMSCs group or pcDNA3·1 group at day 14 or 21 after injection (P<0·01). The number of bFGF-positive neurons in rats of pcDNA3·1-bFGF group was significantly higher than those of groups 1-3 at any time point (P<0·05). The optical density values of NF200-positive neurons and MBP-positive MBP axons in rats of pcDNA3·1-bFGF group were significantly higher than those of groups 1-3 at day 7 or 14 after injection (P<0·05).

CONCLUSIONS:
bFGF gene-modified BMSCs not only effectively promoted axonal outgrowth but also enhanced recovery of neurological function after SCI in rats, and may be a good candidate to evaluate gene therapy of SCI in man.

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**Gene Expression Profile of the Synovium and Cartilage in a Chronic Arthritis Rat Model.**


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Abstract: Background: Primary osteoarthritis (OA) is a polygenic disease. To investigate the gene expression profile of cartilage and synovium from osteoarthritis and healthy rats using cDNA microarray is beneficial to recognize the pathogenesis of osteoarthritis and provide evidence for gene therapy of osteoarthritis. Objective: The present study aimed to investigate the gene expression profile of the cartilage and synovium of chronic arthritis and healthy rats through cDNA microarray assay, and identify the differentially expressed genes. This study may be helpful for understanding the role of differentially expressed genes in osteoarthritis and the gene polymorphism of osteoarthritis. Methods: A total of 24 male Wistar rats were randomly divided into control group and osteoarthritis group (n = 12 per group). The synovial and cartilage were obtained and total RNA was extracted. cDNA microarray assay was performed to identify the differentially expressed genes, and cluster analysis was conducted. Results and Conclusion: A total of 82 differentially expressed genes were identified, among which 27 were up-regulated and 55 down-regulated. Gene microarray assay is effective to identify differentially expressed genes and may find out novel osteoarthritis associated genes. Multiple genes are involved in the pathogenesis of osteoarthritis. The differentially expressed genes provide important information for further studies on the pathogenesis of osteoarthritis and gene therapy of osteoarthritis.

**Glioma Gene Therapy Using Induced Pluripotent Stem Cell Derived Neural Stem Cells.**

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Using neural stem cells (NSCs) with tumor tropic migratory capacity to deliver therapeutic genes is an attractive strategy in eliminating metastatic or disseminated tumors. While different methods have been developed to isolate or generate NSCs, it has not been assessed whether induced pluripotent stem (iPS) cells, a type of pluripotent stem cells that hold great potential for regenerative medicine, can be used as a source for derivation of NSCs with tumor tropism. In this study, we used a conventional lentivirus transduction method to derive iPS cells from primary mouse embryonic fibroblasts and then generated NSCs from the iPS cells. To investigate whether the iPS cell derived NSCs can be used in the treatment of disseminated brain tumors, the cells were transduced with a baculoviral vector containing the herpes simplex virus thymidine kinase suicide gene and injected into the cerebral hemisphere contralateral to a tumor inoculation site in a mouse intracranial human glioma xenograft model. We observed that NSCs expressing the suicide gene were, in the presence of ganciclovir, effective in inhibiting the growth of the glioma xenografts and prolonging survival of tumor-bearing mice. Our findings provide evidence for the feasibility of using iPS cell derived NSCs as cellular vehicles for targeted anticancer gene therapy.
Stem Cell Based Cancer Gene Therapy.
Cihova M, Altanerova V, Altaner C.
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The attractiveness of prodrug cancer gene therapy by stem cells targeted to tumors lies in activating the prodrug directly within the tumor mass, thus avoiding systemic toxicity. Suicide gene therapy using genetically engineered mesenchymal stem cells has the advantage of being safe, because prodrug administration not only eliminates tumor cells but consequently kills the more resistant therapeutic stem cells as well. This review provides an explanation of the stem cell-targeted prodrug cancer gene therapy principle, with focus on the choice of prodrug, properties of bone marrow and adipose tissue-derived mesenchymal stem and neural stem cells as well as the mechanisms of their tumor homing ability. Therapeutic achievements of the cytosine deaminase/5-fluorocytosine prodrug system and Herpes simplex virus thymidine kinase/ganciclovir are discussed. In addition, delivery of immunostimulatory cytokines, apoptosis inducing genes, nanoparticles and antiangiogenic proteins by stem cells to tumors and metastases is discussed as a promising approach for antitumor therapy. Combinations of traditional, targeted and stem cell-directed gene therapy could significantly advance the treatment of cancer.

