



CLINIGENE CURRENT GENE THERAPY WEEKLY

From May 3rd to May 10th 2010

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**PMID:
20449052**

Chem Soc Rev. 2009 Dec;38(12):3326-38. Epub 2009 Jul 8.

Cationic amphiphiles: promising carriers of genetic materials in gene therapy.

Srinivas R, Samanta S, Chaudhuri A.

Division of Lipid Science and Technology, Indian Institute of Chemical Technology, Hyderabad-500 607, India.

The clinical success of gene therapy critically depends on the use of efficient and safe gene delivery reagents. The present tutorial review is aimed at inspiring young researchers and students to take up the unsolved challenges in using cationic amphiphiles as safe gene transfer reagents. The review highlights important structure-activity studies in the field to date including the use of cationic amphiphiles for receptor specific targeted gene therapy and for delivery of siRNAs in the emerging field of RNA interference.

**PMID:
20448672**

Cancer Gene Ther. 2010 May 7. [Epub ahead of print]

Persistent expression of biologically active anti-HER2 antibody by AAVrh.10-mediated gene transfer.

Wang G, Qiu J, Wang R, Krause A, Boyer JL, Hackett NR, Crystal RG.

Department of Genetic Medicine, Weill Cornell Medical College, New York, NY, USA.

Trastuzumab (Herceptin) is a recombinant humanized monoclonal antibody (mAb) directed against an extracellular region of the human epidermal growth-factor receptor type 2 (HER2) protein. We hypothesized that a single adeno-associated virus (AAV)-mediated genetic delivery of an anti-HER2 antibody should be effective in mediating long-term production of anti-HER2 and in suppressing the growth of human tumors in a xenograft model in nude mice. The adeno-associated virus gene transfer vector AAVrh.10alphaHER2 was constructed based on a non-human primate AAV serotype rh.10 to express the complementary DNAs for the heavy and light chains of mAb 4D5, the murine precursor to trastuzumab. The data show that genetically transferred anti-HER2 selectively bound human HER2 protein and suppressed the proliferation of HER2(+) tumor cell lines. A single administration of AAVrh.10alphaHER2 provided long-term therapeutic levels of anti-HER2 antibody expression without inducing an anti-idiotypic response, suppressed the growth of HER2(+) tumors and increased the survival of tumor bearing mice. In the context that trastuzumab therapy requires frequent and repeated administration, this strategy might be developed as an alternate platform for delivery of anti-HER2 therapy.

PMID:
20448671

Cancer Gene Ther. 2010 May 7. [Epub ahead of print]

Armoring CRAds with p21/Waf-1 shRNAs: the next generation of oncolytic adenoviruses.

Höti N, Chowdhury WH, Mustafa S, Ribas J, Castanares M, Johnson T, Liu M, Lupold SE, Rodriguez R.

Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Conditionally replicating adenoviruses (CRAds) represent a promising modality for the treatment of neoplastic diseases, including Prostate Cancer. Selectively replicating viruses can be generated by placing a tissue or cancer-specific promoter upstream of one or more of the viral genes required for replication (for example, E1A, E1B). We have previously reported multiple cellular processes that can attenuate viral replication, which in turn compromises viral oncolysis and tumor kill. In this study, we investigated the importance of the cyclin-dependent kinase inhibitor p21/Waf-1, on viral replication and tumor growth. To our knowledge, this is the first report describing the importance of p21/Waf-1shRNA on the induction of an androgen responsive element (ARE) based promoter driving the E1A gene. As a proof of concept, the study emphasizes the use of RNA interference technology to overcome promoter weaknesses for tissue-specific oncolytic viruses, as well as the cellular inhibitor pathways on viral life cycle. Using RNA interference against p21/Waf-1, we were able to show an increase in viral replication and viral oncolysis of prostate cancer cells. Similarly, CRAd viruses that carry p21/Waf-1 shRNA (Ad5-RV004.21) were able to prevent tumor outgrowth that resulted in a marked increase in the mean survival time of tumor-bearing mice compared with CRAd without p21/Waf-1 shRNA (Ad5-RV004). In studies combining Ad5-RV004.21 with Adriamycin, a suprar-additive effect was observed only in CRAds that harbor shRNA against p21/Waf-1. Taken together, these findings of enhanced viral replication in prostate cancer cells by using RNA interference against the cdk inhibitor p21/Waf-1 have significant implications in the development of prostate-specific CRAd therapies.

PMID:
20448670

Cancer Gene Ther. 2010 May 7. [Epub ahead of print]

Targeting MMP-9, uPAR, and cathepsin B inhibits invasion, migration and activates apoptosis in prostate cancer cells.

