



CLINIGENE CURRENT GENE THERAPY WEEKLY

From August 15th to August 22nd 2011

Table of contents:

Formation of AAV Single Stranded DNA Genome from a Circular Plasmid in <i>Saccharomyces cerevisiae</i>	3
Oncolytic adenovirus armed with human papillomavirus E2 gene in combination with radiation demonstrates synergistic enhancements of antitumor efficacy.	3
Influence of osteopontin expression on the metastatic growth of CC531 rat colorectal carcinoma cells in rat liver.....	4
S100A1 Genetically Targeted Therapy Reverses Dysfunction of Human Failing Cardiomyocytes.....	5
Development of novel efficient SIN vectors with improved safety features for Wiskott-Aldrich Syndrome stem cell based gene therapy.....	6
Peptide Ligands Incorporated into the Threefold Spike Capsid Domain to Re-Direct Gene Transduction of AAV8 and AAV9 In Vivo.....	6
HPV-16 E1, E2 and E6 each complement the Ad5 helper gene set, increasing rAAV2 and wt AAV2 production.	7
Sustained correction of OTC deficiency in spf(ash) mice using optimized self-complementary AAV2/8 vectors.....	7
AAV vectors transduce hepatocytes in vivo as efficiently in cirrhotic as in healthy rat livers.	8
Epidermal growth factor improves lentivirus vector gene transfer into primary mouse hepatocytes.	8
Computational analysis of two species C human adenoviruses provides evidence of a novel virus.	9
Stem cell-mediated transfer of a human artificial chromosome ameliorates muscular dystrophy.	9
Biodistribution and blood clearance of plasmid DNA administered in arginine peptide complexes.....	10
Lentivirus-Mediated Overexpression of MicroRNA-199a Inhibits Cell Proliferation of Human Hepatocellular Carcinoma.....	10
A polymerase chain reaction-based methodology to detect gene doping.	11
Toward a Durable Treatment of HIV-1 Infection Using RNA Interference.	11
Anti-tumor effects of canine adipose tissue-derived mesenchymal stromal cell-based interferon- β gene therapy and cisplatin in a mouse melanoma model.	12
Regulatory and Ethical Issues for Phase I In Utero Gene Transfer Studies.....	12
Leukocyte integrin activation mediates transient neutropenia following G-CSF administration.....	13
Combination therapy utilizing shRNA knockdown and an optimized resistant transgene for rescue of diseases caused by misfolded proteins.	13
Conjugative DNA transfer into human cells by the VirB/VirD4 type IV secretion system of the bacterial pathogen <i>Bartonella henselae</i>	14
The C-terminal region of Bfl-1 sensitizes non-small cell lung cancer to gemcitabine-induced apoptosis by suppressing NF- κ B activity and down-regulating Bfl-1.....	14
Discovery of Cationic Polymers for Non-viral Gene Delivery using Combinatorial Approaches.....	15
The future of incretin-based therapy: novel avenues-novel targets.	15
Single-Molecule Imaging of BMP4 Dimerization on Human Periodontal Ligament Cells.	16
Magnetically enhanced adeno-associated viral vector delivery for human neural stem cell infection.	16

Potential efficacy of cell-penetrating peptides for nucleic acid and drug delivery in cancer. 17

Combination gene therapy using VEGF-shRNA and fusion suicide gene γ CDglyTK inhibits gastric carcinoma growth..... 17

Dopamine signaling as a neural correlate of consciousness. 18

Anthracyclines and ellipticines as DNA-damaging anticancer drugs: Recent advances. 18

Current Options and Future Prospects for the Treatment of Dyskinesia and Motor Fluctuations in Parkinson's Disease..... 19

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21853137

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Formation of AAV Single Stranded DNA Genome from a Circular Plasmid in *Saccharomyces cerevisiae*.

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Adeno-associated virus (AAV)-based vectors are promising tools for targeted transfer in gene therapy studies. Many efforts have been accomplished to improve production and purification methods. We thought to develop a simple eukaryotic system allowing AAV replication which could provide an excellent opportunity for studying AAV biology and, more importantly, for AAV vector production. It has been shown that yeast *Saccharomyces cerevisiae* is able to replicate and form the capsid of many viruses. We investigated the ability of the yeast *Saccharomyces cerevisiae* to carry out the replication of a recombinant AAV (rAAV). When a plasmid containing a rAAV genome in which the cap gene was replaced with the *S. cerevisiae* URA3 gene, was co-transformed in yeast with a plasmid expressing Rep68, a significant number of URA3(+) clones were scored (more than 30-fold over controls). Molecular analysis of low molecular weight DNA by Southern blotting revealed that single stranded DNA is formed and that the plasmid is entirely replicated. The ssDNA contains the ITRs, URA3 gene and also vector sequences suggesting the presence of two distinct molecules. Its formation was dependent on Rep68 expression and ITR. These data indicate that DNA is not obtained by the canonical AAV replication pathway.

PMID:
21852812

Cancer Gene Ther. 2011 Aug 19. doi: 10.1038/cgt.2011.53. [Epub ahead of print]

Oncolytic adenovirus armed with human papillomavirus E2 gene in combination with radiation demonstrates synergistic enhancements of antitumor efficacy.

