



CLINIGENE CURRENT GENE THERAPY WEEKLY

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Embryonic stem cell (ESC)-mediated transgene delivery induces growth suppression, apoptosis and radiosensitization, and overcomes temozolomide resistance in malignant gliomas.

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High-grade gliomas are among the most lethal of all cancers. Despite considerable advances in multimodality treatment, including surgery, radiotherapy and chemotherapy, the overall prognosis for patients with this disease remains dismal. Currently available treatments necessitate the development of more effective tumor-selective therapies. The use of gene therapy for malignant gliomas is promising, as it allows in situ delivery and selectively targets brain tumor cells while sparing the adjacent normal brain tissue. Viral vectors that deliver proapoptotic genes to malignant glioma cells have been investigated. Although tangible results on patients' survival remain to be further documented, significant advances in therapeutic gene transfer strategies have been made. Recently, cell-based gene delivery has been sought as an alternative method. In this paper, we report the proapoptotic effects of embryonic stem cell (ESC)-mediated mda-7/IL-24 delivery to malignant glioma cell lines. Our data show that these are similar to those observed using a viral vector. In addition, acknowledging the heterogeneity of malignant glioma cells and their signaling pathways, we assessed the effects of conventional treatment for high-grade gliomas, ionizing radiation and temozolomide, when combined with ESC-mediated transgene delivery. This combination resulted in synergistic effects on tumor cell death. The mechanisms involved in this beneficial effect included activation of both apoptosis and autophagy. Our in vitro data support the concept that ESC-mediated gene delivery might offer therapeutic advantages over standard approaches to malignant gliomas. Our results corroborate the theory that combined treatments exploiting different signaling pathways are needed to succeed in the treatment of malignant gliomas.

PMID:
20522982

Biol Pharm Bull. 2010;33(6):1073-6.

Adenovirus vector covalently conjugated to polyethylene glycol with a cancer-specific promoter suppresses the tumor growth through systemic administration.

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Cancer gene therapy with adenovirus vectors (Adv) is limited to local administration because systemic administration of Adv produces a weak therapeutic effect and severe side effects. Previously, we generated a dual cancer-specific Adv system by using Adv covalently conjugated to polyethylene glycol (PEG) for transductional targeting and the telomere reverse transcriptase (TERT) promoter as a cancer-specific promoter for transcriptional targeting (PEG-Ad-TERT). We demonstrated that systemic administration of PEG-Ad-TERT showed superior antitumor effects against lung metastatic cancer with negligible side effects. Here, we investigated the therapeutic efficacy of systemic administration of PEG-Ad-TERT for the treatment of primary tumors. We first evaluated the transgene expression of PEG-Ad-TERT containing the luciferase gene (PEG-Ad-TERT/Luc) in primary tumors. Systemic administration of PEG-Ad-TERT/Luc resulted high transgene expression, similar to that observed in tumors for the conventional cytomegalovirus (CMV) promoter-driven Adv containing the luciferase gene (Ad-CMV/Luc). By comparison, transgene expression was 2500-fold lower than that of Ad-CMV/Luc in liver. We then examined the therapeutic effect of systemic administration of PEG-Ad-TERT containing the herpes simplex virus thymidine kinase (HSVtk) gene (PEG-Ad-TERT/HSVtk) for the treatment of primary tumors. We showed that PEG-Ad-TERT/HSVtk produced a notable antitumor effect against primary tumors with negligible side effects. These results demonstrated that PEG-Ad-TERT can be regarded as a prototype Adv with suitable efficacy and safety for systemic cancer gene therapy against both metastatic and primary tumors.

Ocular angiogenesis: mechanisms and recent advances in therapy.