Nalla AK, Gorantla B, Gondi CS, Lakka SS, Rao JS.

Department of Cancer Biology and Pharmacology, University of Illinois College of Medicine, Peoria, IL, USA.

Prostate cancer is one of the most commonly diagnosed cancers and the second leading cause of cancer deaths in Americans. The high mortality rate is mainly attributed to the invasiveness and metastasis of advanced prostate cancer. Targeting the molecules involved in metastasis could be an effective mode of treatment for prostate cancer. In this study, the therapeutic potential of siRNA-mediated targeting of matrix metalloproteinase-9 (MMP-9), urokinase plasminogen activator receptor (uPAR), and cathepsin B (CB) in prostate cancer was carried out using single and bi-cistronic siRNA-expressing constructs. Downregulation of MMP-9, uPAR, and CB inhibited matrigel invasion, in vitro angiogenesis and wound-healing migration ability of PC3 and DU145 prostate cancer cell lines. In addition, the siRNA treatments induced apoptosis in the tumor cells as determined by TUNEL and DNA laddering assays. An attempt to elucidate the apoptotic pathway showed the involvement of FAS-mediated activation of caspases-8 and -7. Further, mice with orthotopic prostate tumors treated with siRNA-expressing vectors showed significant inhibition in tumor growth and migration. In conclusion, we report that the siRNA-mediated knockdown of MMP-9, uPAR, and CB inhibits invasiveness and migration of prostate cancer cells and leads to apoptosis both in vitro and in vivo.

PMID:
20448669

Cancer Gene Ther. 2010 May 7. [Epub ahead of print]

Synergistic growth inhibition of anaplastic large cell lymphoma cells by combining cellular ALK gene silencing and a low dose of the kinase inhibitor U0126.

Ito M, Zhao N, Zeng Z, Chang CC, Zu Y.

Department of Pathology, The Methodist Hospital and the Methodist Hospital Research Institute, Houston, TX, USA.

Abnormal expression of anaplastic lymphoma kinase (ALK) gene is an important pathogenic factor for anaplastic large cell lymphoma (ALCL). To study the function of ALK, an inducible short hairpin RNA (shRNA) system was stably introduced into cultured human ALCL cells. Inducing shRNA expression in the generated cells resulted in cellular ALK gene silencing and led to inactivation of multiple signaling pathways and growth arrest. Interestingly, a combination of ALK gene silencing with U0126, a kinase inhibitor specific for the extracellular signal-regulated kinases 1/2 pathway, resulted in an augmented reduction in cellular JunB expression. Functional studies indicated that combining ALK gene silencing with U0126 treatment provided a synergistic growth inhibition, which occurred faster and was more profound than with either treatment alone. This synergistic effect was also observed when measuring cell proliferation, apoptosis, and in vitro cell colony formation. Importantly, the combination of ALK gene silencing and U0126 had a prolonged inhibitory effect, preventing recovery of ALCL cell growth even after treatments were removed. Moreover, this synergistic inhibitory effect was confirmed in vivo using a mouse model with xenografted ALCL tumors. Our findings indicate that combining cellular ALK gene silencing with a low dose of U0126 may prove to be an effective and more specific therapeutic approach to treating ALCL.

PMID:
20448220

Clin Cancer Res. 2010 May 4. [Epub ahead of print]

AdCD40L Immunogene Therapy for Bladder Carcinoma - The First Phase I/IIa Trial.

Malmstrom PU, Loskog AS, Lindqvist CA, Mangsbo SM, Fransson M, Wanders A, Gardmark T, Totterman TH.

Akademiska Sjukhuset/University Hospital.

PURPOSE: Immunotherapy with bacillus Calmette-Guérin (BCG) instillation is recommended for high-risk non-muscle invasive bladder cancer. BCG is not effective in advanced tumors, and better alternatives are warranted. Immunostimulating gene therapy with adenoviral vectors expressing CD40L (AdCD40L) has shown efficacy in tumor models. CD40L stimulates systemic immunity and may be effective in local and invasive human disease. **EXPERIMENTAL DESIGN:** Patients with invasive bladder cancer scheduled for cystectomy or patients with Ta tumors were enrolled in a Phase I/IIa trial. Patients were treated with three cycles of intra-bladder Clorpactin WCS-90 prewash followed by AdCD40L instillation one week apart. Safety, gene transfer, immune effects and anti-tumor responses were monitored. **RESULTS:** All eight recruited patients were treated as scheduled, and therapy was well tolerated. The main adverse effect was transient local pain during prewash. Postoperatively, urinary tract infections and one case of late septicemia with elevated potassium were reported. No adverse events were ascribed to vector therapy. Gene transfer was detected in biopsies and bladders were heavily infiltrated with T-cells. The effector marker IFN γ increased in biopsies while levels of circulating T regulatory cells were reduced. Histological evaluation indicated that AdCD40L therapy reduced the load of malignant cells. **CONCLUSION:** To our knowledge, this is the first report on immunogene therapy in bladder cancer and the first utilizing AdCD40L in vivo. Local AdCD40L gene therapy was safe, boosted immune activation and should be further evaluated as single or adjuvant therapy for urothelial malignancies.