Wang W, Xia X, Wang S, Sima N, Li Y, Han Z, Gao Q, Luo A, Li K, Meng L, Zhou J, Wang C, Shen K, Ma D.

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High-risk human papillomavirus (hr-HPV) E6 and E7 oncogenes are associated with resistance to radiotherapy in cervical cancer. Efforts have been taken to employ HPV E2, a crucial negative transcriptional modulator of HPV E6 and E7 oncogenes, and also an apoptosis-inducing agent, for therapeutic intervention. Despite being conceptually attractive, the potency and feasibility of current hr-HPV E2-based therapies remain limited. Here, we designed a novel recombinant adenovirus, named M5, with a 27-bp deletion in E1A conserved region-2 by which to realize tumor-specific replication, and a total HPV type 16 (HPV16) E2 gene complementary DNA inserted into the E3 coding region. In this design, M5 exploited the adenovirus E3 promoters to express HPV16 E2 gene in a viral replication-dependent manner and preferentially silenced the hr-HPV E6 and E7 oncogenes in HPV-positive cervical cancer cells. In vitro and in vivo assays confirmed that M5 exhibited potent antitumoral efficacy. Moreover, the effects of combined treatment with M5 and radiation treatment resulted in synergistically enhanced potency ($P < 0.01$). The increase in killing efficacy of M5 was also found in HPV-negative cervical cancer cells, for which the pro-apoptotic activity of HPV16 E2 was thus responsible. Our results indicated that the use of M5 that locally delivers HPV16 E2 to cancers has broad therapeutic windows and that the combination therapy with radiation for cervical cancer will be the more effective way of improving survival.

Influence of osteopontin expression on the metastatic growth of CC531 rat colorectal carcinoma cells in rat liver.

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Development of hepatic metastasis is responsible for most of colorectal cancer-related deaths. Osteopontin (OPN) is a small integrin-binding N-linked glycoprotein, which plays a crucial role in the formation of hepatic metastasis. This study aimed to suppress Opn expression by an antisense-oligonucleotide (ASO(Opn)) to decrease liver metastasis in vivo. The effect of ASO(Opn) was investigated in vitro in CC531(lacZ) colorectal cancer cells in comparison to sense (SO) or nonsense (NSO) oligomers, by determining mRNA and protein expression levels, as well as cell survival. For in vivo treatment, CC531(lacZ) cells were intraportally inoculated into rats to compare the effects of ASO, SO and NSO oligomers, following prolonged subcutaneous administration by osmotic mini-pumps. The resulting CC531(lacZ) tumor cell load in the liver was measured by a β -galactosidase assay. Proliferation of CC531(lacZ) cells in vitro was significantly decreased after ASO(Opn) and SO treatment ($P < 0.001$). Liver metastasis development was reduced as long as ASO(Opn) was administered, but this effect was rapidly blunted following the end of the ASO(Opn) administration. In contrast, administration of the SO resulted in a tumor load reduction, which surprisingly surpassed the ASO(Opn) effect in vivo in terms of a long-lasting metastasis suppression, which was accompanied with increased survival of the animals. Administration of the ASO(Opn) in rats was effective in decreasing their liver metastasis. The short-lived effect might be extended by modifications suited to increase the ASOs' half-life. In addition, there was a superior anti-metastatic effect caused by the SO, which has not been reported previously.

S100A1 Genetically Targeted Therapy Reverses Dysfunction of Human Failing Cardiomyocytes.

Brinks H, Rohde D, Voelkers M, Qiu G, Pleger ST, Herzog N, Rabinowitz J, Ruhparwar A, Silvestry S, Lerchenmüller C, Mather PJ, Eckhart AD, Katus HA, Carrel T, Koch WJ, Most P. Department of Cardiac and Vascular Surgery, University Hospital Berne, Bern, Switzerland; George Zallie and Family Laboratory for Cardiovascular Gene Therapy, Thomas Jefferson University, Philadelphia, Pennsylvania.

OBJECTIVES:

This study investigated the hypothesis whether S100A1 gene therapy can improve pathological key features in human failing ventricular cardiomyocytes (HFCMs).

BACKGROUND:

Depletion of the Ca(2+)-sensor protein S100A1 drives deterioration of cardiac performance toward heart failure (HF) in experimental animal models. Targeted repair of this molecular defect by cardiac-specific S100A1 gene therapy rescued cardiac performance, raising the immanent question of its effects in human failing myocardium.

METHODS:

Enzymatically isolated HFCMs from hearts with severe systolic HF were subjected to S100A1 and control adenoviral gene transfer and contractile performance, calcium handling, signaling, and energy homeostasis were analyzed by video-edge-detection, FURA2-based epifluorescent microscopy, phosphorylation site-specific antibodies, and mitochondrial assays, respectively.

RESULTS:

Genetically targeted therapy employing the human S100A1 cDNA normalized decreased S100A1 protein levels in HFCMs, reversed both contractile dysfunction and negative force-frequency relationship, and improved contractile reserve under beta-adrenergic receptor (β -AR) stimulation independent of cAMP-dependent (PKA) and calmodulin-dependent (CaMKII) kinase activity. S100A1 reversed underlying Ca(2+) handling abnormalities basally and under β -AR stimulation shown by improved SR Ca(2+) handling, intracellular Ca(2+) transients, diastolic Ca(2+) overload, and diminished susceptibility to arrhythmogenic SR Ca(2+) leak, respectively. Moreover, S100A1 ameliorated compromised mitochondrial function and restored the phosphocreatine/adenosine-triphosphate ratio.