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Ocular angiogenesis, the formation of new blood vessels from the existing vascular tree, is an important cause for severe loss of vision. It can occur in a spectrum of ocular disorders such as age-related macular degeneration (AMD), diabetic retinopathy, retinal artery or vein occlusion, and retinopathy of prematurity (ROP). One of the underlying causes of vision loss in proliferative retinal diseases is the increased vascular permeability leading to retinal edema, vascular fragility resulting in hemorrhage, or fibrovascular proliferation with tractional and rhegmatogenous retinal detachment. Pro- and antiangiogenic factors regulate an "angiogenic switch," which when turned on, leads to the pathogenesis of the above ocular diseases. Although neovascularization tends to occur at a relatively late stage in the course of many ocular disorders, it is an attractive target for therapeutic intervention, since it represents a final common pathway in processes that are multifactorial in etiology and is the event that typically leads directly to visual loss. Identification of these angiogenesis regulators has enabled the development of novel therapeutic approaches. In this light, antibodies directed against common markers of neovasculature, expressed in different diseases, may open up a very general and widely applicable approach for diagnostic and therapeutic interventions. Local gene transfer, that is, the intraocular delivery of recombinant viruses carrying genes encoding angiostatic proteins and small interfering RNA (siRNA) against vascular endothelial growth factor (VEGF) and VEGF receptors, offers the possibility of targeted, sustained, and regulatable delivery of angiostatic proteins and other angiogenic regulators to the retina. Recent progress has enabled the planning of clinical trials of gene therapy for ocular neovascularization.

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20521177

Stem Cell Rev. 2010 Jun 3. [Epub ahead of print]

Neural Stem Cell-based Gene Therapy for Brain Tumors.

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Advances in gene-based medicine since 1990s have ushered in new therapeutic strategy of gene therapy for inborn error genetic diseases and cancer. Malignant brain tumors such as glioblastoma multiforme and medulloblastoma remain virtually untreatable and lethal. Currently available treatment for brain tumors including radical surgical resection followed by radiation and chemotherapy, have substantially improved the survival rate in patients suffering from these brain tumors; however, it remains incurable in large proportion of patients. Therefore, there is substantial need for effective, low-toxicity therapies for patients with malignant brain tumors, and gene therapy targeting brain tumors should fulfill this requirement. Gene therapy for brain tumors includes many therapeutic strategies and these strategies can be grouped in two major categories: molecular and immunologic. The widely used molecular gene therapy approach is suicide gene therapy based on the conversion of non-toxic prodrugs into active anticancer agents via introduction of enzymes and genetic immunotherapy involves the gene transfer of immune-stimulating cytokines including IL-4, IL-12 and TRAIL. For both molecular and immune gene therapy, neural stem cells (NSCs) can be used as delivery vehicle of therapeutic genes. NSCs possess an inherent tumor tropism that supports their use as a reliable delivery vehicle to target therapeutic gene products to primary brain tumors and metastatic cancers throughout the brain. Significance of the NSC-based gene therapy for brain tumor is that it is possible to exploit the tumor-tropic property of NSCs to mediate effective, tumor-selective therapy for primary and metastatic cancers in the brain and outside, for which no tolerated curative treatments are currently available.

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20520650

Gene Ther. 2010 Jun 3. [Epub ahead of print]

Efficient delivery of liposome-mediated MGMT-siRNA reinforces the cytotoxicity of temozolomide in GBM-initiating cells.

Kato T, Natsume A, Toda H, Iwamizu H, Sugita T, Hachisu R, Watanabe R, Yuki K, Motomura K, Bankiewicz K, Wakabayashi T.