PMID:
20447504

Dermatol Clin. 2010 Apr;28(2):361-6, xii.

Gene therapy for recessive dystrophic epidermolysis bullosa.

Titeux M, Pendaries V, Hovnanian A.

Institut National de la santé et de la recherche médicale, U563, Toulouse France.

Among the severe genetic disorders of the skin that are suitable for gene and cell therapy, most efforts have been made in the treatment of blistering diseases including dystrophic epidermolysis bullosa. This condition can be recessively or dominantly inherited, depending on the nature and position of the mutation or mutations in the gene encoding type VII collagen. At present, there is no specific treatment for recessive dystrophic epidermolysis bullosa, and gene and cell therapy approaches hold great promise. This article discusses the different gene therapy approaches that have been used for the treatment of this disease and the new perspectives that they open. Copyright 2010 Elsevier Inc. All rights reserved.

PMID:
20447404

J Mol Biol. 2010 May 3. [Epub ahead of print]

Directed evolution of an enhanced and highly efficient FokI cleavage domain for Zinc Finger Nucleases.

Guo J, Gaj T, Barbas CF 3rd.

The Skaggs Institute for Chemical Biology and the Departments of Molecular Biology and Chemistry, The Scripps Research Institute, La Jolla, California, USA.

Zinc finger nucleases (ZFNs) are powerful tools for gene therapy and genetic engineering. The high specificity and affinity of these chimeric enzymes are based on custom-designed zinc finger proteins (ZFPs). In order to improve the performance of existing ZFN technology, we developed an in vivo evolution-based approach to improve the efficacy of the FokI cleavage domain (FCD). After multiple rounds of cycling mutagenesis and DNA shuffling, a more efficient nuclease variant (Sharkey) was generated. In vivo analyses indicated that Sharkey is >15-fold more active than wild-type FCD on a diverse panel of cleavage sites. Further, a mammalian cell-based assay showed a 3 to 6-fold improvement in targeted mutagenesis for ZFNs containing derivatives of the Sharkey cleavage domain. We also identified mutations that impart sequence specificity to the FCD that might be utilized in future studies to further refine ZFNs through cooperative specificity. In addition, Sharkey was observed to enhance the cleavage profiles of previously published and newly selected heterodimer ZFN architectures. This enhanced and highly efficient cleavage domain will aid in a variety of ZFN applications in medicine and biology. Copyright © 2010. Published by Elsevier Ltd.

**PMID:
20447344**

Cell Transplant. 2010 May 4. [Epub ahead of print]

Efficient genetic modification of cynomolgus monkey embryonic stem cells with lentiviral vectors.

Li W, Liu C, Qin J, Zhang L, Chen R, Chen J, Yu X, Wu G, Lahn BT, Fu Y, Xiang AP.

Embryonic stem (ES) cells have the ability to undergo indefinite self-renewal in vitro and give rise during development to derivatives of all three primary germ layers: ectoderm, endoderm and mesoderm, which make them a highly prized reagent in cell and gene therapy. Efficient introduction of various genes of interest into primate ES cells has proven to be difficult. Here, we demonstrated that the self-inactivating HIV-1-based lentiviral vectors constructed by MultiSite gateway technology are efficient tools for the transduction of Cynomolgus monkey (*Macaca fascicularis*) ES (cmES) cells. After antibiotic selection, all of the transduced cells can stably express the reporter gene (humanized Renilla GFP or dTomato) while maintaining their stem cell properties, including continuous expression of stem cell markers, Alkaline phosphatase (AKP), OCT-4, SSEA-4 and TRA-1-60; formation of embryoid bodies in vitro and teratomas in vivo containing derivatives of three embryonic germ layers. This approach will provide a useful tool for both gene function studies and in vivo cell tracking of stem cells.

**PMID:
20446811**

Stem Cells Dev. 2010 May 6. [Epub ahead of print]

Hepatocyte growth factor modification promotes the amelioration effects of human umbilical cord mesenchymal stem cells on rat acute kidney injury.

Chen Y, Qian H, Zhu W, Zhang X, Yan Y, Ye S, Peng X, Li W, Xu W.

Jiangsu University, School of Medical Science and Laboratory Medicine, Zhenjiang, China; suellen_1111@sohu.com.

