



CLINIGENE CURRENT GENE THERAPY WEEKLY

From June 14th to June 21st 2010

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PMID:
20559776

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Delivery of Gene and Cellular Therapies for Heart Disease.

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Although there has been considerable interest in the utilization of gene and cellular therapy for heart disease in recent years, there remain critical questions prior to widespread promotion of therapy, and key among these issues is the delivery method used for both gene therapy and cellular therapy. Much of the failure of gene and cellular therapy can be explained by the biological therapy itself; however, certainly there is a critical role played by the delivery technique, in particular, those that have been adapted from routine clinical use such as intravenous and intracoronary injection. Development of novel techniques to deliver gene and cellular therapy has ensued with some preclinical and even clinical success, though questions regarding safety, invasiveness, and repeatability remain. Here, we review techniques for gene and cellular therapy delivery, both existing and adapted techniques, and novel techniques that have emerged recently at promoting improved efficacy of therapy without the cost of systemic distribution. We also highlight key issues that need to be addressed to improve the chances of success of delivery techniques to enhance therapeutic benefit.

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20559334

Cancer Gene Ther. 2010 Jun 18. [Epub ahead of print]

Direct and indirect antitumor effects by human peripheral blood lymphocytes expressing both chimeric immune receptor and interleukin-2 in ovarian cancer xenograft model.

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Human peripheral blood lymphocytes (PBLs) electroporated with RNA encoding anti-Her-2/neu-specific chimeric immune receptor (CIR) have been reported to elicit potent immune responses against SKOV3 tumors in a nude mouse model. However, CIR-electroporated PBL (CIR-PBL) did not proliferate, and the cell number rapidly decreased in the absence of exogenous interleukin-2 (IL-2). In this study, PBLs electroporated with both CIR and IL-2 RNA (CIR/IL-2-PBL) were studied to determine whether antitumor effects could be improved by adoptive immunotherapy. CIR and IL-2 were expressed in CIR/IL-2-PBL at levels similar to PBLs electroporated, with IL-2 RNA (IL-2-PBL) or CIR-PBL. Transfer of IL-2 RNA induced proliferation and prolonged survival of PBLs in vitro. In a xenograft model, both IL-2-PBL and CIR/IL-2-PBL showed significantly higher antitumor effects than CIR-PBL. The number of tumor-infiltrating natural killer (NK) cells was significantly increased in IL-2-PBL and CIR/IL-2-PBL. After NK cell depletion, IL-2-PBL showed significantly lower antitumor effects than CIR/IL-2-PBL. These results suggest that transfer of IL-2 RNA to CIR-PBL can promote NK cell infiltration of tumors and prolong survival of infused PBLs in vivo. RNA electroporated PBLs may represent efficient tools for delivery of functional molecules to tumors by multiple gene transfer.

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Cancer Gene Ther. 2010 Jun 18. [Epub ahead of print]

The soluble fragment of VE-cadherin inhibits angiogenesis by reducing endothelial cell proliferation and tube capillary formation.

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Vascular endothelial-specific cadherin (VE-cadherin) is an endothelial cell-specific adhesion molecule, localized at cell-cell contact sites. It is involved in physiological and pathological angiogenesis. In this study, we showed that in vitro a soluble N-terminal fragment of VE-cadherin (EC1-3) corresponding to cadherin 1-3 ectodomains inhibited vascular endothelial growth factor-stimulated endothelial cell proliferation and capillary tube structure formation in the matrigel model. In vivo, EC1-3 was tested in a murine colon cancer model. EC1-3-expressing colon cancer C51 cells were subcutaneously grafted into nude mice, and tumor growth and angiogenesis were evaluated. At day 33, the mean volume of the tumors developed was reduced (510+/-104 versus 990+/-120 mm³) for control). Similarly, injection of EC1-3 virus-producing cells into established C51 tumors resulted in an inhibition by 33% of tumor growth. Immunohistological staining of vessels on tumor sections showed a significantly reduced intratumoral angiogenesis. Furthermore, EC1-3 did not induce vessel injury in the lung, liver, spleen, heart and brain in the mice. These results suggest that the soluble N-terminal fragment of VE-cadherin EC1-3 could exert an antitumoral effect by targeting tumor angiogenesis, which included blocking endothelial cell proliferation and capillary tube formation with no obvious toxicity on normal organs.

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20559332

Cancer Gene Ther. 2010 Jun 18. [Epub ahead of print]

Tumor cells engineered to codisplay on their surface 4-1BBL and LIGHT costimulatory proteins as a novel vaccine approach for cancer immunotherapy.

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Primary tumor cells genetically modified to express a collection of immunological ligands on their surface may have the utility as therapeutic autologous cancer vaccines. However, genetic modification of primary tumor cells is not only cost, labor and time intensive, but also has safety repercussions. As an alternative, we developed the ProtEx technology that involves generation of immunological ligands with core streptavidin (SA) and their display on biotinylated cells in a rapid and efficient manner. We herein demonstrate that TC-1 tumor cells can be rapidly and efficiently engineered to codisplay on their surface two costimulatory proteins, SA-4-1BBL and SA-LIGHT, simultaneously. Vaccination with irradiated TC-1 cells codisplaying both chimeric proteins showed 100% efficacy in a prophylactic and >55% efficacy in a therapeutic tumor setting. In contrast, vaccination with TC-1 cells engineered with either protein alone showed significantly reduced efficacy in the prophylactic setting. Vaccine efficacy was associated with the generation of primary and memory T-cell and antibody responses against the tumor without detectable signs of autoimmunity. Engineering tumor cells in a rapid and effective manner to simultaneously display on their surface a collection of immunostimulatory proteins with additive/synergistic functions presents a novel alternative approach to gene therapy with considerable potential for cancer immunotherapy.