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Glioblastoma multiforme (GBM) is one of the most formidable brain tumors with a mean survival period of approximately 12 months. To date, a combination of radiotherapy and chemotherapy with an oral alkylating agent, temozolomide (TMZ), has been used as first-line therapy for glioma. However, the efficacy of chemotherapy for treating GBM is very limited; this is partly because of the high activity levels of the DNA repair protein O(6)-methylguanine-DNA methyltransferase (MGMT) in tumor cells, which creates a resistant phenotype by blunting the therapeutic effect of alkylating agents. Thus, MGMT may be an important determinant of treatment failure and should be considered as a suitable target for intervention, in an effort to improve the therapeutic efficacy of TMZ. In this study, we showed that small-interfering RNA (siRNA)-based downregulation of MGMT could enhance the chemosensitivity of malignant gliomas against TMZ. Notably, TMZ-resistant glioma-initiating cells with increased DNA repair and drug efflux capabilities could be efficiently transduced with MGMT-siRNA by using a novel liposome, LipoTrust. Accordingly, such transduced glioma-initiating cells could be sensitized to TMZ in both in vitro and in vivo tumor models. Taken together, this study provides an experimental basis for the clinical use of such therapeutic combinations.

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20520649

Gene Ther. 2010 Jun 3. [Epub ahead of print]

Intra-articular lentivirus-mediated delivery of galectin-3 shRNA and galectin-1 gene ameliorates collagen-induced arthritis.

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Different members of the galectin family may have inhibitory or stimulatory roles in controlling immune responses and regulating inflammatory reactions in autoimmune diseases such as rheumatoid arthritis (RA). A hypothetical model of a cross talk between galectin-1 and galectin-3 has been established in the circumstance of rheumatoid joints. As galectin-3 is a positive regulator and galectin-1 is a negative regulator of inflammation and autoimmune responses, in this study we evaluated the effects of local knockdown of galectin-3 or overexpression of galectin-1 on ameliorating collagen-induced arthritis (CIA) in rats. Lentiviral vectors encoding galectin-3 small hairpin RNA (shRNA) and galectin-1, as well as two control vectors expressing luciferase shRNA and green fluorescent protein, were individually injected intra-articularly into the ankle joints of rats with CIA, and their treatment responses were monitored by measuring the clinical, radiological and histological changes. Our results show that both knockdown of galectin-3 and overexpression of galectin-1 induced higher percentages of antigen-induced T-cell death in the lymph node cells from arthritic rats. Furthermore, these treatments significantly reduced articular index scores, radiographic scores and histological scores, accompanied with decreased T-cell infiltrates and reduced microvessel density in the ankle joints. Our findings implicate galectin-3 and galectin-1 as potential therapeutic targets for the treatment of RA.

PMID:
20519210

J R Soc Interface. 2010 Jun 2. [Epub ahead of print]

Size mapping of electric field-assisted production of polycaprolactone particles.

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In this investigation, biodegradable polycaprolactone polymeric particles (300-4500 nm in diameter) were prepared by jetting a solution in an electric field. An extensive study has been carried out to determine how the size and size distribution of the particles generated can be controlled by systematically varying the polymer concentration in solution (and thereby its viscosity and electrical conductivity), and also the selected flow rate (2-50 microl min⁻¹) and applied voltage (0-15 kV) during particle generation. Change in these parameters affects the mode of jetting, and within the stable cone-jet mode window, an increase in the applied voltage (approx. 15 kV) resulted in a reduction in particle size and this was more pronounced at high flow rates (such as; 30, 40 and 50 microl min⁻¹) in the same region. The carrier particles were more polydisperse at the peripheral regions of the stable cone-jet mode, as defined in the applied voltage-flow rate parametric map. The effect of loading a drug on the particle size, size distribution and encapsulation efficiency was also studied. Release from drug-loaded particles was investigated using UV spectrophotometry over 45 days. This work demonstrates a powerful method of generating drug-loaded polymeric particles, with the ability to control size and polydispersivity, which has great potential in several categories of biotechnology requiring carrier particles, such as drug delivery and gene therapy.

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20519199

Nucleic Acids Res. 2010 Jun 2. [Epub ahead of print]

Enhancement of gene targeting in human cells by intranuclear permeation of the *Saccharomyces cerevisiae* Rad52 protein.