Human umbilical cord mesenchymal stem cells (hucMSCs) are particularly attractive cells for cellular and gene therapy in acute kidney injury (AKI). Adenovirus-mediated gene therapy has been limited by immune reaction and target genes selection. However, in the present study, we investigated the therapeutic effects of hepatocyte growth factor (HGF) modified hucMSCs (HGF-hucMSCs) in ischemia/reperfusion-induced AKI rat models. In vivo animal models were generated by subjecting to 60 min of bilateral renal injury by clamping the renal pedicles, and then introduced HGF-hucMSCs via the left carotid artery. Our results revealed that serum creatinine and urea nitrogen levels decreased to the baseline more quickly in HGF-hucMSCs-treated group than that in hucMSCs- or GFP-hucMSCs-treated groups at 72h after injury. The percent of proliferating cell nuclear antigen (PCNA)-positive cells in HGF-hucMSCs-treated group was higher than that in the hucMSCs or GFP-hucMSCs-treated groups. Moreover, injured renal tissues treated with HGF-hucMSCs also exhibited less hyperemia and renal tubule cast during the recovery process. Immunohistochemistry and living body imaging confirmed that HGF-hucMSCs localize to areas of renal injury. Real-time PCR result showed that HGF-hucMSCs also inhibited caspase-3 and IL-1 β mRNA expression in injured renal tissues. Western blot also showed HGF-hucMSCs-treated groups had lower expression of IL-1 β . Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) method indicated that HGF-hucMSCs-treated group had the least apoptosis cells. In conclusion, our findings suggest that HGF modification promotes the amelioration of I/R-induced rat renal injury via anti-apoptotic and anti-inflammatory mechanisms; thus, providing a novel therapeutic application for human umbilical cords mesenchymal stem cells in acute kidney injury..

PMID:
20445581

Gene Ther. 2010 May 6. [Epub ahead of print]

Signed outside: a surface marker system for transgenic cytoplasmic proteins.

Wohlgensinger V, Seger R, Ryan MD, Reichenbach J, Siler U.

Division of Immunology/Hematology/BMT, University Children's Hospital Zürich, Zürich, Switzerland.

Chronic granulomatous disease is a primary immunodeficiency, comprising five molecular defects, characterized by an impaired respiratory burst activity of myeloid cells. We are currently developing a gene therapy vector for the p47phox-deficient form of chronic granulomatous disease. Classic intracellular immunostaining of the cytoplasmic p47phox transgene product, however, interferes with respiratory burst activity. In this study we report a new system for measuring p47phox expression: A single open reading frame encoding the surface marker protein DeltaLNGFR (truncated low-affinity nerve growth factor receptor) linked to the p47phox transgene by the 2A oligopeptide coexpression technology. Translation generates two discrete products: p47phox localizing to the cytoplasm and 'DeltaLNGFR-2A' localizing to the cell surface. Six weeks after transplantation of transduced autologous hematopoietic stem cells into p47^{-/-} mice, the intracellular p47phox fluorescence-activated cell sorting (FACS) signal intensities corresponded to surface DeltaLNGFR staining in monocytes, B cells, T cells and Sca 1⁺ bone marrow cells in vivo. The p47phox cleavage product restored nicotinamide adenine dinucleotide phosphate-oxidase activity in granulocytes differentiated from transduced p47phox^{-/-} murine hematopoietic stem cells ex vivo, in murine granulocytes/monocytes in vivo, and in transduced human monocyte derived macrophages from p47phox-deficient chronic granulomatous disease patients. In conclusion, this new marker system allows highly efficient, indirect detection of cytoplasmic transgene products by FACS surface staining.

PMID:
20445580

Gene Ther. 2010 May 6. [Epub ahead of print]

Locoregional intravascular viral therapy of cancer: precision guidance for Paris's arrow?

Pencavel T, Seth R, Hayes A, Melcher A, Pandha H, Vile R, Harrington KJ.

Targeted Therapy Team, The Institute of Cancer Research, and Sarcoma/Melanoma Unit, Royal Marsden Hospital, London, UK.