PMID: 20558975 Dermatology. 2010 Jun 18. [Epub ahead of print]

A Novel Transdermal Plasmid-Dimethylsulfoxide Delivery Technique for Treatment of Psoriasis.

Zhang Y, Li J, Liu CY, Zhou XK, Qiu J, Zhang YB, Huang NY, Li Y, Chen XJ, Li XL, Wang YS, Yang HS, Chen XC, Kan B, Mao YQ, Deng HX, Yang L, Wen YJ, Zhao X, Wei YQ.
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Background: Psoriasis is a chronic and relapsing inflammatory skin disease associated with various immunologic abnormalities. Repeated subcutaneous injection of interleukin-4 (IL-4) has been established as an effective treatment to counteract psoriasis. **Objective:** We investigated whether gene therapy using IL-4 expression plasmid (pIL-4) via transdermal delivery was an alternative treatment for psoriasis. In our experiment, dimethylsulfoxide (DMSO) was used as a penetration enhancer. **Methods:** At first, the penetration efficiency of the complex of reporter plasmid accompanied by DMSO was investigated both in vitro and in vivo. Then, the antipsoriasis efficiency of the treatment with pIL-4-DMSO was tested in mice. **Results:** The expression of the reporter gene was detected in epidermis and dermis both in vitro and in vivo. More importantly, the psoriasis symptoms were relieved, and significant reductions in some psoriasis-associated factors were observed after pIL-4-DMSO treatment. **Conclusion:** We conclude that the topical application of pIL-4-DMSO can treat psoriasis to a significant extent.

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Electroporation Gene Therapy Preclinical and Clinical Trials for Melanoma.

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In vivo electroporation (EP) is a versatile delivery method for gene transfer which can be applied to any accessible tissue. Delivery of plasmid DNA encoding therapeutic genes or cDNAs with in vivo EP has been tested extensively in preclinical melanoma models. Direct delivery to the tumor has been shown to generate a direct antitumor effect. Delivery to alternative sites may generate additional therapeutic options, for example the production of cancer vaccines, the reduction of tumor angiogenesis, or the induction of tumor cell apoptosis. Several of the preclinical therapies tested have a demonstrated therapeutic effect against melanomas. Two immunotherapies have advanced to melanoma clinical trials. Delivery of a plasmid DNA encoding interleukin-12 (IL-12) or interleukin-2 (IL-2) using electroporation was demonstrated to be a safe with no grade 3 or 4 toxicities reported. Delivery of IL-12 with electroporation resulted in significant necrosis of melanoma cells in the majority of treated tumors and significant lymphocytic infiltrate in biopsies from patients in several cohorts. In addition, clinical evidence of responses in untreated lesions suggested the induction of a systemic response following therapy. This review discusses preclinically tested electroporation gene therapies for melanoma with clinical potential and the conversion of these therapies to clinical trials.

PMID:
20556588

J Huazhong Univ Sci Technolog Med Sci. 2010 Jun;30(3):391-6. Epub 2010 Jun 17.

Effects of combined siRNA-TR and -TERT on telomerase activity and growth of bladder transitional cell cancer BIU-87 cells.

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The effects of combined RNA interference (RNAi) of human telomerase RNA (hTR) and human telomerase reverse transcriptase (hTERT) genes on telomerase activity in a bladder cancer cell line (BIU-87 cells) were investigated by using gene chip technology in vitro with an attempt to evaluate the role of RNAi in the gene therapy of bladder transitional cell cancer (BTCC). Three TR-specific double-stranded small interfering RNAs (siRNAs) and three TERT-specific double-stranded siRNAs were designed to target different regions of TR and TERT mRNA. The phTR-siRNA, pHTERT-siRNA, and the combination of both plasmids phTR+phTERT-siRNA were transfected into BIU-87 cells. The expression of hTR and hTERT mRNA was detected by quantitative fluorescent reverse transcription-polymerase chain reaction, and a telomeric repeat amplification protocol was applied to detect telomerase activity. Growth inhibition of BIU-87 cells was measured by MTT assay. Gene chip analysis was performed to evaluate the effects of the combined RNAi of hTR+hTERT genes on telomerase activity and growth of BIU-87 cells in vitro. The results showed that the expression of hTERT and hTR mRNA was inhibited by pRNAT-hTERT-III, pRNAT-hTR-III, and pRNAT-hTR-III+hTERT-III in BIU-87 cells. The inhibition efficiency of pRNAT-hTERT-III, pRNAT-hTR-III, pRNAT-hTERT-III+pRNAT-hTR-III was 67% for TERT mRNA, 41% for TR mRNA, 57% for TR mRNA and 70% for TERT mRNA in BIU-87 cells respectively. The growth of BIU-87 cells was inhibited and telomerase activity was considerably decreased, especially in the cells treated with combined RNAi-hTR and -hTERT. Gene chip analysis revealed that 21 genes were down-regulated (ATM, BAX, BCL2, BCL2L1, BIRC5, CD44, CTNNA1, E2F1, JUN, MCAM, MTA1, MYC, NFKB1, NFKBIA, NME4, PNN, PNN, SERPINE1, THBS1, TNFRSF1A, and UCC1). The results indicated that hTR-siRNA and hTERT-siRNA, especially their combination, siRNA hTR+hTERT, specifically and effectively suppressed the expression of both hTR and hTERT mRNA and telomerase activity. Molecular biological mechanism by which combined siRNA-TR and -TERT inhibited telomerase activity and growth of BIU-87 cells in vitro may involve the down-regulation of the 21 genes.