CONCLUSIONS:

Our results demonstrate for the first time the therapeutic efficacy of genetically reconstituted S100A1 protein levels in HFCMs by reversing pathophysiological features that characterize human failing myocardium. Our findings close a gap in our understanding of S100A1's effects in human cardiomyocytes and strengthen the rationale for future molecular-guided therapy of human HF.

PMID:
21851067

Mol Pharm. 2011 Aug 18. [Epub ahead of print]

Development of novel efficient SIN vectors with improved safety features for Wiskott-Aldrich Syndrome stem cell based gene therapy.

Avedillo Diez I, Zychlinski D, Coci EG, Galla M, Modlich U, Dewey RA, Schwarzer A, Maetzig T, Mpofo N, Jaeckel E, Boztug K, Baum C, Klein C, Schambach A.

Gene therapy is a promising therapeutic approach to treat primary immunodeficiencies. Indeed, the clinical trial for the Wiskott-Aldrich Syndrome (WAS) that is currently ongoing at the Hannover Medical School (Germany) has recently reported the correction of all affected cell lineages of the hematopoietic system in the first treated patients. However, an extensive study of the clonal inventory of those patients reveals that LMO2, CCND2 and MDS1/EVI1 were preferentially prevalent. Moreover, a first leukemia case was observed in this study, thus reinforcing the need of developing safer vectors for gene transfer into HSC in general. Here we present a novel self-inactivating (SIN) vector for the gene therapy of WAS that combines improved safety features. We used the elongation factor 1 alpha (EFS) promoter, which has been extensively evaluated in terms of safety profile, to drive a codon-optimized human WASP cDNA. To test vector performance in a more clinically relevant setting, we transduced murine HSPC as well as human CD34+ cells and also analyzed vector efficacy in their differentiated myeloid progeny. Our results show that our novel vector generates comparable WAS protein levels and is as effective as the clinically used LTR-driven vector. Therefore, the described SIN vectors appear to be good candidates for potential use in a safer new gene therapy protocol for WAS, with decreased risk of insertional mutagenesis.

PMID:
21850255

PLoS One. 2011;6(8):e23101. Epub 2011 Aug 5.

Peptide Ligands Incorporated into the Threefold Spike Capsid Domain to Re-Direct Gene Transduction of AAV8 and AAV9 In Vivo.

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Efficiency and specificity of viral vectors are vital issues in gene therapy. Insertion of peptide ligands into the adeno-associated viral (AAV) capsid at receptor binding sites can re-target AAV2-derived vectors to alternative cell types. Also, the use of serotypes AAV8 and -9 is more efficient than AAV2 for gene transfer to certain tissues in vivo. Consequently, re-targeting of these serotypes by ligand insertion could be a promising approach but has not been explored so far. Here, we generated AAV8 and -9 vectors displaying peptides in the threefold spike capsid domain. These peptides had been selected from peptide libraries displayed on capsids of AAV serotype 2 to optimize systemic gene delivery to murine lung tissue and to breast cancer tissue in PymT transgenic mice (PymT). Such peptide insertions at position 590 of the AAV8 capsid and position 589 of the AAV9 capsid changed the transduction properties of both serotypes. However, both peptides inserted in AAV8 did not result in the same changes of tissue tropism as they did in AAV2. While the AAV2 peptides selected on murine lung tissue did not alter tropism of serotypes 8 and -9, insertion of the AAV2-derived peptide selected on breast cancer tissue augmented tumor gene delivery in both serotypes. Further, this peptide mediated a strong but unspecific in vivo gene transfer for AAV8 and abrogated transduction of various control tissues for AAV9. Our findings indicate that peptide insertion into defined sites of AAV8 and -9 capsids can change and improve their efficiency and specificity compared to their wild type variants and to AAV2, making these insertion sites attractive for the generation of novel targeted vectors in these serotypes.

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21850053**

Gene Ther. 2011 Aug 18. doi: 10.1038/gt.2011.115. [Epub ahead of print]

HPV-16 E1, E2 and E6 each complement the Ad5 helper gene set, increasing rAAV2 and wt AAV2 production.