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The introduction of exogenous DNA in human somatic cells results in a frequency of random integration at least 100-fold higher than gene targeting (GT), posing a seemingly insurmountable limitation for gene therapy applications. We previously reported that, in human cells, the stable over-expression of the *Saccharomyces cerevisiae* Rad52 gene (yRAD52), which plays the major role in yeast homologous recombination (HR), caused an up to 37-fold increase in the frequency of GT, indicating that yRAD52 interacts with the double-strand break repair pathway(s) of human cells favoring homologous integration. In the present study, we tested the effect of the yRad52 protein by delivering it directly to the human cells. To this purpose, we fused the yRAD52 cDNA to the arginine-rich domain of the TAT protein of HIV (tat11) that is known to permeate the cell membranes. We observed that a recombinant yRad52tat11 fusion protein produced in *Escherichia coli*, which maintains its ability to bind single-stranded DNA (ssDNA), enters the cells and the nuclei, where it is able to increase both intrachromosomal recombination and GT up to 63- and 50-fold, respectively. Moreover, the non-homologous plasmid DNA integration decreased by 4-fold. yRAD52tat11 proteins carrying point mutations in the ssDNA binding domain caused a lower or nil increase in recombination proficiency. Thus, the yRad52tat11 could be instrumental to increase GT in human cells and a 'protein delivery approach' offers a new tool for developing novel strategies for genome modification and gene therapy applications.

PMID:
20519042

J Laryngol Otol. 2010 Jun 2:1-7. [Epub ahead of print]

Inhibitory effect of HuR gene small interfering RNA segment on laryngeal carcinoma Hep-2 cell growth.

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Objectives:To investigate the effect of the HuR gene on laryngeal carcinoma Hep-2 cell growth, and to analyse correlations between the HuR, cyclooxygenase-2 and survivin genes.**Study design:**Experiment study.**Setting:**Department of Otolaryngology-Head and Neck Surgery, Lihuili Hospital of Ningbo University, Ningbo, a tertiary care centre in China.**Methods:**Copies of a small interfering RNA segment directed against the HuR gene were transfected into Hep-2 cells using LipofectamineTM 2000. The effect of the small interfering RNA segment on Hep-2 cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Changes in the expression of the HuR, cyclooxygenase-2 and survivin genes were detected by semi-quantitative reverse transcription polymerase chain reaction analysis. Concentrations of the HuR, cyclooxygenase-2 and survivin proteins were evaluated using Western blotting.**Results:**Expression of the HuR, cyclooxygenase-2 and survivin genes, as indicated by messenger RNA and protein levels, was suppressed by the HuR gene small interfering RNA segment in a dose-dependent manner. The proliferation indices of all treated groups were significantly lower than those of control groups ($p < 0.05$).**Conclusions:**Impairment of HuR gene expression, using interfering RNA technology, can significantly suppress Hep-2 cell proliferation and induce apoptosis. The HuR gene may be an effective target for gene therapy in patients with laryngeal carcinoma.

PMID:
20517938

Ann Neurol. 2010 Jun;67(6):771-80.

Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy.

Malik V, Rodino-Klapac LR, Viollet L, Wall C, King W, Al-Dahhak R, Lewis S, Shilling CJ, Kota J, Serrano-Munuera C, Hayes J, Mahan JD, Campbell KJ, Banwell B, Dasouki M, Watts V, Sivakumar K, Bien-Willner R, Flanigan KM, Sahenk Z, Barohn RJ, Walker CM, Mendell JR.