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20556583

J Huazhong Univ Sci Technolog Med Sci. 2010 Jun;30(3):365-9. Epub 2010 Jun 17.

Cloning of WWOX gene and its growth-inhibiting effects on ovarian cancer cells.

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The growth-inhibiting and apoptosis-inducing effects of WW domain-containing oxidoreductase (WWOX) gene on ovarian cancer cell line A2780 were investigated. The full length cDNA of human WWOX gene was amplified from normal human ovary tissues. The correct cDNA of full length WWOX was subcloned into eukaryotic expression vector pCMV. After introduction of WWOX gene into cancer cells with liposome, the WWOX mRNA and protein level in the cancer cells were detected by reverse transcription polymerase chain reaction (RT-PCR) and immunoblotting. The growth activities of cancer cells were detected by Trypan blue staining. The clone formation assay in soft agar was employed to observe the proliferation of the cancer cells. Apoptosis was examined by DNA ladder and acridine orange-ethidium bromide fluorescent staining. The results showed that 72 h after WWOX gene transfection, the WWOX expression was increased significantly ($P < 0.01$). The growth of ovarian cancer cells was decreased by 16.41% to 38.49% ($P < 0.01$). The clone formation abilities were reduced ($P < 0.01$). Some cancer cells presented the characteristic morphological changes of apoptosis with obvious ladder bands on electrophoresis. The apoptosis rate was $(20.7 \pm 6.0)\%$ ($P < 0.01$). It was concluded that over-expression of WWOX gene could induce apoptosis and inhibit the growth of ovarian cancer cells, which might be potentially useful in the gene therapy of ovarian cancers.

PMID:
2055360

Gene Ther. 2010 Jun 17. [Epub ahead of print]

Adoptive immunotherapy with genetically engineered T cells: modification of the IgG1 Fc 'spacer' domain in the extracellular moiety of chimeric antigen receptors avoids 'off-target' activation and unintended initiation of an innate immune response.

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Chimeric antigen receptors (CARs, immunoreceptors) are frequently used to redirect T cells with pre-defined specificity, in particular towards tumour cells for use in adoptive immunotherapy of malignant diseases. Specific targeting is mediated by an extracellularly located antibody-derived binding domain, which is joined to the transmembrane and intracellular CD3zeta moiety for T-cell activation. Stable CAR expression in T cells, however, requires a spacer domain interposed between the binding and the transmembrane domain and which is commonly the constant IgG1 Fc domain. We here revealed that CARs with Fc spacer domain bind to IgG Fc gamma receptors (FcgammaRs), thereby unintentionally activating innate immune cells, including monocytes and natural killer (NK) cells, which consequently secrete high amounts of pro-inflammatory cytokines. Engineered T cells, on the other hand, are likewise activated by FcgammaR binding resulting in cytokine secretion and lysis of monocytes and NK cells independently of the redirected specificity. To reduce FcgammaR binding, we modified the spacer domain without affecting CAR expression and antigen binding. Engineered with the modified CAR, T cells are not activated in presence of FcgammaR(+) cells, thereby minimizing the risk of off-target activation while preserving their redirected targeting specificity.

PMID: 20555022
Sci Transl Med. 2010 Jun 16;2(36):36ra43.

RNA-based gene therapy for HIV with lentiviral vector-modified CD34(+) cells in patients undergoing transplantation for AIDS-related lymphoma.

DiGiusto DL, Krishnan A, Li L, Li H, Li S, Rao A, Mi S, Yam P, Stinson S, Kalos M, Alvarnas J, Lacey SF, Yee JK, Li M, Couture L, Hsu D, Forman SJ, Rossi JJ, Zaia JA.
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AIDS patients who develop lymphoma are often treated with transplanted hematopoietic progenitor cells. As a first step in developing a hematopoietic cell-based gene therapy treatment, four patients undergoing treatment with these transplanted cells were also given gene-modified peripheral blood-derived (CD34(+)) hematopoietic progenitor cells expressing three RNA-based anti-HIV moieties (tat/rev short hairpin RNA, TAR decoy, and CCR5 ribozyme). In vitro analysis of these gene-modified cells showed no differences in their hematopoietic potential compared with nontransduced cells. In vitro estimates of successful expression of the anti-HIV moieties were initially as high as 22% but declined to approximately 1% over 4 weeks of culture. Ethical study design required that patients be transplanted with both gene-modified and unmanipulated hematopoietic progenitor cells obtained from the patient by apheresis. Transfected cells were successfully engrafted in all four infused patients by day 11, and there were no unexpected infusion-related toxicities. Persistent vector expression in multiple cell lineages was observed at low levels for up to 24 months, as was expression of the introduced small interfering RNA and ribozyme. Therefore, we have demonstrated stable vector expression in human blood cells after transplantation of autologous gene-modified hematopoietic progenitor cells. These results support the development of an RNA-based cell therapy platform for HIV.