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Adeno-associated virus type 2 (AAV) is a popular vector for human gene therapy, because of its safety record and ability to express genes long term. Yet large-scale recombinant (r) AAV production remains problematic because of low particle yield. The adenovirus (Ad) and herpes (simplex) virus helper genes for AAV have been widely used and studied, but the helper genes of human papillomavirus (HPV) have not. HPV-16 E1, E2 and E6 help wild-type (wt) AAV productive infection in differentiating keratinocytes, however, HEK293 cells are the standard cell line used for generating rAAV. Here we demonstrate that the three HPV genes were unable to stimulate significant rAAV replication in HEK293 cells when used alone. However, when used in conjunction (complementation) with the standard Ad5 helper gene set, E1, E2 and E6 were each capable of significantly boosting rAAV DNA replication and virus particle yield. Moreover, wt AAV DNA replication and virion yield were also significantly boosted by each HPV gene along with wt Ad5 virus co-infection. Mild-to-moderate changes in rep- and cap-encoded protein levels were evident in the presence of the E1, E2 and E6 genes. Higher wt AAV DNA replication was not matched by similar increases in the levels of rep-encoded protein. Moreover, although rep mRNA was upregulated, cap mRNA was upregulated more. Higher virus yields did correlate most consistently with increased Rep52-, VP3- and VP-related 21/31 kDa species. The observed boost in wt and rAAV production by HPV genes was not unexpected, as the Ad and HPV helper gene sets do not seem to recapitulate each other. These results raise the possibility of generating improved helper gene sets derived from both the Ad and HPV helper gene sets.

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21850052**

Gene Ther. 2011 Aug 18. doi: 10.1038/gt.2011.111. [Epub ahead of print]

Sustained correction of OTC deficiency in spf(ash) mice using optimized self-complementary AAV2/8 vectors.

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Ornithine transcarbamylase deficiency (OTCD) is the most common inborn error of urea synthesis. Complete OTCD can result in hyperammonemic coma in the neonatal period, which can rapidly become fatal. Current acute therapy involves dialysis; chronic therapy involves the stimulation of alternate nitrogen clearance pathways; and the only curative approach is liver transplantation. Adeno-associated virus (AAV) vector-based gene therapy would add to current treatment options provided the vector delivers high level and stable transgene expression in liver without dose-limiting toxicity. In this study, we employed an AAV2/8-based self-complementary (sc) vector expressing the murine OTC (mOTC) gene under a liver-specific thyroxine-binding globulin promoter and examined the therapeutic effects in a mouse model of OTCD, the spf(ash) mouse. Seven days after a single intravenous injection of vector, treated mice showed complete normalization of urinary orotic acid, a measure of OTC activity. We further improved vector efficacy by incorporating a Kozak or Kozak-like sequence into mOTC complementary DNA, which increased the OTC activity by five or twofold and achieved sustained correction of orotic aciduria for up to 7 months. Our results demonstrate that vector optimizations can significantly improve the efficacy of gene therapy.

PMID:
21850051

Gene Ther. 2011 Aug 18. doi: 10.1038/gt.2011.119. [Epub ahead of print]

AAV vectors transduce hepatocytes in vivo as efficiently in cirrhotic as in healthy rat livers.

Sobrevals L, Enguita M, Rodriguez C, Gonzalez-Rojas J, Alzaguren P, Razquin N, Prieto J, Fortes P.

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In liver cirrhosis, abnormal liver architecture impairs efficient transduction of hepatocytes with large viral vectors such as adenoviruses. Here we evaluated the ability of adeno-associated virus (AAV) vectors, small viral vectors, to transduce normal and cirrhotic rat livers. Using AAV serotype-1 (AAV1) encoding luciferase (AAV1Luc) we analyzed luciferase expression with a CCD camera. AAV1Luc was injected through the hepatic artery (intra-arterial (IA)), the portal vein (intra-portal (IP)), directly into the liver (intra-hepatic (IH)) or infused into the biliary tree (intra-biliar). We found that AAV1Luc allows long-term and constant luciferase expression in rat livers. Interestingly, IP administration leads to higher expression levels in healthy than in cirrhotic livers, whereas the opposite occurs when using IA injection. IH administration leads to similar transgene expression in cirrhotic and healthy rats, whereas intra-biliar infusion is the least effective route. After 70% partial hepatectomy, luciferase expression decreased in the regenerating liver, suggesting lack of efficient integration of AAV1 DNA into the host genome. AAV1Luc transduced mainly the liver but also the testes and spleen. Within the liver, transgene expression was found mainly in hepatocytes. Using a liver-specific promoter, transgene expression was detected in hepatocytes but not in other organs. Our results indicate that AAVs are convenient vectors for the treatment of liver cirrhosis.

PMID:
21850050

Gene Ther. 2011 Aug 18. doi: 10.1038/gt.2011.117. [Epub ahead of print]

Epidermal growth factor improves lentivirus vector gene transfer into primary mouse hepatocytes.

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Partial resistance of primary mouse hepatocytes to lentiviral (LV) vector transduction poses a challenge for ex vivo gene therapy protocols in models of monogenetic liver disease. We thus sought to optimize ex vivo LV gene transfer while preserving the hepatocyte integrity for subsequent transplantation into recipient animals. We found that culture media supplemented with epidermal growth factor (EGF) and, to a lesser extent, hepatocyte growth factor (HGF) markedly improved transduction efficacy at various multiplicities of infection. Up to 87% of primary hepatocytes were transduced in the presence of 10 ng EGF, compared with ~30% in standard culture medium (SCMs). The increased number of transgene-expressing cells correlated with increased nuclear import and more integrated pro-viral copies per cell. Higher LV transduction efficacy was not associated with proliferation, as transduction capacity of gammaretroviral vectors remained low (<1%). Finally, we developed an LV transduction protocol for short-term (maximum 24 h) adherent hepatocyte cultures. LV-transduced hepatocytes showed liver repopulation capacities similar to freshly isolated hepatocytes in alb-uPA mouse recipients. Our findings highlight the importance of EGF for efficient LV transduction of primary hepatocytes in culture and should facilitate studies of LV gene transfer in mouse models of monogenetic liver disease.