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OBJECTIVE: The objective of this study was to establish the feasibility of long-term gentamicin dosing to achieve stop codon readthrough and produce full-length dystrophin. Mutation suppression of stop codons, successfully achieved in the mdx mouse using gentamicin, represents an important evolving treatment strategy in Duchenne muscular dystrophy (DMD). **METHODS:** Two DMD cohorts received 14-day gentamicin (7.5mg/kg/day): Cohort 1 (n = 10) stop codon patients and Cohort 2 (n = 8) frameshift controls. Two additional stop codon DMD cohorts were gentamicin treated (7.5mg/kg) for 6 months: Cohort 3 (n = 12) dosed weekly and Cohort 4 (n = 4) dosed twice weekly. Pre- and post-treatment biopsies were assessed for dystrophin levels, as were clinical outcomes. **RESULTS:** In the 14-day study, serum creatine kinase (CK) dropped by 50%, which was not seen in frameshift DMD controls. After 6 months of gentamicin, dystrophin levels significantly increased (p = 0.027); the highest levels reached 13 to 15% of normal (1 in Cohort 3, and 2 in Cohort 4), accompanied by reduced serum CK favoring drug-induced readthrough of stop codons. This was supported by stabilization of strength and a slight increase in forced vital capacity. Pretreatment stable transcripts predicted an increase of dystrophin after gentamicin. Readthrough efficiency was not affected by the stop codon or its surrounding fourth nucleotide. In 1 subject, antigen-specific interferon-gamma enzyme-linked immunospot assay detected an immunogenic dystrophin epitope. **INTERPRETATION:** The results support efforts to achieve drug-induced mutation suppression of stop codons. The immunogenic epitope resulting from readthrough emphasizes the importance of monitoring T-cell immunity during clinical studies that suppress stop codons. Similar principles apply to other molecular strategies, including exon skipping and gene therapy.

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20516646

J Clin Invest. 2010 Jun 1. pii: 42297. doi: 10.1172/JCI42297. [Epub ahead of print]

Human keratinocytes are efficiently immortalized by a Rho kinase inhibitor.

Chapman S, Liu X, Meyers C, Schlegel R, McBride AA.

Primary human keratinocytes are useful for studying the pathogenesis of many different diseases of the cutaneous and mucosal epithelia. In addition, they can form organotypic tissue equivalents in culture that can be used as epidermal autografts for wound repair as well as for the delivery of gene therapy. However, primary keratinocytes have a finite lifespan in culture that limits their proliferative capacity and clinical use. Here, we report that treatment of primary keratinocytes (originating from 3 different anatomical sites) with Y-27632, a Rho kinase inhibitor, greatly increased their proliferative capacity and resulted in efficient immortalization without detectable cell crisis. More importantly, the immortalized cells displayed characteristics typical of primary keratinocytes; they had a normal karyotype and an intact DNA damage response and were able to differentiate into a stratified epithelium. This is the first example to our knowledge of a defined chemical compound mediating efficient cell immortalization, and this finding could have wide-ranging and profound investigational and medical applications.

PMID:
20516173

Cold Spring Harb Protoc. 2010 Jun 1;2010(6):pdb.prot5435.

Generation of retroviral particles for the spleen necrosis virus (SNV)-based vector system and their use in transduction of various cell types.

Parveen Z, Mukhtar M, Pomerantz RJ.

Genetically engineered retroviruses are widely used for gene delivery into human cells. A number of investigators have studied spleen necrosis virus (SNV) as a vehicle for gene delivery. Vectors developed from SNV and its closely associated avian reticuloendotheliosis virus strain A (REV-A) can be used for gene transfer into a variety of cells, including primary hematopoietic cells and human brain and post-mitotic neuronal cells that are difficult to transduce with other vector systems. SNV-based vector systems have the advantage of being quite safe, because wild-type SNV is unable to infect human cells and has less preference for integration into transcriptionally active sites or genes. However, the generation of retroviral vectors requires cotransfection of more than one plasmid into a packaging cell line, which is a tedious process. The development of stable packaging cell lines expressing envelope (Env) proteins and the structural proteins Gag-Pol will enhance mass production of retroviral vectors for future gene therapy experiments both in vitro and in vivo. This protocol describes the generation of retroviral particles for the SNV-based vector system. These particles can then be used for transduction of various cell types; as an example, a technique for transduction of post-mitotic neurons is also presented.

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20515607

Discov Med. 2010 May;9(48):399-403.

Reengineered AAV vectors: old dog, new tricks.