Viral therapy of cancer includes strategies such as viral transduction of tumour cells with 'suicide genes', using viral infection to trigger immune-mediated tumour cell death and using oncolytic viruses for their direct anti-tumour action. However, problems still remain in terms of adequate viral delivery to tumours. A role is also emerging for single-organ isolation and perfusion. Having begun with the advent of isolated limb perfusion for extremity malignancy, experimental systems have been developed for the perfusion of other organs, particularly the liver, kidneys and lungs. These are beginning to be adopted into clinical treatment pathways. The combination of these two modalities is potentially significant. Locoregional perfusion increases the exposure of tumour cells to viral agents. In addition, the avoidance of systemic elimination through the immune and reticulo-endothelial systems should provide a mechanism for increased transduction/infection of target cells. The translation of laboratory research to clinical practice would occur within the context of perfusion programmes, which are already established in the clinic. Many of these programmes include the use of vasoactive cytokines such as tumour necrosis factor-alpha, which may have an effect on viral uptake. Evidence of activation of specific anti-tumour immunological responses by intratumoural and other existing methods of viral administration raises the intriguing possibility of a locoregional therapy, with the ability to affect distant sites of disease. In this review, we examined the state of the literature in this area and summarized current findings before indicating likely areas of continuing interest.

PMID:
20445579

Gene Ther. 2010 May 6. [Epub ahead of print]

Noninvasive assessment of regulable transferred-p53 gene expression and evaluation of therapeutic response with FDG-PET in tumor model.

Aung W, Hasegawa S, Koshikawa-Yano M, Tsuji AB, Sogawa C, Sudo H, Sugyo A, Koizumi M, Furukawa T, Saga T.

Diagnostic Imaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan.

The use of tumor-suppressor gene p53 as an anticancer therapeutic has been vigorously investigated. However, progress has met with limited success to date. Some major drawbacks are the difficulty in achieving controllable and efficient gene transfer as well as in analyzing the transferred gene expression in real time and the treatment response in a timely manner. Thus, development of novel gene transfer vector with a regulative gene expression system coupled with the reporter gene, by which transgene can be monitored simultaneously, is critical. Moreover, noninvasive imaging-based assessment of the therapeutic response to exogenous wild-type p53 gene transfer is crucial for refining treatment protocols. In this study, as a simple preclinical model, we constructed a doxycycline-regulated bidirectional vector harboring a reporter gene encoding red fluorescence protein and p53. Then, we determined the controllable and simultaneously coordinated expression of both proteins and the p53-mediated anticancer effects in vitro and in vivo. Next, we observed that cells or tumors with induced p53 overexpression exhibited decreased uptake of [(14)C]FDG in cellular assay and [(18)F]FDG in positron emission tomography (PET) imaging. Thus, by coupling with bidirectional vector, controllable p53 transfer was achieved and the capability of fluoro-2-deoxy-D-glucose (FDG)-PET to assess the therapeutic response to p53 gene therapy was evidently confirmed, which may have an impact on the improvement of p53 gene therapy.

PMID:
20445020

Blood. 2010 May 5. [Epub ahead of print]

Factor IX ectopically expressed in platelets can be stored in {alpha}-granules and corrects the phenotype of hemophilia B mice.

Zhang G, Shi Q, Fahs SA, Kuether EL, Walsh CE, Montgomery RR.

Dept. of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin, United States;

We developed 2bF9 transgenic mice in a hemophilia B mouse model with the expression of human factor IX (FIX) under control of the platelet-specific integrin alphaIIb promoter, to determine whether ectopically expressing FIX in megakaryocytes can enable the storage of FIX in platelet alpha-granules and corrects murine hemophilia B phenotype. FIX was detected in the platelets and plasma of 2bF9 transgenic mice by both antigen and activity assays. About 90% of total FIX in blood was stored in platelets, most of which is releasable upon activation of platelets. Immuno-staining demonstrated that FIX was expressed in platelets and megakaryocytes and stored in alpha-granules. All 2bF9 transgenic mice survived tail clipping, suggesting that platelet-derived FIX normalizes hemostasis in the hemophilia B mouse model. This protection can be transferred by bone marrow transplantation or platelet transfusion. However, unlike our experience with platelet FVIII, the efficacy of platelet-derived FIX was limited in the presence of anti-FIX inhibitory antibodies. These results demonstrate that releasable FIX can be expressed and stored in platelet alpha-granules, and that platelet-derived FIX can correct the bleeding phenotype in hemophilia B mice. Our studies suggest that targeting FIX expression to platelets could be a new gene therapy strategy for hemophilia B.

PMID:
20444480

Virology. 2010 May 3. [Epub ahead of print]

The structure of adeno-associated virus serotype 3B (AAV-3B): Insights into receptor binding and immune evasion.

Lerch TF, Xie Q, Chapman MS.

Department of Biochemistry and Molecular Biology, School of Medicine, Oregon Health & Science University, Portland, OR 97239-3098, USA.