PMID:
21849694

J Clin Microbiol. 2011 Aug 17. [Epub ahead of print]

Computational analysis of two species C human adenoviruses provides evidence of a novel virus.

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Human adenovirus (HAdV) C species are a common cause of respiratory infections, and can occasionally produce severe clinical manifestations. Deeper understanding of the variation and evolution in species HAdV-C is especially important as these viruses, including HAdV-C6, are used as gene delivery vectors for human gene therapy and in other biotechnological applications. Here, the full-genome analysis of the prototype HAdV-C6 and a recently identified virus provisionally termed HAdV-C57 are reported. Although the genomes of all species HAdV-C members are very similar to each other, the E3 region, hexon and fiber (ten proteins total) present a wide range of identity values at the amino acid level. Studies of these viruses in comparison with the other three HAdV-C prototypes (1, 2 and 5) comprise a comprehensive analysis of the diversity and conservation within species HAdV-C. HAdV-C6 contains a recombination event within the constant region of the hexon gene. HAdV-C57 is a recombinant virus with a fiber gene nearly identical to HAdV-C6 and a unique hexon distinguished by its loop 2 motif.

PMID:
21849666

Sci Transl Med. 2011 Aug 17;3(96):96ra78.

Stem cell-mediated transfer of a human artificial chromosome ameliorates muscular dystrophy.

Tedesco FS, Hoshiya H, D'Antona G, Gerli MF, Messina G, Antonini S, Tonlorenzi R, Benedetti S, Berghella L, Torrente Y, Kazuki Y, Bottinelli R, Oshimura M, Cossu G.

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In contrast to conventional gene therapy vectors, human artificial chromosomes (HACs) are episomal vectors that can carry large regions of the genome containing regulatory elements. So far, HACs have not been used as vectors in gene therapy for treating genetic disorders. Here, we report the amelioration of the dystrophic phenotype in the mdx mouse model of Duchenne muscular dystrophy (DMD) using a combination of HAC-mediated gene replacement and transplantation with blood vessel-associated stem cells (mesoangioblasts). We first genetically corrected mesoangioblasts from dystrophic mdx mice with a HAC vector containing the entire (2.4 Mb) human dystrophin genetic locus. Genetically corrected mesoangioblasts engrafted robustly and gave rise to many dystrophin-positive muscle fibers and muscle satellite cells in dystrophic mice, leading to morphological and functional amelioration of the phenotype that lasted for up to 8 months after transplantation. Thus, HAC-mediated gene transfer shows efficacy in a preclinical model of DMD and offers potential for future clinical translation.

PMID:
21849058

Genet Vaccines Ther. 2011 Aug 17;9(1):13. [Epub ahead of print]

Biodistribution and blood clearance of plasmid DNA administered in arginine peptide complexes.

Woo JG, Kim NY, Yang JM, Shin S.

ABSTRACT: Background Peptide/DNA complexes have great potential as non-viral methods for gene delivery. Despite promising results for peptide-mediated gene delivery technology, an effective systemic peptide-based gene delivery system has not yet been developed. Methods This study used pCMV-Luc as a model gene to investigate the biodistribution and the in vivo efficacy of arginine peptide-mediated gene delivery by polymerase chain reaction (PCR). Results Plasmid DNA was detected in all organs tested 1 h after intraperitoneal administration of arginine/DNA complexes, indicating that the arginine/DNA complexes disseminated widely through the body. The plasmid was primarily detected in the spleen, kidney, and diaphragm 24 h post administration. The mRNA expression of plasmid DNA was noted in the spleen, kidney, and diaphragm for up to 2 weeks, and in the other major organs, for at least 1 week. Blood clearance studies showed that injected DNA was found in the blood as long as 6 h after injection. Conclusions Taken together, our results demonstrated that arginine/DNA complexes are stable in blood and are effective for in vivo gene delivery. These findings suggest that intraperitoneal administration of arginine/DNA complexes is a promising tool in gene therapy.

PMID:
21847633

Cell Biochem Biophys. 2011 Aug 17. [Epub ahead of print]

Lentivirus-Mediated Overexpression of MicroRNA-199a Inhibits Cell Proliferation of Human Hepatocellular Carcinoma.