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Adeno-associated viral (AAV) vectors have emerged in recent years as powerful tools for therapeutic gene transfer. Successes in clinical trials and the discovery of several hundreds of naturally occurring AAV isolates have triggered efforts to understand and manipulate this deceptively simple parvovirus for a myriad of gene therapy applications. Exciting breakthroughs based on directed evolution of novel tissue-specific variants from combinatorial AAV libraries have been reported. Recent approaches driven by the availability of structural information have yielded a new generation of reengineered AAV vectors.

PMID:
20515474

BMC Genomics. 2010 Jun 1;11(1):345. [Epub ahead of print]

Transcriptomic analysis of dystrophin RNAi knockdown reveals a central role for dystrophin in muscle differentiation and contractile apparatus organization.

Ghahramani Seno MM, Trollet C, Athanasopoulos T, Graham IR, Hu P, Dickson G.

ABSTRACT: BACKGROUND: Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disorder caused by mutations in the dystrophin gene. DMD has a complex and as yet incompletely defined molecular pathophysiology hindering development of effective ameliorative approaches. Transcriptomic studies so far conducted on dystrophic cells and tissues suffer from non-specific changes and background noise due to heterogeneous comparisons and secondary pathologies. A study design in which a perfectly matched control cell population is used as reference for transcriptomic studies will give a much more specific insight into the effects of dystrophin deficiency and DMD pathophysiology. **RESULTS:** Using RNA interference (RNAi) to knock down dystrophin in myotubes from C57BL10 mice, we created a homogenous model to study the transcriptome of dystrophin-deficient myotubes. We noted significant differences in the global gene expression pattern between these myotubes and their matched control cultures. In particular, categorical analyses of the dysregulated genes demonstrated significant enrichment of molecules associated with the components of muscle cell contractile unit, ion channels, metabolic pathways and kinases. Additionally, some of the dysregulated genes could potentially explain conditions and endophenotypes associated with dystrophin deficiency, such as dysregulation of calcium homeostasis (Pvalb and Casq1), or cardiomyopathy (Obscurin, Tcap). In addition to be validated by qPCR, our data gains another level of validity by affirmatively reproducing several independent studies conducted previously at genes and/or protein levels in vivo and in vitro. **CONCLUSION:** Our results suggest that in striated muscles, dystrophin is involved in orchestrating proper development and organization of myofibers as contractile units, depicting a novel pathophysiology for DMD where the absence of dystrophin results in maldeveloped myofibers prone to physical stress and damage. Therefore, it becomes apparent that any gene therapy approaches for DMD should target early stages in muscle development to attain a maximum clinical benefit. With a clear and specific definition of the transcriptome of dystrophin deficiency, manipulation of identified dysregulated molecules downstream of dystrophin may lead to novel ameliorative approaches for DMD.

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20512821

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Preorganized, Macromolecular, Gene-Delivery Systems.

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Viruses represent a paradigmatic example of multicomponent, self-organized supramolecular systems specialized in the delivery and replication of their genetic material. Mimicking their functioning by artificial synthetic molecules represents a fantastic challenge that will lead to the future development of gene therapy. This is only possible if general approaches towards the construction of nanoscale vehicles for DNA are developed and the key rules governing their capacity to compact genetic material and its active transport/delivery through cell membranes are understood. In this area of research, synthetic organic chemistry plays an important role by providing tools to create tailor-made molecules of increasing complexity. Preorganization of functional elements onto macromolecular platforms has the potential to allow control of the self-assembling behavior of discrete architectures to produce nanometric objects that can be programmed to complex, compact, deliver, and release plasmid DNA in a target cell.

PMID:
20512535

Mol Biotechnol. 2010 May 29. [Epub ahead of print]

Activation of the CMV-IE Promoter by Hyperthermia In Vitro and In Vivo: Biphasic Heat Induction of Cytosine Deaminase Suicide Gene Expression.