Adeno-associated viruses (AAVs) are leading candidate vectors for human gene therapy. AAV serotypes have broad cellular tropism and use a variety of cellular receptors. AAV serotype 3 binds to heparan sulfate proteoglycan prior to cell entry and is serologically distinct from other serotypes. The capsid features that distinguish AAV-3B from other serotypes are poorly understood. The structure of AAV-3B has been determined to 2.6Å resolution from twinned crystals of an infectious virus. The most distinctive structural features are located in regions implicated in receptor and antibody binding, providing insights into the cell entry mechanisms and antigenic nature of AAVs. We show that AAV-3B has a lower affinity for heparin than AAV-2, which can be rationalized by the distinct features of the AAV-3B capsid. The structure of AAV-3B provides an additional foundation for the future engineering of improved gene therapy vectors with modified receptor binding or antigenic characteristics.

PMID:
20444457

J Chromatogr A. 2010 Apr 8. [Epub ahead of print]

Modeling electrostatic interactions of baculovirus vectors for ion-exchange process development.

Vicente T, Peixoto C, Alves PM, Carrondo MJ.

IBET, Apartado 12, P-2781-901 Oeiras, Portugal; ITQB-UNL, P-2780-157 Oeiras, Portugal.

Product-related impurities constitute a major burden in the production of recombinant viral vectors for gene therapy and vaccination; it impairs not only the biological efficacy of the preparation but the process yield/productivity. Recombinant baculovirus was used as an enveloped virus model to address this issue. Given that ion-exchange chromatography is a process of choice for purification of viral vectors, the analysis of the electrostatic behavior can be instrumental for the improvement of impurity removal. The main species, product (infective virus particle) and product-derived impurities (dsDNA-, glycoprotein-, and envelope-deprived baculovirus particles), were isolated and correspondent zeta potentials were analyzed through dynamic light scattering. A model of the virus based on the viral components critical for biological function is proposed. The contribution of these viral components to the overall particle electrostatic interaction energy profile (calculated between the particle and a putative ion-exchange surface) was assessed as a function of ionic strength and pH. This resulted in a deterministic tool capable of distinguishing the electrostatic properties of the infective virus particle from the major virus-related impurities. Within an ion-exchange bind-elute process, this knowledge helps narrow the optimization space in early stage process development for viral vectors by predicting the best selectivity conditions.

PMID:
20440752

J Gene Med. 2010 May;12(5):413-22.

Combining keratinocyte growth factor transfection into the airways and tracheal occlusion in a fetal sheep model of congenital diaphragmatic hernia.

Saada J, Oudrhiri N, Bonnard A, de Lagausie P, Aissaoui A, Hauchecorne M, Oury JF, Aigrain Y, Peuchmaur M, Lehn JM, Lehn P, Luton D.
EA 3102, Hôpital Robert Debré, AP-HP, Paris, France.

BACKGROUND: In utero tracheal occlusion (TO) has been developed to improve the lung hypoplasia associated with congenital diaphragmatic hernia (CDH). However, although TO stimulates fetal lung growth, it results in a decrease of alveolar type II cells (ATII) and surfactant production. Because keratinocyte growth factor (KGF) is a potent stimulus of ATII proliferation and maturation, we evaluated, in a fetal lamb model of CDH, a gene therapy strategy combining TO and ovine KGF transfection into the fetal airways using bisguanidium-tren-cholesterol/dioleoyl-phosphatidylethanolamine (BGTC/DOPE) cationic liposomes. **METHODS:** Three groups of sheep fetuses with CDH and a group of normal fetuses were studied. The fetuses of the three groups with CDH (KGF, Medium and Hernia groups) underwent surgery at 85 days of gestation to create a diaphragmatic hernia. The KGF and medium group fetuses underwent a second surgery step at day 125 to perform TO associated with injection of the KGF transfection mixture (KGF group) or control medium (Medium group), whereas the fetuses of the Hernia group were left untreated. Normal fetuses were used as a control (Normal group). All fetuses were euthanized at 132 days of gestation and various analytical studies [lung weight, radial alveolar count (RAC), KGF and surfactant protein B (SPB) expression, number of ATII cells] were performed to assess the efficiency of KGF transfection and its effects on fetal lung development. **RESULTS:** TO was associated with lung hyperplasia and increased RAC in the Medium and KGF groups versus the Hernia group. Expression of KGF was increased in the KGF group compared to all other groups and was associated with an increased synthesis of SPB by alveolar cells and an ectopic synthesis of SPB by bronchiolar cells compared to TO treatment alone. **CONCLUSIONS:** Thus, BGTC/DOPE liposomes can mediate efficient KGF transfection into the airways in a fetal sheep model of CDH. Furthermore, combining KGF transfection and TO resulted not only (as did TO alone) in the correction of the CDH-associated lung hypoplasia and decreased RAC, but also in increased SPB synthesis, suggesting a better maturation of the re-growing lung (compared to TO alone). Additional studies are required to further explore the therapeutic potential of such a combined strategy; in particular, studies evaluating the lung function of in utero-treated CDH lamb newborns.