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microRNA-199a (miR-199a) is a highly conserved miRNA, always deregulated in numerous human tumors. The results of microarray analysis indicated that miR-199a was frequently downregulated in hepatocellular carcinoma (HCC). The expression levels of miR-199a in 11 pairs of matched HCC neoplastic and adjacent non-neoplastic tissues, 5 HCC cell lines and liver cell line L02 were examined by quantitative real-time PCR analysis. We found miR-199a was significantly down-regulated in HCC tissues when compared with pair-matched adjacent non-tumor tissues. We also found the expression level of miR-199a was also substantially decreased in several human HCC cell lines including SMMC-7721, BEL-7402, BEL-7701, MHCC97H, and HepG2. To investigate the role of miR-199a in tumorigenesis, we developed a lentiviral vector for the expression of pre-miR-199a (Lenti-miR-199a). Lenti-miR-199a inhibited HCC cell proliferation in vitro and in vivo. Compared to parental cells or cells transfected with a control vector, the overexpression of microRNA-199a in the HCC cell lines HepG2 stably was showed to reduce cell proliferation in vitro and in vivo. Luciferase reporter assay revealed the regulation of miR-199a on 3'-UTR of HIF-1 α . Further investigation confirmed that miR-199a significantly reduced the endogenous protein level of HIF-1 α in hypoxia. MiR-199a inhibits cell proliferation in vitro and in vivo partly through down-regulation of HIF-1 α in human HCC. Thus, these studies provide an important new insight into the pathogenesis of human HCC and it may open a new perspective for the development of effective gene therapy for human HCC.

PMID:
21847575

Eur J Appl Physiol. 2011 Aug 17. [Epub ahead of print]

A polymerase chain reaction-based methodology to detect gene doping.

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The non-therapeutic use of genes to enhance athletic performance (gene doping) is a novel threat to the World of Sports. Skeletal muscle is a prime target of gene therapy and we asked whether we can develop a test system to produce and detect gene doping. Towards this end, we introduced a plasmid (pCMV-FAK, 3.8 kb, 50 µg) for constitutive expression of the chicken homologue for the regulator of muscle growth, focal adhesion kinase (FAK), via gene electro transfer in the anti-gravitational muscle, m. soleus, or gastrocnemius medialis of rats. Activation of hypertrophy signalling was monitored by assessing the ribosomal kinase p70S6K and muscle fibre cross section. Detectability of the introduced plasmid was monitored with polymerase chain reaction in deoxyribonucleic acids (DNA) from transfected muscle and serum. Muscle transfection with pCMV-FAK elevated FAK expression 7- and 73-fold, respectively, and increased mean cross section by 52 and 16% in targeted muscle fibres of soleus and gastrocnemius muscle 7 days after gene electro transfer. Concomitantly p70S6K content was increased in transfected soleus muscle (+110%). Detection of the exogenous plasmid sequence was possible in DNA and cDNA of muscle until 7 days after transfection, but not in serum except close to the site of plasmid deposition, 1 h after injection and surgery. The findings suggest that the reliable detection of gene doping in the immoral athlete is not possible unless a change in the current practice of tissue sampling is applied involving the collection of muscle biopsy close to the site of gene injection.

PMID:
21846571

Prog Mol Biol Transl Sci. 2011;102:141-63.

Toward a Durable Treatment of HIV-1 Infection Using RNA Interference.

Eekels JJ, Berkhout B.

RNA interference (RNAi) is a cellular mechanism that mediates sequence-specific gene silencing at the posttranscriptional level. RNAi can be used as an antiviral approach against human pathogens. An attractive target for RNAi therapeutics is the human immunodeficiency virus type 1 (HIV-1), and the first clinical trial using a lentiviral gene therapy was initiated in early 2008. In this chapter, we focus on some basic principles of such an RNAi-based gene therapy against HIV-1. This includes the subjects of target site selection within the viral RNA genome, the phenomenon of viral escape, and therapeutic strategies to prevent viral escape. The latter antiescape strategies include diverse combinatorial RNAi approaches that are all directed against the HIV-1 RNA genome. As an alternative strategy, we also discuss the possibilities and restrictions of targeting cellular cofactors that are essential for virus replication, but less important for cell physiology.

PMID:
21846298

Cytotherapy. 2011 Sep;13(8):944-55.

Anti-tumor effects of canine adipose tissue-derived mesenchymal stromal cell-based interferon- β gene therapy and cisplatin in a mouse melanoma model.

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Abstract Background aims. Adipose tissue (AT)-derived mesenchymal stromal cells (MSC) (AT-MSC) represent a novel tool for delivering therapeutic genes to tumor cells. Interferon (IFN)- β is a cytokine with pleiotropic cellular functions, including anti-proliferative, immunomodulatory and anti-angiogenic activities. The purpose of this study was to engineer canine AT-MSC (cAT-MSC) producing IFN- β and to evaluate the anti-tumor effect of cAT-MSC-IFN- β combined with cisplatin in mouse melanoma model. **Methods.** cAT-MSC engineered to express mouse IFN- β were generated using a lentiviral vector (cAT-MSC-IFN- β) and the secreted IFN- β -induced inhibition of tumor cell growth and apoptosis on B16F10 cells was investigated in vitro prior to in vivo studies. Melanoma-bearing mouse was developed by injecting B16F10 cells subcutaneously into 6-week-old C57BL/6 mice. After 14 days, cisplatin (10 mg/kg) was injected intratumorally, and 3 days later the engineered cAT-MSC were injected subcutaneously every 3 days to death. Tumor volume and survival times were measured. **Results.** The combination treatment of cAT-MSC-IFN- β with cisplatin was more effective in inhibiting the growth of melanoma and resulted in significantly extended survival time than both an unengineered cAT-MSC-cisplatin combination group and a cisplatin-alone group. Interestingly, subcutaneously injected cAT-MSC-IFN- β were migrated to tumor sites. **Conclusions.** Our data suggest that canine AT-MSC could serve as a powerful cell-based delivery vehicle for releasing therapeutic proteins to tumor lesions. Maximal anti-tumor effects were seen when this therapy was combined with a DNA-damaging chemotherapeutic agent. This study demonstrates the possible applicability of AT-MSC-mediated IFN- β in treating canine and human cancer patients.