Kobelt D, Aumann J, Fichtner I, Stein U, Schlag PM, Walther W.

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The cytomegalovirus-immediate early (CMV-IE) promoter is widely used as a strong and constitutively active promoter. Although the CMV-IE promoter does not harbor heat-responsive sequences, we determined its heat inducibility. We analyzed in vitro and in vivo heat responsiveness and possible mechanisms of heat induction of the CMV-IE promoter. We used transfected SW480 human colon carcinoma cells (SW480/CMVCD), expressing CMV-IE promoter-driven bacterial cytosine deaminase (CD) gene. These cells were heated at 42 degrees C. The SW480/CMVCD cells were also used for in vivo studies, in which tumor-bearing animals were treated with hyperthermia at 41.5 degrees C. As controls, SW480 (SW480/HSPCD) cells were used, in which CD expression is driven by the HSP70-promoter. In vitro, we observed a biphasic, up to 25-fold heat induction of CMV-IE-driven CD expression after hyperthermia in SW480/CMVCD cells. In vivo, we found a 2.5-fold induction of CD expression after hyperthermia in SW480/CMVCD tumor-bearing animals. The analysis of the CMV-IE promoter sequence revealed several transcription factor-binding sites, which mediate stress responsiveness. YB-1 and C/EBP-beta might mediate heat responsiveness of the CMV-IE promoter. These data point to limitations in heat-induction gene therapy studies, in which the CMV-IE promoter is used as control system. In addition, the CMV-IE promoter itself could well be used for construction of heat-inducible vectors.

PMID:
20511387

J Biomater Appl. 2010 May 28. [Epub ahead of print]

Review Paper: DNA Delivery Strategies to Promote Periodontal Regeneration.

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Periodontal diseases are caused by bacteria with an inflammatory component that result in the loss of bone and soft tissue around the neck of the teeth. Recent therapies allow clinicians to regenerate some of the lost structures of the periodontium. Regeneration of these lost supporting structures is a highly orchestrated process, involving various cellular and molecular players, leading to the complete restoration of the periodontium (the tooth-supporting apparatus). The introduction of growth factors has positively influenced the clinical outcome of the existing regenerative procedures but the supra-physiological doses and the high cost associated with these growth factors can be drawbacks. Gene therapy may offer some interesting advantages to current therapies. In the field of periodontology, several studies have been conducted to explore the efficacy of delivering the DNA of key growth factors using viral vectors in both periodontal and peri-implant bone regeneration. Relatively few studies have explored the application of nonviral gene therapy in periodontal regeneration. This article is aimed at reviewing the studies conducted so far using viral and nonviral gene delivery approaches to achieve periodontal and peri-implant bone regeneration.

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20509975

BMC Cancer. 2010 May 31;10(1):245. [Epub ahead of print]

Inhibition of experimental lung metastasis by systemic lentiviral delivery of kallistatin.

Shiau AL, Teo ML, Chen SY, Wang CR, Hsieh JL, Chang MY, Chang CJ, Chao J, Chao L, Wu CL, Lee CH.

ABSTRACT: **BACKGROUND:** Angiogenesis plays an important role in the development and progression of tumors. Kallistatin exerts anti-angiogenic and anti-inflammatory activities that may be effective in inhibiting tumor metastasis. We investigated the antitumor effect of lentivirus-mediated kallistatin gene transfer in a syngeneic murine tumor model. **METHODS:** Lentiviral vector encoding kallistatin (LV-Kallistatin) was constructed. The expression of kallistatin was verified by enzyme-linked immunosorbent assay (ELISA), and the bioactivity of kallistatin was determined by using cell proliferation, migration, and invasion assays. In addition, antitumor effects of LV-Kallistatin were evaluated by the intravenous injection of virus into tumor-bearing mice. **RESULTS:** The conditioned medium from LV-Kallistatin-treated cells inhibited the migration and proliferation of endothelial cells. Meanwhile, it also reduced the migration and invasion of tumor cells. In the experimental lung metastatic model, tumor-bearing mice receiving LV-Kallistatin had lower tumor nodules and longer survival than those receiving control virus or saline. Moreover, the microvessel densities, the levels of vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF-alpha), and nuclear factor kappa B (NF-kappaB) transcriptional activity were reduced in the LV-Kallistatin-treated mice. **CONCLUSION:** Results of this study showed that systemic administration of lentiviral vectors encoding kallistatin inhibited the growth of metastatic tumor and prolonged the survival of tumor-bearing mice. These results suggest that gene therapy using lentiviruses carrying the kallistatin gene, which exerts anti-angiogenic and anti-inflammatory activities, represents a promising strategy for the treatment of lung cancer.