Single-chain VαVβ T-cell receptors function without mispairing with endogenous TCR chains.
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Transduction of exogenous T-cell receptor (TCR) genes into patients' activated peripheral blood T cells is a potent strategy to generate large numbers of specific T cells for adoptive therapy of cancer and viral diseases. However, the remarkable clinical promise of this powerful approach is still being overshadowed by a serious potential consequence: mispairing of the exogenous TCR chains with endogenous TCR chains. These 'mixed' heterodimers can generate new specificities that result in graft-versus-host reactions. Engineering TCR constant regions of the exogenous chains with a cysteine promotes proper pairing and reduces the mispairing, but, as we show here, does not eliminate the formation of mixed heterodimers. By contrast, deletion of the constant regions, through use of a stabilized Vα/Vβ single-chain TCR (scTv), avoided mispairing completely. By linking a high-affinity scTv to intracellular signaling domains, such as Lck and CD28, the scTv was capable of activating functional T-cell responses in the absence of either the CD3 subunits or the co-receptors, and circumvented mispairing with endogenous TCRs. Such transduced T cells can respond to the targeted antigen independent of CD3 subunits via the introduced scTv, without the transduced T cells acquiring any new undefined and potentially dangerous specificities.
**Pinhole micro-SPECT/CT for noninvasive monitoring and quantitation of oncolytic virus dispersion and percent infection in solid tumors.**

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Department of Molecular Medicine, Mayo Clinic, Rochester, MN, USA.

The purpose of our study was to validate the ability of pinhole micro-single-photon emission computed tomography/computed tomography (SPECT/CT) to: 1) accurately resolve the intratumoral dispersion pattern and 2) quantify the infection percentage in solid tumors of an oncolytic measles virus encoding the human sodium iodide symporter (MV-NIS). Sodium iodide symporter (NIS) RNA level and dispersion pattern were determined in control and MV-NIS-infected BxPC-3 pancreatic tumor cells and mouse xenografts using quantitative, real-time, reverse transcriptase, polymerase chain reaction, autoradiography and immunohistochemistry (IHC). Mice with BxPC-3 xenografts were imaged with (123)I or (99)TcO(4) micro-SPECT/CT. Tumor dimensions and radionuclide localization were determined with imaging software. Linear regression and correlation analyses were performed to determine the relationship between tumor infection percentage and radionuclide uptake (% injected dose per gram) above background and a highly significant correlation was observed (r(2)=0.947). A detection threshold of 1.5-fold above the control tumor uptake (background) yielded a sensitivity of 2.7% MV-NIS-infected tumor cells. We reliably resolved multiple distinct intratumoral zones of infection from non-infected regions. Pinhole micro-SPECT/CT imaging using the NIS reporter demonstrated precise localization and quantitation of oncolytic MV-NIS infection, and can replace more time-consuming and expensive analyses (for example, autoradiography and IHC) that require animal killing.

**Scale-up and manufacturing of clinical-grade self-inactivating γ-retroviral vectors by transient transfection.**

Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.

The need for γ-retroviral (gRV) vectors with a self-inactivating (SIN) design for clinical application has prompted a shift in methodology of vector manufacturing from the traditional use of stable producer lines to transient transfection-based techniques. Herein, we set out to define and optimize a scalable manufacturing process for the production of gRV vectors using transfection in a closed-system bioreactor in compliance with current good manufacturing practices (cGMP). The process was based on transient transfection of 293T cells on Fibra-Cel disks in the Wave Bioreactor. Cells were harvested from tissue culture flasks and transferred to the bioreactor containing Fibra-Cel in the presence of vector plasmid, packaging plasmids and calcium-phosphate in Dulbecco's modified Eagle's medium and 10% fetal bovine serum. Virus supernatant was harvested at 10-14 h intervals. Using optimized procedures, a total of five ecotropic cGMP-grade gRV vectors were produced (9 liters each) with titers up to 3.6 × 10(7) infectious units per milliliter on 3T3 cells. One GMP preparation of vector-like particles was also produced. These results describe an optimized process for the generation of SIN viral vectors by transfection using a disposable platform that allows for the generation of clinical-grade viral vectors without the need for cleaning validation in a cost-effective manner.
Noninvasive optical imaging of nitroreductase gene-directed enzyme prodrug therapy system in living animals.
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Gene-directed enzyme prodrug therapy (GDEPT) is a promising and emerging strategy that attempts to limit the systemic toxicity inherent to cancer chemotherapy by means of tumor-targeted delivery and expression of an exogenous gene whose product converts nontoxic prodrug(s) into activated cytotoxic agent(s). The bacterial nitroreductase (NTR) enzyme, coupled with its substrate prodrug 5-(azaridin-1-yl)-2,4-dinitrobenzamide (CB1954), is a promising GDEPT strategy that has reached clinical trials. However, no strategy exists to visually monitor and quantitatively evaluate the therapeutic efficacy of NTR/CB1954 prodrug therapy in cells and imaging in living animals. As the success of any GDEPT is dependent upon the efficiency of transgene expression in vivo, we developed a safe, sensitive and reproducible noninvasive imaging method to monitor NTR transgene expression that would allow quantitative assessment of both therapeutic efficacy and diagnostic outcome of NTR/CB1954 prodrug therapy in the future. Here, we investigate the use of a novel fluorescent imaging dye CytoCy5S (a Cy5-labeled quenched substrate of NTR enzyme) on various cancer cell lines in vitro and in NTR-transfected tumor-bearing animals in vivo. CytoCy5S-labeled cells become fluorescent at 'red-shifted' wavelengths (638 nm) when reduced by cellular NTR enzyme and remains trapped within the cells for extended periods of time. The conversion and entrapment was dynamically recorded using a time-lapsed microscopy. Systemic and intratumoral delivery of CytoCy5S to NTR-expressing tumors in animals indicated steady and reproducible signals even 16 h after delivery (P<0.001). This is the first study to address visual monitoring and quantitative evaluation of NTR activity in small animals using CytoCy5S, and establishes the capability of NTR to function as an imageable reporter gene.