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21846200

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Regulatory and Ethical Issues for Phase I In Utero Gene Transfer Studies.

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Clinical gene transfer research has involved adult and child subjects, and it is expected that gene transfer in fetal subjects will occur in the future. Some genetic diseases have serious adverse effects on the fetus prior to birth, and there is hope that prenatal gene therapy could prevent such disease progression. Research in animal models of prenatal gene transfer is actively being pursued. The prospect of human Phase I in utero gene transfer studies raises important regulatory and ethical issues. One issue not previously addressed arises in applying U.S. research regulations to such studies. Specifically, current regulations state that research involving greater than minimal risk to the fetus and no prospect of direct benefit to the fetus or pregnant woman is not permitted. Phase I studies will involve interventions such as needle insertions through the uterus, which carry risks to the fetus including spontaneous abortion and preterm birth. It is possible that these risks will be regarded as exceeding minimal. Also, some regard the probability of therapeutic benefit in Phase I studies to be so low that these studies do not satisfy the regulatory requirement that they "hold out the prospect of direct benefit" to subjects. Based on these considerations, investigators and Institutional Review Boards might reasonably conclude that some Phase I in utero studies are not permitted. This paper identifies considerations that are relevant to such judgments and explores ethically acceptable ways in which Phase I studies can be designed so that they are permitted by the regulations.

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21844566

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Leukocyte integrin activation mediates transient neutropenia following G-CSF administration.

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Following administration of granulocyte colony-stimulating factor (G-CSF) there is a marked, albeit transient, drop in circulating neutrophils. To determine the role of leukocyte integrins in this disappearance, a dog having canine leukocyte adhesion deficiency (CLAD) or CLAD dogs who had undergone gene correction either by matched littermate allogeneic transplant or autologous gene therapy were evaluated. Shortly following G-CSF administration, a dramatic and yet transient neutropenia was observed in the control littermates. This neutropenia was not as marked in the CLAD dogs. In all instances, it was CD18+ neutrophils which preferentially egressed from the circulation. The association of CD18 with this rapid loss suggested leukocyte integrin activation following G-CSF administration. To determine the activation status of the integrin, a monoclonal antibody recognizing the activated α -subunit cation binding domain (mAb 24) was used to evaluate human leukocytes following G-CSF administration. Mirroring the dramatic decrease in circulating neutrophil numbers, there was a dramatic and specific increase in the activation of the α -subunit following G-CSF expression on polymorphonuclear leukocytes. This activation, like the drop in neutrophil count, was transient. These results demonstrate that the leukocyte integrin on circulating neutrophils is transiently activated following G-CSF administration and mediates the transient neutropenia observed following G-CSF administration.

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21844342

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Combination therapy utilizing shRNA knockdown and an optimized resistant transgene for rescue of diseases caused by misfolded proteins.

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Molecular knockdown of disease proteins and restoration of wild-type activity represent a promising but challenging strategy for the treatment of diseases that result from the accumulation of misfolded proteins (i.e., Huntington disease, amyotrophic lateral sclerosis, and α -1 antitrypsin deficiency). In this study we used alpha-1 antitrypsin (AAT) deficiency with the piZZ mutant phenotype as a model system to evaluate the efficiency of gene-delivery approaches that both silence the piZZ transcript (e.g., shRNA) and restore circulating wild-type AAT expression from resistant codon-optimized AAT (AAT-opt) transgene cassette using adeno-associated virus (AAV) vector delivery. After systemic injection of a self-complimentary AAV serotype 8 (scAAV8) vector encoding shRNA in piZZ transgenic mice, both mutant AAT mRNA in the liver and defected serum protein level were inhibited by 95%, whereas liver pathology, as monitored by dPAS and fibrosis staining, reversed. To restore blood AAT levels in AAV8/shRNA-treated mice, several strategies to restore functional AAT levels were tested, including using AAV AAT-opt transgene cassettes targeted to muscle and liver, or combination vectors carrying piZZ shRNA and AAT-opt transgenes separately, or a single bicistronic AAV vector. With these molecular approaches, we observed over 90% knockdown of mutant AAT with a 13- to 30-fold increase of circulating wild-type AAT protein from the shRNA-resistant AAT-opt cassette. The molecular approaches applied in this study can simultaneously prevent liver pathology and restore blood AAT concentration in AAT deficiencies. Based on these observations, similar gene-therapy strategies could be considered for any diseases caused by accumulation of misfolded proteins.

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21844337

Proc Natl Acad Sci U S A. 2011 Aug 15. [Epub ahead of print]

Conjugative DNA transfer into human cells by the VirB/VirD4 type IV secretion system of the bacterial pathogen *Bartonella henselae*.