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Sci Transl Med. 2010 Jun 16;2(36):36ps30.

Gene therapy takes a cue from HAART: combinatorial antiviral therapeutics reach the clinic.

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For the first time, scientists have tested a combination of three RNA-based gene therapies, delivered via a lentiviral vector, to target HIV in patients. This study not only demonstrates the safety and long-term viability of this approach, but also highlights areas in which focused improvements in gene therapy strategies may provide the most impact in increasingly translating promise in the laboratory to efficacy in the clinic.

PMID:
20552345

Methods Mol Biol. 2010;635:133-45.

Photochemical internalization (PCI): a technology for drug delivery.

Berg K, Weyergang A, Prasmickaite L, Bonsted A, Høgset A, Strand MT, Wagner E, Selbo PK.

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The utilization of macromolecules in therapy of cancer and other diseases is becoming increasingly relevant. Recent advances in molecular biology and biotechnology have made it possible to improve targeting and design of cytotoxic agents, DNA complexes, and other macromolecules for clinical applications. To achieve the expected biological effect of these macromolecules, in many cases, internalization to the cell cytosol is crucial. At an intracellular level, the most fundamental obstruction for cytosolic release of the therapeutic molecule is the membrane-barrier of the endocytic vesicles. Photochemical internalization (PCI) is a novel technology for release of endocytosed macromolecules into the cytosol. The technology is based on the use of photosensitizers located in endocytic vesicles that upon activation by light induces a release of macromolecules from their compartmentalization in endocytic vesicles. PCI has been shown to potentiate the biological activity of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane, including type I ribosome-inactivating proteins (RIPs), gene-encoding plasmids, adenovirus, oligonucleotides, and the chemotherapeutic bleomycin. PCI has also been shown to enhance the treatment effect of targeted therapeutic macromolecules. The present protocol describes PCI of an epidermal growth factor receptor (EGFR)-targeted protein toxin (Cetuximab-saporin) linked via streptavidin-biotin for screening of targeted toxins as well as PCI of nonviral polyplex-based gene therapy. Although describing in detail PCI of targeted protein toxins and DNA polyplexes, the methodology presented in these protocols are also applicable for PCI of other gene therapy vectors (e.g., viral vectors), peptide nucleic acids (PNA), small interfering RNA (siRNA), polymers, nanoparticles, and some chemotherapeutic agents.

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20551917

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Ex Vivo Transduction and Transplantation of Bone Marrow Cells for Liver Gene Delivery of alpha1-Antitrypsin.

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Adult stem cell-based gene therapy holds several unique advantages including avoidance of germline or other undesirable cell transductions. We have previously shown that liver progenitor (oval) cells can be used as a platform for liver gene delivery of human alpha1-antitrypsin (hAAT). However, this cell source cannot be used in humans for autologous transplantation. In the present study, we tested the feasibility of bone marrow (BM) cell-based liver gene delivery of hAAT. In vitro studies showed that BM cells can be transduced by lentiviral vector (Lenti-CB-hAAT) and recombinant adeno-associated viral vectors (rAAV1-CB-hAAT, and rAAV8-CB-hAAT). Transplantation studies showed that transplanted BM cells homed into liver, differentiated into hepatocytes and expressed hAAT in the liver. Importantly, we showed that transplantation of rAAV8-CB-hAAT vector-transduced BM cells resulted in sustained levels of hAAT in the systemic circulation of recipient mice. These results demonstrated that rAAV vector-mediated BM cell-based liver gene therapy is feasible for the treatment of AAT deficiency and implies a novel therapy for the treatment of liver diseases.

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20551916

Recognition of Virus Infection and Innate Host Responses to Viral Gene Therapy Vectors.

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The innate immune and inflammatory response represents one of the key stumbling blocks limiting the efficacy of viral-based therapies. Numerous human diseases could be corrected or ameliorated if viruses were harnessed to safely and effectively deliver therapeutic genes to diseased cells and tissues in vivo. Recent studies have shown that host cells recognize viruses using an elaborate network of sensor proteins localized at the plasma membrane, in endosomes, or in the cytosol. Three classes of sensors have been implicated in sensing viruses in mammalian cells-Toll-like receptors (TLRs), retinoid acid-inducible gene (RIG)-I-like receptors (RLRs), and nucleotide oligomerization domain (NOD)-like receptors (NLRs). The interaction of virus-associated nucleic acids with these sensor molecules triggers a signaling cascade that activates the principal host defense program aimed to limit or eliminate virus infection and restore tissue homeostasis. In addition, recent data strongly suggest that host cells can mount innate immune responses to viruses without prior recognition of their nucleic acids. To deliver therapeutic genes into the nuclei of diseased cells, viral gene therapy vectors must be efficient at penetrating either the plasma or endosomal membrane. The therapeutic use of high numbers of virus particles disturbs cellular homeostasis, triggering cell damage and stress pathways, or "sensing of modified self". Accumulating data indicate that the sensing of modified self might represent a powerful framework explaining the innate immune response activation by viral gene therapy vectors.

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20551908

Exon Skipping and Duchenne Muscular Dystrophy Therapy: Selection of the Most Active U1 snRNA Antisense Able to Induce Dystrophin Exon 51 Skipping.