Chemical modification and design of anti-miRNA oligonucleotides.
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Antisense techniques have been employed for over 30 years to suppress expression of target RNAs. Recently, microRNAs (miRNAs) have emerged as a new class of small, non-coding, regulatory RNA molecules that widely impact gene regulation, differentiation and disease states in both plants and animals. Antisense techniques that employ synthetic oligonucleotides have been used to study miRNA function and some of these compounds may have potential as novel drug candidates to intervene in diseases where miRNAs contribute to the underlying pathophysiology. Anti-miRNA oligonucleotides (AMOs) appear to work primarily through a steric blocking mechanism of action; these compounds are synthetic reverse complements that tightly bind and inactivate the miRNA. A variety of chemical modifications can be used to improve the performance and potency of AMOs. In general, modifications that confer nuclease stability and increase binding affinity improve AMO performance. Chemical modifications and/or certain structural features of the AMO may also facilitate invasion into the miRNA-induced silencing complex. In particular, it is essential that the AMO binds with high affinity to the miRNA 'seed region', which spans bases 2-8 from the 5'-end of the miRNA.
**PMID: 21753742**


**Hypoxia-induced expression of VEGF in the organotypic spinal cord slice culture.**

An SS, Pennant WA, Ha Y, Oh JS, Kim HJ, Gwak SJ, Yoon do H, Kim KN.
Department of Neurosurgery, Spine and Spinal Cord Institute, Yonsei University College of Medicine, Seodaemoon-gu, Seoul, Korea.

We used the erythropoietin enhancer and Simian virus-40 promoter to create a hypoxia-inducible gene expression system to investigate the effect of vascular endothelial growth factor (VEGF) gene therapy on neuroprotection and neurogenesis in organotypic spinal cord slice culture. The organotypic spinal cord slice culture transfected with pEpo-SV-VEGF expressed the highest amount of VEGF under hypoxic conditions and showed decreased apoptosis and increased proliferation, and evidence of neurogenesis. Our results show that the hypoxia-induced VEGF expression in an organotypic spinal cord slice culture may lead to an optimal treatment for spinal cord injury.

**PMID: 21750689**


**Suicide gene therapy to increase the safety of chimeric antigen receptor-redirected T lymphocytes.**

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Chimeric antigen receptors (CARs) are generated by fusing the antigen-binding motif of a monoclonal antibody (mAb) with the signal transduction machinery of the T-cell receptor (TCR). The genetic modification of T lymphocytes with chimeric receptors specific for tumor-associated antigens (TAAs) allows for the redirection towards tumor cells. Clinical experience with CAR-redirected T cells suggests that antitumor efficacy associates with some degree of toxicity, especially when TAA expression is shared with healthy tissues. This situation closely resembles the case of allogeneic hematopoietic stem cell transplantation (HSCT), wherein allore cognition causes both the graft-versus-leukemia (GVL) effect and graft-versus-host disease (GVHD). Suicide gene therapy, i.e. the genetic induction of a conditional suicide phenotype into donor T cells, enables dissociating the GVL effect from GVHD. Applying suicide gene modification to CAR-redirected T cells may therefore greatly increase their safety profile and facilitate their clinical development.
Glycosidic enzymes enhance retinal transduction following intravitreal delivery of AAV2.
Cehajic-Kapetanovic J, Le Goff MM, Allen A, Lucas RJ, Bishop PN.

PURPOSE:
To determine whether the co-injection of extracellular matrix degrading enzymes improves retinal transduction following intravitreal delivery of adeno-associated virus-2 (AAV2).

METHODS:
AAV2 containing cDNA encoding enhanced green fluorescent protein (GFP), under the control of a chicken β-actin promoter, was delivered by intravitreal injection to adult mice in conjunction with enzymes including collagenase, hyaluronan lyase, heparinase III, or chondroitin ABC lyase. Two weeks later, retinal flatmounts were examined for GFP expression using confocal microscopy.

RESULTS:
Without the addition of enzymes, transduction was limited to occasional cells in the retinal ganglion cell layer. The addition of heparinase III or chondroitin ABC lyase greatly enhanced transduction of the retinal ganglion cell layer and increased the depth of transduction into the outer retina. Hyaluronan lyase had a limited effect and collagenase was ineffective. Electroretinograms survived with higher concentrations of heparinase III and chondroitin ABC lyase than were required for optimal retinal transduction.