PMID:
20440755

J Gene Med. 2010 May;12(5):446-52.

Functional HIV-2- and SIVsmmPBj- derived lentiviral vectors generated by a novel polymerase chain reaction-based approach.

Kloke BP, Schüle S, Mühlebach MD, Wolfrum N, Cichutek K, Schweizer M.

Division of Medical Biotechnology, Paul-Ehrlich-Institut, Paul-Ehrlich-Strasse 51-59, Langen, Germany.

BACKGROUND: Lentiviral vectors allow stable gene transfer into nonreplicating cells and are increasingly used in clinical gene therapy approaches. Vectors derived from different origins can show distinct target cell transduction properties. Therefore, the construction of modern vector systems of different viral origin remains desirable. The generation of safe and efficient lentivirus-derived transfer vectors by gradual enhancing cloning steps is a time-consuming process that depends on the presence of suitable restriction sites. Multiple-step cloning protocols also enhance the risk of acquisition of mutations or other genetic instabilities. **METHODS:** We constructed novel HIV-2 and SIVsmmPBj-derived transfer vectors by amplification of three essential segments of the viral genome [5'-long terminal repeat (LTR), rev responsive element, DeltaU3-3'-LTR] on the template of the lentiviral full-length genome by a highly flexible three-step fusion polymerase chain reaction approach. Further necessary vector elements, as well as a multiple cloning site, were included into the resulting vector by extension of the primer sequences. The respective vesicular stomatitis virus G pseudotyped lentiviral vector particles were generated and analysed. **RESULTS:** Two novel transfer vectors of different lentiviral origin were successfully generated. Titers for the corresponding SIVsmmPBj- and HIV-2-derived vectors reached up to 9.9×10^7 transforming units (TU)/ml and 1.2×10^8 TU/ml, respectively. The specific capacity to transduce primary human monocytes was maintained in both newly-generated vector systems. **CONCLUSIONS:** We anticipate that this novel and fast way of generating any lentiviral transfer vector will improve the generation of such vectors. The HIV-2- and SIVsmmPBj-derived vectors described will prove valuable for future gene therapy strategies. Copyright (c) 2010 John Wiley & Sons, Ltd.

PMID:
20440155

Plast Reconstr Surg. 2010 May;125(5):1343-51.

Effect of gene therapy with vascular endothelial growth factor after abdominoplasty on TRAM flap viability in a rat model.

de Freitas AL, Gomes HC, Lisboa BC, Arias V, Han SW, Ferreira LM.

Division of Plastic Surgery, the Interdisciplinary Center for Gene Therapy, and the Department of Pathology, Federal University of São Paulo, São Paulo, Brazil. sandra.dcir@epm.br

BACKGROUND: The transverse rectus abdominis musculocutaneous (TRAM) flap may develop necrosis, especially in patients with risk factors such as previous abdominoplasty, caused by damage to perforating vessels during surgical procedures. This study was designed from the perspective of using vascular endothelial growth factor (VEGF) gene therapy with plasmid vector after abdominoplasty to stimulate neovascularization of the TRAM flap, thus increasing flap viability. **METHODS:** Thirty-two Wistar rats were divided into four groups (n = 8). A right inferiorly based TRAM flap was constructed in all animals and was the only procedure performed in group I (TRAM flap). Animals from groups II (abdominoplasty) and III (plasmid) underwent abdominoplasty and were injected intramuscularly with physiologic saline solution and empty plasmid, respectively. Group IV (VEGF) received intramuscular injection of naked plasmid DNA encoding VEGF-165 during abdominoplasty. The TRAM flap was created 30 days after abdominoplasty. **RESULTS:** The mean necrosis was 24.65 +/- 18.13 percent in group I, 62.49 +/- 28.06 percent in group II, 57.80 +/- 25.43 percent in group III, and 18.33 +/- 16.20 percent in group IV. The number of vessels in the TRAM flap was determined by immunohistochemistry using the antibody human heart factor. Groups I and IV had a similar number of vessels, as did groups II and III. Groups I and IV had greater viability and number of vessels than groups II and III. **CONCLUSION:** VEGF gene therapy increased viability and vessel number in the TRAM flap created after abdominoplasty in a rat model.

PMID:
20437208

Appl Biochem Biotechnol. 2010 May 2. [Epub ahead of print]

Monitoring of Cell Viability and Proliferation in Hydrogel-Encapsulated System by Resazurin Assay.

Xiao J, Zhang Y, Wang J, Yu W, Wang W, Ma X.