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One promising approach for the gene therapy of Duchenne muscular dystrophy (DMD) is exon skipping. When thinking of possible intervention on human, it is very crucial to identify the most appropriate antisense sequences able to provide the highest possible skipping efficiency. In this article, we compared the exon 51 skipping activity of 10 different antisense molecules, raised against splice junctions and/or exonic splicing enhancers (ESEs), expressed as part of the U1 small nuclear RNA (snRNA). The effectiveness of each construct was tested in human DMD myoblasts carrying the deletion of exons 48-50, which can be treated with skipping of exon 51. Our results show that the highest skipping activity and dystrophin rescue is achieved upon expression of a U1 snRNA-derived antisense molecule targeting exon 51 splice sites in combination with an internal exon sequence. The efficacy of this molecule was further proven on an exon 45-50 deletion background, utilizing patient's fibroblasts transdifferentiated into myoblasts. In this system, we showed that the selected antisense was able to produce 50% skipping of exon 51.

PMID:
20551514

J Clin Invest. 2010 Jul 1;120(7):2345-54. doi: 10.1172/JCI40767. Epub 2010 Jun 14.

Efficient and stable MGMT-mediated selection of long-term repopulating stem cells in nonhuman primates.

Beard BC, Trobridge GD, Ironside C, McCune JS, Adair JE, Kiem HP.

HSC transplantation using genetically modified autologous cells is a promising therapeutic strategy for various genetic diseases, cancer, and HIV. However, for many of these conditions, the current efficiency of gene transfer to HSCs is not sufficient for clinical use. The ability to increase the percentage of gene-modified cells following transplantation is critical to overcoming this obstacle. In vivo selection with mutant methylguanine methyltransferase (MGMT^{P140K}) has been proposed to overcome low gene transfer efficiency to HSCs. Previous studies have shown efficient in vivo selection in mice and dogs but only transient selection in primates. Here, we report efficient and stable MGMT^{P140K}-mediated multilineage selection in both macaque and baboon nonhuman primate models. Treatment consisting of both O⁶-benzylguanine (O⁶BG) and N,N'-bis(2-chloroethyl)-N-nitroso-urea (BCNU) stably increased the percentage of transgene-expressing cells from a range of initial levels of engrafted genetically modified cells, with the longest follow-up after drug treatment occurring over 2.2 years. Drug treatment was well tolerated, and selection occurred in myeloid, lymphoid, and erythroid cells as well as platelets. Retrovirus integration site analysis before and after drug treatments confirmed the presence of multiple clones. These nonhuman primate studies closely model a clinical setting and should have broad applications for HSC gene therapy targeting human diseases of malignant, genetic, and infectious nature, including HIV.

PMID:
20550436

Drug Chem Toxicol. 2010 Jun 15. [Epub ahead of print]

Comparative studies on the genotoxicity and cytotoxicity of polymeric gene carriers polyethylenimine (PEI) and polyamidoamine (PAMAM) dendrimer in Jurkat T-cells.

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A safe alternative to the viral system used in gene therapy is a nonviral gene delivery system. Although polyethylenimine (PEI) and polyamidoamine (PAMAM) dendrimer are among the most promising gene-carrier candidates for efficient nonviral gene delivery, safety concerns regarding their toxicity remain. The aim of this study was to scrutinize the underlying mechanism of the cytotoxicity and genotoxicity of PEI (25 kDa) and PAMAM (G4). To our knowledge, this is the first study to explore the genotoxic effect of polymeric gene carriers. To evaluate cell death by PEI and PAMAM, we performed propidium-iodide staining and lactate-dehydrogenase release assays. The genotoxicity of the polymers was measured by comet assay and cytokinesis-block micronucleus assay. PEI- and PAMAM-treated groups induced both necrotic and apoptotic cell death. In the comet assay and micronuclei formation, significant increases in DNA damage were observed in both treatments. We conclude that PEI and PAMAM dendrimer can induce not only a relatively weak apoptotic and a strong necrotic effect, but also a moderate genotoxic effect.

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20549818

Cancer. 2010 Jun 14. [Epub ahead of print]

Adenovirus-mediated expression of truncated E2F-1 suppresses tumor growth in vitro and in vivo.

Gomez-Gutierrez JG, Garcia-Garcia A, Hao H, Rao XM, Montes de Oca-Luna R, Zhou HS, McMasters KM.

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BACKGROUND:: Adenovirus (Ad)-mediated E2F-1 gene transfer induces apoptosis in cancer cells in vitro and in vivo, but clinical application of E2F-1 in cancer gene therapy remains controversial because of the oncogenic potential of E2F-1. This barrier can be circumvented by using the truncated form of the E2F-1 gene (E2Ftr) (amino acids 1 through 375), which lacks the E2F-1 transactivation domain and cell cycle-promoting effects. **METHODS::** The authors constructed 3 adenoviral vectors that expressed E2Ftr under regulation of the tetracycline (Tet)-off system (AdTet-E2Ftr1, AdTet-E2Ftr2, and AdTet-E2Ftr3). These vectors were compared for E2Ftr expression and apoptosis induction in cancer cells and normal cells. E2Ftr antitumor activity in vivo also was assessed in a melanoma xenograft model. **RESULTS::** One of the 3 vectors, AdTet-E2Ftr3, had the highest E2Ftr protein expression levels, which were correlated with the greatest induction of apoptosis and inhibition of cancer cell growth. E2Ftr induced apoptosis in a variety of cancer cell lines independent of p53 status with little cytotoxicity in normal cell lines. In a mouse melanoma xenograft model, AdTet-E2Ftr3 exhibited an approximately 80% decrease in tumor size compared with controls in vivo. **CONCLUSIONS::** The current results indicated that AdTet-E2Ftr3 is a novel anticancer agent that has significant therapeutic activity in vitro and in vivo. Cancer 2010. (c) 2010 American Cancer Society.