CONCLUSIONS:
AAV2-mediated retinal transduction is improved by co-injection of heparinase III or chondroitin ABC lyase. Improved transduction efficiency may allow intravitreal injection to become the preferred route for delivering gene therapy to both the inner and outer retina.

Correction of Murine SCID-X1 by Lentiviral Gene Therapy Using a Codon-optimized IL2RG Gene and Minimal Pretransplant Conditioning.
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Clinical trials have demonstrated the potential of ex vivo hematopoietic stem cell gene therapy to treat X-linked severe combined immunodeficiency (SCID-X1) using γ-retroviral vectors, leading to immune system functionality in the majority of treated patients without pretransplant conditioning. The success was tempered by insertional oncogenesis in a proportion of the patients. To reduce the genotoxicity risk, a self-inactivating (SIN) lentiviral vector (LV) with improved expression of a codon optimized human interleukin-2 receptor γ gene (IL2RG) cDNA (coγc), regulated by its 1.1 kb promoter region (ycPr), was compared in efficacy to the viral spleen focus forming virus (SF) and the cellular phosphoglycerate kinase (PGK) promoters. Pretransplant conditioning of Il2rg(-/-) mice resulted in long-term reconstitution of T and B lymphocytes, normalized natural antibody titers, humoral immune responses, ConA/IL-2 stimulated spleen cell proliferation, and polyclonal T-cell receptor gene rearrangements with a clear integration preference of the SF vector for proto-oncogenes, contrary to the PGK and ycPr vectors. We conclude that SIN lentiviral gene therapy using coγc driven by the ycPr or PGK promoter corrects the SCID phenotype, potentially with an improved safety profile, and that low-dose conditioning proved essential for immune competence, allowing for a reduced threshold of cell numbers required.
Liver engraftment potential of hepatic cells derived from patient-specific induced pluripotent stem cells.


Stem Cell Biology Laboratory; Sidney Kimmel Comprehensive Cancer Center; Johns Hopkins University School of Medicine; Baltimore, MD USA.

Human induced pluripotent stem cells (iPSCs) are potential renewable sources of hepatocytes for drug development and cell therapy. Differentiation of human iPSCs into different developmental stages of hepatic cells has been achieved and improved during the last several years. We have recently demonstrated the liver engraftment and regenerative capabilities of human iPSC-derived multistage hepatic cells in vivo. Here we describe the in vitro and in vivo activities of hepatic cells derived from patient specific iPSCs, including multiple lines established from either inherited or acquired liver diseases, and discuss basic and clinical applications of these cells for disease modeling, drug screening and discovery, gene therapy and cell replacement therapy.

CD3 limits the efficacy of TCR gene therapy in vivo.


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The function of T cell receptor (TCR) gene modified T cells is dependent on efficient surface expression of the introduced TCR α/β heterodimer. We tested whether endogenous CD3 chains are rate-limiting for TCR expression and antigen-specific T cell function. We show that co-transfer of CD3 and TCR genes into primary murine T cells enhanced TCR expression and antigen-specific T cell function in vitro. Peptide titration experiments showed that T cells expressing introduced CD3 and TCR genes recognised lower concentration of antigen than T cells expressing TCR only. In vivo imaging revealed that TCR+CD3 gene modified T cells infiltrated tumors faster and in larger numbers, which resulted in more rapid tumor elimination compared to T cells modified by TCR only. Following tumor clearance, TCR+CD3 engineered T cells persisted in larger numbers than TCR-only T cells and mounted a more effective memory response when re-challenged with antigen. The data demonstrate that provision of additional CD3 molecules is an effective strategy to enhance the avidity, anti-tumor activity and functional memory formation of TCR gene modified T cells in vivo.

**PLGA-based gene delivering nanoparticle enhance suppression effect of miRNA in HePG2 cells.**

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ABSTRACT: The biggest challenge in the field of gene therapy is how to effectively deliver target genes to special cells. This study aimed to develop a new type of poly(D,L-lactide-co-glycolide) (PLGA)-based nanoparticles for gene delivery, which are capable of overcoming the disadvantages of polyethylenimine (PEI)- or cationic liposome-based gene carrier, such as the cytotoxicity induced by excess positive charge, as well as the aggregation on the cell surface. The PLGA-based nanoparticles presented in this study were synthesized by emulsion evaporation method and characterized by transmission electron microscopy, dynamic light scattering, and energy dispersive spectroscopy. The size of PLGA/PEI nanoparticles in phosphate-buffered saline (PBS) was about 60 nm at the optimal charge ratio. Without observable aggregation, the nanoparticles showed a better monodispersity. The PLGA-based nanoparticles were used as vector carrier for miRNA transfection in HepG2 cells. It exhibited a higher transfection efficiency and lower cytotoxicity in HepG2 cells compared to the PEI/DNA complex. The N/P ratio (ratio of the polymer nitrogen to the DNA phosphate) 6 of the PLGA/PEI/DNA nanocomplex displays the best property among various N/P proportions, yielding similar transfection efficiency when compared to Lipofectamine/DNA lipoplexes. Moreover, nanocomplex shows better serum compatibility than commercial liposome. PLGA nanocomplexes obviously accumulate in tumor cells after transfection, which indicate that the complexes contribute to cellular uptake of pDNA and pronouncedly enhance the treatment effect of miR-26a by inducing cell cycle arrest. Therefore, these results demonstrate that PLGA/PEI nanoparticles are promising non-viral vectors for gene delivery.