PMID:
20509929

J Biomed Sci. 2010 May 28;17(1):42. [Epub ahead of print]

Combinatorial gene therapy renders increased survival in cirrhotic rats.

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ABSTRACT: **BACKGROUND:** Liver fibrosis ranks as the second cause of death in Mexico's productive-age population. This pathology is characterized by accumulation of fibrillar proteins in hepatic parenchyma causing synthetic and metabolic dysfunction. Removal of excessive fibrous proteins might result in benefit for subjects increasing survival index. The goal of this work was to find whether the already known therapeutical effect of human urokinase Plasminogen Activator and human Matrix Metalloprotease 8 extends survival index in cirrhotic animals. **METHODS:** Wistar rats (80 g) underwent chronic intoxication with CCl4: mineral oil for 8 weeks. Cirrhotic animals were injected with a combined dose of Ad-delta-huPA plus Ad-MMP8 (3×10^{11} and 1.5×10^{11} vp/Kg, respectively) or with Ad-beta-Gal (4.5×10^{11}) and were killed after 2, 4, 6, 8 and 10 days. Then, liver and serum were collected. An additional set of cirrhotic animals injected with combined gene therapy was also monitored for their probability of survival. **RESULTS:** Only the cirrhotic animals treated with therapeutical genes (Ad-delta-huPA+Ad-MMP-8) showed improvement in liver fibrosis. These results correlated with hydroxyproline determinations. A significant decrement in alpha-SMA and TGF-beta1 gene expression was also observed. Cirrhotic rats treated with Ad-delta-huPA plus Ad-MMP8 had a higher probability of survival at 60 days with respect to Ad-beta-Gal-injected animals. **CONCLUSION:** A single administration of Ad-delta-huPA plus Ad-MMP-8 is efficient to induce fibrosis regression and increase survival in experimental liver fibrosis.

Novel and Current Treatment Concepts Using Pulmonary Drug Delivery.

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The novel technologies in pulmonary drug delivery propelled the development of new strategies for pharmacological intervention in human diseases. In particular, this review will focus on pulmonary parameters which influence the delivery of inhaled therapeutics and summarize novel applications and recent innovations. The central issues of pulmonary drug application are optimal effectiveness under conditions of greatest safety. They not only depend on the properties of the drug but also feature the application vehicle and drug formulation. The optimization of the whole system (drug, formulation and vehicle) is therefore a necessary prerequisite for reliable inhaling medicines. Depending on the desired locus of drug action, the inhaled medicine has to be adjusted to particle size, concentration and chemical composition to guarantee a local or systemic drug action. Local asthma therapy represents the established concept for inhalation therapy. Due to the disease status, deposition of drugs is therefore often seen in central rather than peripheral airways. Recent developments in ultrafine therapeutic particles should therefore provide enough drug deposition even in the deeper airways. Recent approvals and interesting new therapy concepts will be discussed. Beside a pulmonary drug action there is an accumulating number of applications also for systemic drug action after pulmonary drug delivery. These involve among others inhaled insulin, glucagon-like-peptide 1 or growth hormone. But also novel therapeutic systems for gene therapy and vaccination are currently under investigation. Successful feasibility of these novel concepts will be expected in the near future.