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Using a fed-batch culture strategy to enhance rAAV production in the baculovirus/insect cell system.

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Recombinant adeno-associated virus (rAAV) is one of the most promising vectors for human gene therapy. However, the production systems that are currently available have a limited capacity and cannot provide sufficient quantities of rAAV for preclinical or clinical trials. Many novel methods for improving rAAV production have been developed, but few researchers have focused on the culture process. In this study, we use a fed-batch culture system to enhance rAAV yield in the baculovirus/insect cell system. When the insect cells were co-infected with MOI=5 of Bac-GFP at a ratio of 1:9:9 (Bac-GFP: Bac-Rep: Bac-VP), the fed-batch culture achieved optimal rAAV yields. In batch culture, the optimal cell density for producing rAAV was found to be 1×10^6 cells/ml, and the highest rAAV yield (1.22×10^8 IVP/ml, 122 IVP/cell) occurred at day 5 post-infection. In the fed-batch culture, rAAV yield reached 2.13×10^8 IVP/ml at day 4 post-infection, and the highest rAAV yield was 2.40×10^8 IVP/ml (240 IVP/cell) at day 5 post-infection. The cost of the batch and fed-batch cultures is similar; however, the rAAV yield was 2.6-fold higher in the fed-batch culture system compared with that in the batch culture system. Therefore, here we demonstrated an economical and efficient strategy for rAAV production. Copyright © 2010 The Society for Biotechnology, Japan. Published by Elsevier B.V. All rights reserved.

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Clinical value of signal transducers and activators of transcription 3 (STAT3) gene expression in human osteosarcoma.

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The dysregulation of signal transducers and activators of transcription 3 (STAT3) has been reported to be associated with tumor progression, angiogenesis and metastasis. The purpose of this study was to analyze the clinical value of STAT3 expression in human osteosarcoma. First, semi-quantitative RT-PCR was performed to detect the expression of STAT3 mRNA in normal bone tissues, chondroma tissues and osteosarcoma tissues. Then, immunohistochemistry was performed to detect the expression of STAT3 protein in 76 osteosarcoma tissues and the relationship of STAT3 protein expression with clinicopathologic factors or prognosis of osteosarcoma patients. RNA interference (RNAi) technology was employed to inhibit STAT3 expression. MTT and flow cytometric assays were performed to analyze the effect of STAT3 inhibition on proliferation and apoptosis of osteosarcoma cells. Finally, the expression of STAT3-related target genes were also determined. Results showed that osteosarcoma tissues showed significantly higher expression levels of STAT3 mRNA than normal bone or chondroma tissues ($P < 0.05$). Immunohistochemistry showed that the staining of STAT3 protein was mainly located in cytoplasm of osteosarcoma cells in osteosarcoma tissue samples. The high level of STAT3 protein was associated with poor tumor differentiation and presentation of metastasis ($P = 0.039$ and 0.022). Moreover, the 5-year overall and relapse-free survival rates for osteosarcoma patients with high STAT3 expression were lower than those for patients with low STAT3 expression. In addition, the status of STAT3 protein expression was an independent prognostic factor for both disease-free survival ($P = 0.0235$) and overall survival ($P = 0.0032$). RNAi-mediated STAT3 inhibition could induce proliferation inhibition and apoptosis enhancement in osteosarcoma cells, which might be associated with inhibition of some anti-apoptosis genes. Overall, STAT3 plays crucial roles in osteosarcoma development and might become a potential molecular target for gene therapy of human osteosarcomas.

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Lentiviral Vectors That Express UGT1A1 in Liver and Contain Mir-142 Target Sequences Normalize Hyperbilirubinemia in Gunn Rats.

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BACKGROUND & AIMS:: Crigler-Najjar type 1 (CN-I) is an inherited liver disease caused by an absence of bilirubin-UDP-glucuronosyltransferase (UGT1A1) activity. It results in life-threatening levels of unconjugated bilirubin; therapeutic options are limited. We used adult Gunn rats (an animal model of the disease) to evaluate the efficiency of lentiviral-based gene therapy to express UGT1A1 in liver. **METHODS::** Gunn rats were given intraportal injections of VSVG-pseudotyped lentiviral vectors that encode UGT1A1 under the control of a liver-specific transthyretin (TTR) promoter (mTTRUGT1A1); this vector does not contain target sequences for miR-142, a miRNA that is expressed specifically in hematopoietic cells. Rats were also injected with the vector mTTR.hUGT1A1.142T, which contains 4 copies of the miR-142 target sequences-its mRNA should be degraded in antigen presenting cells. Bilirubinemia was monitored; the presence of transduced hepatocytes was analyzed by quantitative PCR. Vector expression was tested in vitro, in rat hematopoietic cells. **RESULTS::** In Gunn rats, bilirubin levels normalized 2 weeks after administration of mTTRUGT1A1. However, hyperbilirubinemia resumed 8 weeks after vector administration, concomitant with the induction of an immune response. In contrast, in rats injected with mTTR-UGT1A1.142T, bilirubin levels normalized for up to 6 months and transduced cells were not eliminated. **CONCLUSION::** Lentiviral vectors that express UGT1A1 reduce hyperbilirubinemia in immunocompetent Gunn rats for at least 6 months. The immune response against virally expressed UGT1A1 can be circumvented by inclusion of miR-142 target sequences, which reduce vector expression in antigen presenting cells. This lentiviral-based gene therapy approach might be developed to treat patients with CN-I.