**Light-directed delivery of nucleic acids.**

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A major barrier within the field of non-viral gene therapy toward therapeutic strategies, e.g., tumor therapy, has been lack of appropriate specific delivery strategies to the intended target tissues or cells. In this chapter, we describe a protocol for light-directed delivery of nucleic acids through the use of photochemical internalization (PCI) technology. PCI is based on a photosensitizing compound that localizes to endocytic membranes. Upon illumination, the photosensitizing compound induces damage to the endocytic membranes, resulting in release of endocytosed material, i.e., nucleic acids into cytosol. The main benefit of the strategy described is the possibility for site-specific delivery of nucleic acids to a place of interest.
Glucagon-like Peptide-1 gene therapy.
Rowzee AM, Cawley NX, Chiorini JA, Di Pasquale G.
Molecular Physiology and Therapeutics Branch, National Institute of Dental and Craniofacial Research, Bethesda, MD 20892-2190, USA.

Glucagon-like peptide 1 (GLP-1) is a small peptide component of the prohormone, proglucagon, that is produced in the gut. Exendin-4, a GLP-1 receptor agonist originally isolated from the saliva of H. suspectum or Gila monster, is a peptide that shares sequence and functional homology with GLP-1. Both peptides have been demonstrated to stimulate insulin secretion, inhibit glucagon secretion, promote satiety and slow gastric emptying. As such, GLP-1 and Exendin-4 have become attractive pharmaceutical targets as an adjunctive therapy for individuals with type II diabetes mellitus, with several products currently available clinically. Herein we summarize the cell biology leading to GLP-1 production and secretion from intestinal L-cells and the endocrine functions of this peptide and Exendin-4 in humans. Additionally, gene therapeutic applications of GLP-1 and Exendin-4 are discussed with a focus on recent work using the salivary gland as a gene therapy target organ for the treatment of diabetes mellitus.

Application of magnetic nanoparticles to gene delivery.
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Nanoparticle technology is being incorporated into many areas of molecular science and biomedicine. Because nanoparticles are small enough to enter almost all areas of the body, including the circulatory system and cells, they have been and continue to be exploited for basic biomedical research as well as clinical diagnostic and therapeutic applications. For example, nanoparticles hold great promise for enabling gene therapy to reach its full potential by facilitating targeted delivery of DNA into tissues and cells. Substantial progress has been made in binding DNA to nanoparticles and controlling the behavior of these complexes. In this article, we review research on binding DNAs to nanoparticles as well as our latest study on non-viral gene delivery using polyethylenimine-coated magnetic nanoparticles.
In-utero stem cell transplantation: clinical use and therapeutic potential.
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Early evidence suggests that in-utero stem cell transplantation represents a new therapeutic strategy for different congenital disease. Moreover, gene therapy constitutes one of the most promising new approach to treat a wide spectrum of genetic disorders. It was shown that the fetus could represent an ideal recipient because of his immunologic early naiveté in gestation that reduces the risk of immunoreactions. Clinical experience in human fetus was performed in order to treat immunodeficiency and metabolic disorders, hemoglobinopathies and some other genetic diseases. Use of alternative source (i.e., cord blood, placenta, membrane, amniotic fluid, fetal tissue) of stem cell transplanted has been only one of the several strategies to improve donor cell advantages on host stem cell. The present review focused on the clinical use and therapeutic potential of in-utero stem cell transplantation, reporting the outcome of human cases treated and the limits of this therapy and possible future applications.

E phage gene transfection associated to chemotherapeutic agents increases apoptosis in lung and colon cancer cells.
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The limited ability of conventional therapies to achieve the long-term survival of metastatic lung and colon cancer patients suggests the need for new treatment options. In this respect, genes encoding cytotoxic proteins have been proposed as a new strategy to enhance the activity of drugs, and combined therapies involving such genes and classical antitumoral drugs have been studied intensively. The E gene from phiX174 encodes a membrane protein with a toxic domain that leads to a decrease in tumour cell growth rates. Therefore, in order to improve the anti-tumour effects of currently used chemotherapeutic drugs on cancer cells, we investigated the association of the E suicide gene with these antineoplastic drugs. The E gene has antitumoral effects in both lung and colon cancer cells. In addition, expression of this gene induces ultrastructural changes in lung cancer transfected cells (A-549), although the significance of these changes remains unknown. The effect of combined therapy (gene and cytotoxic therapy) enhances the inhibition of tumour cell proliferation in comparison to single treatments. Indeed, our in vitro results indicate that an experimental therapeutic strategy based on this combination of E gene therapy and cytotoxic drugs may result in a new treatment strategy for patients with advanced lung and colon cancer.
Intravenous administration of AAV2/9 to the fetal and neonatal mouse leads to differential targeting of CNS cell types and extensive transduction of the nervous system.