Laboratory of Biomedical Material Engineering, Department of Science and Technology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian, 116023, People's Republic of China.

Cell microencapsulation is a promising approach for cell implantation, cell-based gene therapy and large-scale cell culture. For better quality control, it is important to accurately measure the microencapsulated cell viability and proliferation in the culture. A number of assays have been used for this purpose, but limitations arise. In this study, we investigated the feasibility and reliability of resazurin as a cell growth indicator in microencapsulated culture system. According to the experiment data, there was a reversible, time- and dose-dependent growth inhibition as observed for resazurin application in encapsulated cells. A positive relationship was observed between reduction of resazurin and CHO cell number in microcapsule. Moreover, the resazurin assay provided an equivalent result to the commonly used MTT method in determining CHO cell proliferation in APA microcapsule with no notable influence on cell distribution and organization pattern. In conclusion, resazurin assay is offered as a simple, rapid and non-invasive method for in vitro microencapsulated cell viability and proliferation measurement.

Future of local bone regeneration - Protein versus gene therapy.

Fischer J, Kolk A, Wolfart S, Pautke C, Warnke PH, Plank C, Smeets R.

Department of Prosthodontics, RWTH Aachen University Hospital, Pauwelsstrasse 30, D-52074 Aachen, Germany.

The most promising attempts to achieve bone regeneration artificially are based on the application of mediators such as bone morphogenetic proteins (BMPs) directly to the deficient tissue site. BMPs, as promoters of the regenerative process, have the ability to induce de novo bone formation in various tissues, and many animal models have demonstrated their high potential for ectopic and orthotopic bone formation. However, the biological activity of the soluble factors that promote bone formation in vivo is limited by diffusion and degradation, leading to a short half-life. Local delivery remains a problem in clinical applications. Several materials, including hydroxyapatite, tricalcium phosphate, demineralised bone matrices, poly-lactic acid homo- and heterodimers, and collagen have been tested as carriers and delivery systems for these factors in a sustained and appropriate manner. Unfortunately these delivery vehicles often have limitations in terms of biodegradability, inflammatory and immunological rejection, disease transmission, and most importantly, an inability to provide a sustained, continuous release of these factors at the region of interest. In coping with these problems, new approaches have been established: genes encoding these growth factor proteins can be delivered to the target cells. In this way the transfected cells serve as local "bioreactors", as they express the exogenous genes and secrete the synthesised proteins into their vicinity. The purpose of this review is to present the different methods of gene versus growth factor delivery in tissue engineering. Our review focuses on these promising and innovative methods that are defined as regional gene therapy and provide an alternative to the direct application of growth factors. Various advantages and disadvantages of non-viral and viral vectors are discussed. This review identifies potential candidate genes and target cells, and in vivo as well as ex vivo approaches for cell transduction and transfection. In explaining the biological basis, this paper also refers to current experimental and clinical applications. Copyright © 2010 European Association for Cranio-Maxillo-Facial Surgery. Published by Elsevier Ltd. All rights reserved.

**PMID:
20434188**

Tissue Cell. 2010 Apr 29. [Epub ahead of print]

Establishment of lentiviral-vector-mediated model of human alpha-1 antitrypsin delivery into hepatocyte-like cells differentiated from mesenchymal stem cells.

Ghaedi M, Lotfi AS, Soleimani M.

Department of Clinical Biochemistry, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran.

Alpha-1 antitrypsin (AAT) deficiency is a lethal hereditary disorder characterized by a severe diminution in plasma levels of AAT leading to progressive liver dysfunction. Since mesenchymal stem cells can differentiate into hepatocyte-like cells they offer a potential unlimited source in autologous transplant procedures. The transfer of genetically modified hepatocyte cells derived from hMSCs into the body constitutes a novel paradigm of coupling cell therapy with gene therapy for this disease. hMSCs were isolated by density gradient centrifugation and plastic adherence. Hepatic differentiation was induced by exposing hMSC to induction medium for up to 21 days. The mRNA levels and protein expression of several important hepatic genes were determined using RT-PCR and immunocytochemistry. The chimeric AAT-Jred transgene was transferred to differentiated cells using a lentiviral vector and its expression was visualized by fluorescent microscopy. Flow cytometric analysis confirmed that hMSCs were obtained. Major hepatocyte marker genes expression were confirmed by RT-PCR and immunocytochemistry. AAT gene was successfully introduced into hepatocyte-like cells differentiated from hMSCs. This established system could be suitable for generation of hMSC derived hepatocyte-like cells containing the normal AAT gene, thus offering a potential in vitro source of cells for transplantation therapy of liver diseases in AAT-deficient patients. Copyright © 2010 Elsevier Ltd. All rights reserved.