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Decorin transfection suppresses profibrogenic genes and myofibroblast formation in human corneal fibroblasts.

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Decorin, a small leucine-rich proteoglycan, is a natural inhibitor of transforming growth factor beta (TGFbeta). Myofibroblast and haze formation in the cornea have been attributed to TGFbeta hyperactivity released from corneal epithelium following injury to eye. This study tested the hypothesis that decorin gene transfer inhibits TGFbeta-driven myofibroblast and haze formation in the cornea. Human corneal fibroblast (HSF) cultures generated from donor human corneas were used. Decorin cDNA was cloned into mammalian expression vector. Restriction enzyme analysis and DNA sequencing confirmed the nucleotide sequence of generated vector construct. The decorin gene cloned into mammalian expression vector was introduced into HSF with lipofectamine transfection kit. Expression of decorin in selected clones was characterized with RT-PCR, immunocytochemistry and western blotting. Phage contrast microscopy and trypan blue exclusion assay evaluated the effects of decorin gene transfer on HSF phenotype and viability, respectively. Real-time PCR, western blot and immunocytochemistry were used to analyze inhibitory effects of decorin gene transfer on TGFbeta-induced myofibroblast formation by measuring differential expression of alpha smooth muscle actin (SMA), a myofibroblast marker, mRNA and protein expression. Analysis of variance (ANOVA) and the Bonferonni-Dunn adjustment for repeated measures were used for statistical analysis. Our data indicate that decorin-gene transfer into HSF do not alter cellular phenotype or viability. Decorin-overexpressing HSF clones grown in the presence of TGFbeta1 under serum-free conditions showed a statistically significant 80-83% decrease in SMA expression (p value <0.01) compared to naked-vector transfected clones or un-transfected HSF controls. Decorin-transfected, naked-vector transfected and un-transfected HSF grown in the absence of TGFbeta1 showed no or extremely low expression of SMA. Furthermore, decorin over-expression did not affect HSF phenotype and decreased TGFbeta-induced RNA levels of profibrogenic genes such as fibronectin, collagen type I, III, and IV that play important role in stromal matrix modulation and corneal wound healing. The results of study suggest that decorin gene transfer effectively prevents TGFbeta-driven transformation of keratocyte and corneal fibroblast to myofibroblasts. We postulate that decorin gene therapy can be used to treat corneal haze in vivo. Copyright © 2010. Published by Elsevier Ltd.

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Radiation-inducible human tumor necrosis factor-related apoptosis inducing ligand (TRAIL) gene therapy: a novel treatment for radioresistant uveal melanoma.

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Summary Uveal melanoma (UM) is one of the most therapy-resistant cancers. Radiotherapy is the preferred treatment for most cases of UM. However, some UM cells, such as the SP6.5 or OM431 cell line, are relatively radioresistant. In this study, we attempted to improve the current UM therapy using an adenovirus radio-inducible gene therapy system. The anti-tumor adenovirus was constructed by inclusion of the radiation-inducible early growth response gene 1 (EGR1) promoter and the anticancer tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene. We demonstrated that the UM SP6.5 and OM431 cell lines were susceptible to the TRAIL-induced anti-tumor effect. TRAIL expression was enhanced in the adenovirus containing EGR1/TRAIL (Ad-ET) treatment group by radiotherapy, while Ad-ET significantly increased cell death and apoptosis caused by radiotherapy. In mice bearing xenograft tumors, apoptotic cells were detected in pathological tumor sections. Adenovirus Ad-ET combined with radiation therapy significantly inhibited tumor growth compared with the other treatment groups ($P < 0.01$). Our findings indicate that radio-responsive gene therapy has the potential to be a more effective and specific therapy for UM because the therapeutic gene can be spatially or temporally controlled by exogenous radiation.

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Regenerative medicine in dermatology: biomaterials, tissue engineering, stem cells, gene transfer and beyond.

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Abstract

Please cite this paper as: Regenerative medicine in dermatology: biomaterials, tissue engineering, stem cells, gene transfer and beyond. *Experimental Dermatology* 2010. Abstract: The term 'regenerative medicine' refers to a new and expanding field in biomedical research that focuses on the development of innovative therapies allowing the body to replace, restore and regenerate damaged or diseased cells, tissues and organs. It combines several technological approaches including the use of soluble molecules, biomaterials, tissue engineering, gene therapy, stem cell transplantation and the reprogramming of cell and tissue types. Because of its easy accessibility, skin is becoming an attractive model organ for regenerative medicine. Here, we review recent developments in regenerative medicine and their potential relevance for dermatology with a particular emphasis on biomaterials, tissue engineering, skin substitutes and stem cell-based therapies for skin reconstitution in patients suffering from chronic wounds and extensive burns.

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Human Hepatocyte Growth Factor Receptor is a Cellular Co-Receptor for AAV3.

Ling C, Lu Y, Kelsi JK, Jayandharan GR, Li B, Ma W, Cheng B, Gee SW, McGoogan KE, Govindasamy L, Agbandje-McKenna M, Zhong L, Srivastava A.