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Several diseases of the nervous system are characterized by neurodegeneration and death in childhood. Conventional medicine is ineffective, but fetal or neonatal gene therapy may provide an alternative route to treatment. We evaluated the ability of single-stranded and self-complementary adeno-associated virus pseudotype 2/9 (AAV2/9) to transduce the nervous system and target gene expression to specific neural cell types following intravenous injection into fetal and neonatal mice, using control uninjected age-matched mice. Fetal and neonatal administration produced global delivery to the central (brain, spinal cord, and all layers of the retina) and peripheral (myenteric plexus and innervating nerves) nervous system but with different expression profiles within the brain; fetal and neonatal administration resulted in expression in neurons and protoplasmic astrocytes, respectively. Neither single-stranded nor self-complementary AAV2/9 triggered a microglia-mediated immune response following either administration. In summary, intravenous AAV2/9 targets gene expression to specific neural cell types dependent on developmental stage. This represents a powerful tool for studying nervous system development and disease. Furthermore, it may provide a therapeutic strategy for treatment of early lethal genetic diseases, such as Gaucher disease, and for disabling neuropathies, such as preterm brain injury.-Rahim, A. A., Wong, A. M. S., Hoefer, K., Buckley, S. M. K., Mattar, C. N., Cheng, S. H., Chan, J. K. Y., Cooper, J. D., Waddington, S. N. Intravenous administration of AAV2/9 to the fetal and neonatal mouse leads to differential targeting of central nervous system cell types and extensive transduction of the nervous system.

Electrospun nanofibrous scaffolds for controlled release of adeno-associated viral vectors.

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The integration of viral gene delivery with key features of biomaterial scaffolds that modulate viral delivery in a controlled manner offers a promising strategy for numerous tissue engineering applications. In this study adeno-associated virus (AAV), which is widely utilized in human gene therapy as a gene carrier due to its safety and efficient gene delivery capability, was encapsulated within electrospun nanofibrous scaffolds composed of blended mixtures of elastin-like polypeptides (ELP) and poly (ε-caprolactone) (PCL) and was employed to transduce fibroblasts adherent on the scaffolds. Combinatorial interactions between ELP and PCL chains upon physical blending significantly altered the mechanical properties (i.e. wettability, elastic modulus, strain, etc.) of the ELP/PCL composites, thus providing key tools to mediate controlled release of AAV vectors and robust cellular transduction on the fibrous scaffolds. The ability of ELP/PCL composites to manipulate the controlled release of AAV-mediated gene delivery for subsequent high-efficiency cellular transduction will provide tremendous opportunities for a variety of tissue engineering applications.
European attitudes to gene therapy and pharmacogenetics.
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Views on pharmacogenetics and gene therapy systematically differ across European countries. But despite a complex regulatory regime there is a balance of support, albeit laced with considerable uncertainty.

Specific antibody-receptor interactions trigger InlAB-independent uptake of listeria monocytogenes into tumor cell lines.
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BACKGROUND:
Specific cell targeting is an important, yet unsolved problem in bacteria-based therapeutic applications, like tumor or gene therapy. Here, we describe the construction of a novel, internalin A and B (InlAB)-deficient Listeria monocytogenes strain (Lm-spa+), which expresses protein A of Staphylococcus aureus (SPA) and anchors SPA in the correct orientation on the bacterial cell surface.

RESULTS:
This listerial strain efficiently binds antibodies allowing specific interaction of the bacterium with the target recognized by the antibody. Binding of Trastuzumab (Herceptin®) or Cetuximab (Erbitux®) to Lm-spa+, two clinically approved monoclonal antibodies directed against HER2/neu and EGFR/HER1, respectively, triggers InlAB-independent internalization into non-phagocytic cancer cell lines overexpressing the respective receptors. Internalization, subsequent escape into the host cell cytosol and intracellular replication of these bacteria are as efficient as of the corresponding InlAB-positive, SPA-negative parental strain. This specific antibody/receptor-mediated internalization of Lm-spa+ is shown in the murine 4T1 tumor cell line, the isogenic 4T1-HER2 cell line as well as the human cancer cell lines SK-BR-3 and SK-OV-3. Importantly, this targeting approach is applicable in a xenograft mouse tumor model after crosslinking the antibody to SPA on the listerial cell surface.