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Adeno-associated viruses (AAV) utilize a variety of cellular receptors/co-receptors to gain entry into cells. A number of AAV serotypes are now available, and the cognate receptors/co-receptors for only a handful of those have been identified thus far. Of the ten commonly used AAV serotypes, AAV3 is by far the least efficient in transducing cells in general. However, in our recent studies, we observed that AAV3 vectors transduced human liver cancer cells remarkably well, which led to the hypothesis that AAV3 utilizes hepatocyte growth factor receptor (HGFR) as a cellular co-receptor for viral entry. AAV3 infection of human liver cancer cell lines was strongly inhibited by hepatocyte growth factor (HGF), HGFR-specific siRNA, and anti-HGFR antibody, which corroborated this hypothesis. However, AAV3 vectors failed to efficiently transduce murine hepatocytes, both in vitro and in vivo, suggesting that AAV3 specifically utilizes human HGFR, but not murine HGFR, as a cellular co-receptor for transduction. AAV3 may prove to be a useful vector for targeting human liver cancers for the potential gene therapy.

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In vitro contrast-enhanced ultrasound measurements of capillary microcirculation: Comparison between polymer- and phospholipid-shelled microbubbles.

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The focus of contrast-enhanced ultrasound research has developed beyond visualizing the blood pool and its flow to new areas such as perfusion imaging, drug and gene therapy, and targeted imaging. In this work comparison between the application of polymer- and phospholipid-shelled ultrasound contrast agents (UCAs) for characterization of the capillary microcirculation is reported. All experiments are carried out using a microtube as a vessel phantom. The first set of experiments evaluates the optimal concentration level where backscattered signal from microbubbles depends on concentration linearly. For the polymer-shelled UCAs the optimal concentration level is reached at a value of about 2×10^4 MB/ml, whereas for the phospholipid-shelled UCAs the optimal level is found at about 1×10^5 MB/ml. Despite the fact that the polymer shell occupies 30% of the radius of microbubble, compared to 0.2% of the phospholipid-shelled bubble, approximately 5-fold lower concentration of the polymer UCA is needed for investigation compared to phospholipid-shelled analogues. In the second set of experiments, destruction/replenishment method with varied time intervals ranging from 2ms to 3s between destructive and monitoring pulses is employed. The dependence of the peak-to-peak amplitude of backscattered wave versus pulse interval is fitted with an exponential function of the time $\gamma = A(1 - \exp(-\beta t))$ where A represents capillary volume and the time constant β represents velocity of the flow. Taking into account that backscattered signal is linearly proportional to the microbubble concentration, for both types of the UCAs it is observed that capillary volume is linearly proportional to the concentration of the microbubbles, but the estimation of the flow velocity is not affected by the change of the concentration. Using the single capillary model, for the phospholipid-shelled UCA a delay of about 0.2-0.3s in evaluation of the perfusion characteristics is found while polymer-shelled UCA provide response immediately. The latter at the concentration lower than 3.6×10^5 MB/ml have no statistically significant delay ($p < 0.01$), do not cause any attenuation of the backscattered signal or saturation of the receiving part of the system. In conclusion, these results suggest that the novel polymer-shelled microbubbles have a potential to be used for perfusion evaluation. Copyright © 2010 Elsevier B.V. All rights reserved.

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Bio and nanotechnological strategies for tumor-targeted gene therapy.

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Gene therapy is a new medical approach for the treatment of tumors. For safe and efficient gene therapy, therapeutic genes need to be delivered efficiently into the target tumor cells. Development of gene delivery systems to specifically recognize and target tumor cells and to distinguish them from normal cells, especially in the same tissue or organ, is one of the most important issues regarding the present gene delivery methodologies. The enhanced permeability and retention (EPR) effect using the characteristics of angiogenic tumor blood vessels, as well as gene delivery systems recognizing hyperactivated receptors or intracellular signals, is broadly applied to tumor-targeted gene therapy. In addition, bacterial vectors can be a useful means for targeting hypoxic or anoxic regions of a tumor.

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Improvement and induction property of radiation-responsive promoter through DNA shuffling of 5'-flanking regions of the human p21 gene.

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A promoter that augments gene expression in response to stimulation of ionizing radiation would be a desired tool for radiogenetic therapy, a combination of radiotherapy and gene therapy. Although various promoters occurring naturally or artificially have been used for researches, one showing higher reactivity to ionizing radiation is desirable. In the present study, we attempted to improve a radiation-responsive promoter of the p21 through a technique called DNA shuffling. A library of DNA fragments was constructed by re-ligation of randomly digested promoter fragments and improved promoters were chosen out of the library. We repeated this process twice to obtain a promoter showing 2.6-fold better reactivity to ionizing radiation compared with its parent, p21 promoter after 10 Gy gamma-ray irradiation. Nucleotide sequence analyses revealed that the obtained promoter was densely packed with some of the cis-acting elements including binding sites for p53, NF-kappaB, NRF-2, AP-1 and NF-Y more than p21 promoter. In addition, it was shown that its induction by ionizing radiation was dependent upon p53 status of a cell line, suggesting that the promoter retained properties of the p21 promoter. This technique is simple and efficient to improve a promoter responsive to other stimulus of interest besides IR. 2009 The Society for Biotechnology, Japan. All rights reserved.