CONCLUSIONS:
Binding of receptor-specific antibodies to SPA-expressing L. monocytogenes may represent a promising approach to target L. monocytogenes to host cells expressing specific receptors triggering internalization.
Non-viral retinal gene-therapy: a review.
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In the developed world, diseases of the retina are common causes of untreatable blindness. In many cases, a genetic component to the aetiology has been identified, making the development of gene-based treatments a logical long-term goal. The clinical strategy for retinal gene therapy broadly encompasses two distinct advantages over systemic drug delivery. First is that gene delivery can limit expression of a therapeutic protein to a specific target cell, which is rarely possible even with local drug delivery methods. Second, by delivering DNA that remains stable and non-degraded, gene expression and hence protein production could in theory be indefinite, obviating the need for repeated tablets or injections. Viruses have evolved distinct mechanisms, such as receptor mediated uptake and genomic integration, which efficiently encompass these two properties. For non-viral gene therapy approaches, however, nuclear localisation and stable long-term transgene expression remain significant hurdles that need to be overcome. The challenge of non-viral gene therapy is therefore to harness current laboratory and molecular based techniques to develop a man-made system that can approach the efficiency of a natural biological process. In the unique environment of the retina, this goal may not be insurmountable and would overcome the major limiting factor of adeno-associated viral vectors, which is the size of gene that can be delivered.

Integrase-Defective Lentiviral Vectors - A Stage for Nonviral Integration Machineries.
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Gene vehicles derived from lentiviruses have become highly esteemed tools for gene transfer and genomic insertion in a wealth of cell types both in vivo and ex vivo. However, accumulating evidence of preferred insertion into actively transcribed genes, driven by biological properties of the parental human immunodeficiency virus type 1, has questioned the safety of this vector technology. As a consequence, integrase-defective lentiviral vectors (IDLVs), carrying an inactive integrase protein, have been developed and used with success for persistent in vivogene transfer to quiescent or slowly dividing cells. We and others have shown that episomal DNA delivered by IDLVs may serve as a substrate for heterologous integration machineries, including recombinases and transposases, and homologous recombination triggered by nuclease-induced DNA damage. New vector systems that combine the best of lentiviral gene delivery and nonviral integration systems are under development. The first prototypes of such hybrid lentiviral vectors facilitate efficient gene transfer and show profiles of insertion that are not dictated by the biological constraints of the normal integration pathway and are, therefore, significantly different from the profile of conventional lentiviral vectors. The stage is set for further exploration of these vectors. In this review, we summarize the background and short history of hybrid IDLV-based vector systems and discuss their applicability in gene therapy and treatment of genetic disease.
Silencing of the human TERT gene by RNAi inhibits A549 lung adenocarcinoma cell growth in vitro and in vivo.
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Human telomerase reverse transcriptase (hTERT) is the catalytic subunit and the activity determinant factor of the telomerase enzyme which maintains the length of human chromosomes. In recent years it has become an attractive molecular target for cancer gene therapy. In the present study, we show that hTERT siRNA effectively suppressed the expression of hTERT mRNA and hTERT protein levels, reduced telomerase activity, and induced apoptosis of A549 lung adenocarcinoma cells (P<0.05). In vivo, tumors treated with the hTERT siRNA were of reduced sizes, indicating that the hTERT siRNA also reduced the tumorigenic potential of lung adenocarcinoma cells (P<0.05). These results demonstrate that hTERT siRNA can cause effective suppression of telomerase and lead to apoptosis in A549 lung adenocarcinoma cells. hTERT siRNA may, therefore, be a strong candidate for highly selective therapy for chemoprevention and treatment of lung adenocarcinoma.
promising strategy for clinical application.

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Treatment of chemotherapy resistant ovarian cancer with a MDR1 targeted oncolytic adenovirus.
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OBJECTIVE:
Multidrug resistance gene 1 (MDR1) mediated resistance to chemotherapeutic agents is a major obstacle for the therapy of various cancer types. The use of conditionally replicating adenoviruses (CRAds) is dependent on molecular differences between tumor cells and non-tumor cells. Transcriptional targeting of CRAd replication is an effective way to control replication regulation. The aim of this study was to evaluate the effect of a MDR1 targeted fiber-modified CRAd against chemotherapy resistant ovarian cancer.

METHODS:
MDR1 expression was evaluated in chemotherapy naïve and pretreated ovarian cancer cells and various control cells. We constructed 2 variants of a fiber-modified CRAd, Ad5/3MDR1E1 and Ad5/3MDR1E1∆24 containing the MDR1 promoter to control viral replication via the E1A gene. The MDR promoter activity and cell killing efficacy were evaluated in vitro. Orthotopic murine models of peritoneally disseminated ovarian cancer were utilized to evaluate the preclinical efficacy of MDR targeted CRAds in vivo. To evaluate the liver toxicity of MDR1 targeted CRAds, we compared Ad5/3MDR1E1 with Ad5/3∆24, a CRAd that replicates in cancer cells inactive in the Rb/p16 pathway by use of an in vivo hepatotoxicity model.

RESULTS:
We demonstrate efficient oncolysis of Ad5/3MDR1E1 in both chemotherapy resistant ovarian cancer cell lines and in primary tumor cells from pretreated patients as well as therapeutic efficacy in an orthotopic mouse model. Ad5/3MDR1E1 demonstrated significantly decreased liver toxicity compared to other 5/3-fiber modified control vectors examined.

CONCLUSIONS:
In summary, Ad5/3MDR1E1 is an efficient and safe gene therapy approach for specific targeting of chemotherapy resistant cancer cells.


Influence of needle gauge on in vivo ultrasound and microbubble-mediated gene transfection.
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Ultrasound and microbubble-mediated gene transfection are potential tools for safe, site-selective gene therapy. However, preclinical trials have demonstrated a low transfection efficiency that has hindered the progression of the technique to clinical application. In this paper it is shown that simple changes to the method of intravenous injection can lead to an increase in transfection efficiency when using 6-MHz diagnostic ultrasound and the ultrasound contrast agent, SonoVue. By using needles of progressively smaller gauge, i.e., larger internal diameter (ID), from 29 G (ID 0.184 mm) to 25 G (ID 0.31 mm), the transfection of a luciferase plasmid (pGL4.13) was significantly increased threefold in heart-targeted female CD1 mice. In vitro work indicated that the concentration and size distribution of SonoVue were affected by increasing needle gauge. These results suggest that the process of systemic delivery alters the bubble population and adversely affects transfection. This is exacerbated by using high-gauge needles. These findings demonstrate that the needle with the largest possible ID should be used for systemic delivery of microbubbles and genetic
Summit on cell therapy for cancer: the importance of the interaction of multiple disciplines to advance clinical therapy.
Melief CJ, O'Shea JJ, Stroncek DF.

ABSTRACT: The field of cellular therapy of cancer is moving quickly and the issues involved with its advancement are complex and wide ranging. The growing clinical applications and success of adoptive cellular therapy of cancer has been due to the rapid evolution of immunology, cancer biology, gene therapy and stem cell biology and the translation of advances in these fields from the research laboratory to the clinic. The continued development of this field is dependent on the exchange of ideas across these diverse disciplines, the testing of new ideas in the research laboratory and in animal models, the development of new cellular therapies and GMP methods to produce these therapies, and the testing of new adoptive cell therapies in clinical trials. The Summit on Cell Therapy for Cancer to held on November 1st and 2nd, 2011 at the National Institutes of Health (NIH) campus will include a mix of perspectives, concepts and ideas related to adoptive cellular therapy that are not normally presented together at any single meeting. This novel assembly will generate new ideas and new collaborations and possibly increase the rate of advancement of this field.

Mining the Adenovirus "Virome" for Systemic Oncolytics.
Barry MA, Weaver EA, Chen CY.

Adenoviruses (Ads) are arguably one of the most potent viruses for in vivo gene therapy, vaccine, and oncolytic applications. The attraction for the use of Ads stems from their ability to infect a wide range of dividing and non-dividing cell types in some cases to efficiencies of nearly 100%. Additional benefits include their stability, the ability to purify the vector to concentrations of up to 10(13) particles/ml, and the fact that viral vectors self-assemble into particles of specific size (≈100 nm). The vast majority of clinical applications of Ad have utilized Ad serotype 5 (Ad5) viruses. Considering that at least half of humans are already immune to Ad5, Ad5 oncolytics may not be optimal for clinical translation. Given this and that there are 54 different serotypes of human Ads, this review considers the utility of "mining" these alternate Ad serotypes for viruses that can evade Ad5 immunity and kill different types of cancer.
Targeted Oncolytic Herpes Simplex Viruses for Aggressive Cancers.
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Herpes simplex virus (HSV) is a well-known vector that is often used for gene therapy to treat cancers. The most attractive feature of HSV is its ability to destroy tumors through a distinctive oncolytic mechanism where the virus can destroy cancer cells via cell lysis, a killing function that no anti-cancer drugs can mimic. Importantly, HSV is a safe and effective virus that can be easily manipulated to preferentially replicate in tumor cells. In the last 20 years of re-engineering efforts, a number of HSV designs, including the classical G207, have been focused on deleting viral genes in order to render the virus tumor specific. Although such designs can successfully destroy tumor xenografts in animal models, with minimal impact on normal tissues, a common trade-off is the marked attenuation of the virus. This problem is most profound in many clinical tumors, where virus dissemination is often hindered by the difficult cellular and molecular terrain of the human tumor mass. In order to harness all of HSV's replication potential to destroy tumor cells, efforts in our lab, as well as others, last several years have been focused on engineering an oncolytic HSV to target tumor cells without deleting any viral genes, and have since generated highly tumor specific viruses including our transcriptional translational dually regulated HSV (TTDR-HSV). In this review, we will discuss the improvements associated with the newer TTDR-HSV design compared to the classical defective HSV designs such as G207 and tk- HSV. Lastly, we will review additional cellular features of aggressive tumors, such as their immense cellular heterogeneity and volatility, which may serve to hinder the dissemination of TTDR-HSV. The challenge for future studies would be to explore how TTDR-HSV could be redesigned and/or employed with combinatorial approaches to better target and destroy the heterogeneous and dynamic cell populations in the aggressive tumor mass.