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Bacterial type IV secretion systems (T4SS) mediate interbacterial conjugative DNA transfer and transkingdom protein transfer into eukaryotic host cells in bacterial pathogenesis. The sole bacterium known to naturally transfer DNA into eukaryotic host cells via a T4SS is the plant pathogen *Agrobacterium tumefaciens*. Here we demonstrate T4SS-mediated DNA transfer from a human bacterial pathogen into human cells. We show that the zoonotic pathogen *Bartonella henselae* can transfer a cryptic plasmid occurring in the bartonellae into the human endothelial cell line EA.hy926 via its T4SS VirB/VirD4. DNA transfer into EA.hy926 cells was demonstrated by using a reporter derivative of this *Bartonella*-specific mobilizable plasmid generated by insertion of a eukaryotic egfp-expression cassette. Fusion of the C-terminal secretion signal of the endogenous VirB/VirD4 protein substrate BepD with the plasmid-encoded DNA-transport protein Mob resulted in a 100-fold increased DNA transfer rate. Expression of the delivered egfp gene in EA.hy926 cells required cell division, suggesting that nuclear envelope breakdown may facilitate passive entry of the transferred ssDNA into the nucleus as prerequisite for complementary strand synthesis and transcription of the egfp gene. Addition of an eukaryotic neomycin phosphotransferase expression cassette to the reporter plasmid facilitated selection of stable transgenic EA.hy926 cell lines that display chromosomal integration of the transferred plasmid DNA. Our data suggest that T4SS-dependent DNA transfer into host cells may occur naturally during human infection with *Bartonella* and that these chronically infecting pathogens have potential for the engineering of in vivo gene-delivery vectors with applications in DNA vaccination and therapeutic gene therapy.

PMID:
21843371

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The C-terminal region of Bfl-1 sensitizes non-small cell lung cancer to gemcitabine-induced apoptosis by suppressing NF- κ B activity and down-regulating Bfl-1.

Kim MK, Jeon YK, Woo JK, Choi Y, Choi DH, Kim YH, Kim CW.

ABSTRACT: Gemcitabine is used to treat several cancers including lung cancer. However, tumor cells often escape gemcitabine-induced cell death via various mechanisms, which include modulating bcl-2 family members and NF- κ B activation. We previously reported that the C-terminal region of Bfl-1 fused with GFP (BC) is sufficient to induce apoptosis in 293T cells. In the present study, we investigated the anti-tumor effect of combined BC gene therapy and gemcitabine chemotherapy in vitro and in vivo using non-small cell lung cancer cell lines and a xenograft model. Cell lines were resistant to low dose gemcitabine (4-40 ng/ml), which induced NF- κ B activation and concomitant up-regulation of Bfl-1 (an NF- κ B-regulated anti-apoptotic protein). BC induced the apoptosis of A549 and H157 cells with caspase-3 activation. Furthermore, cotreatment with BC and low dose gemcitabine synergistically and efficiently induced mitochondria-mediated apoptosis in these cells. When administered alone or with low dose gemcitabine, BC suppressed NF- κ B activity, inhibited the nuclear translocation of p65/relA, and down-regulated Bfl-1 expression. Furthermore, direct suppression of Bfl-1 by RNA interference sensitized cells to gemcitabine-induced cell death, suggesting that Bfl-1 importantly regulates lung cancer cell sensitivity to gemcitabine. BC and gemcitabine co-treatment also showed a strong anti-tumor effect in a nude mouse/A549 xenograft model. These results suggest that lung cancer cells become resistant to gemcitabine via NF- κ B activation and the subsequent overexpression of Bfl-1, and that BC, which has both pro-apoptotic and NF- κ B inhibitory effects, could be harnessed as a gene therapy to complement gemcitabine chemotherapy in non-small cell lung cancer.

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21843141

Comb Chem High Throughput Screen. 2011 Aug 16. [Epub ahead of print]

Discovery of Cationic Polymers for Non-viral Gene Delivery using Combinatorial Approaches.

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Gene therapy is an attractive treatment option for diseases of genetic origin, including several cancers and cardiovascular diseases. While viruses are effective vectors for delivering exogenous genes to cells, concerns related to insertional mutagenesis, immunogenicity, lack of tropism, decay and high production costs necessitate the discovery of non-viral methods. Significant efforts have been focused on cationic polymers as non-viral alternatives for gene delivery. Recent studies have employed combinatorial syntheses and parallel screening methods for enhancing the efficacy of gene delivery, biocompatibility of the delivery vehicle, and overcoming cellular level barriers as they relate to polymer-mediated transgene uptake, transport, transcription, and expression. This review summarizes and discusses recent advances in combinatorial syntheses and parallel screening of cationic polymer libraries for the discovery of efficient and safe gene delivery systems.

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21824270

Diabetes Obes Metab. 2011 Oct;13 Suppl 1:158-66. doi: 10.1111/j.1463-1326.2011.01457.x.

The future of incretin-based therapy: novel avenues-novel targets.

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Incretin-based therapy for type 2 diabetes is based on the antidiabetic effects of glucagon-like peptide-1 (GLP-1) and instituted by GLP-1 receptor agonists and dipeptidyl peptidase-4 inhibitors targeting the key islet defects of the disease. The treatment is clinically efficient and safe, and associated with a low risk of adverse events. It can be used both in early and late stages of the disease and both as monotherapy and add-on to other therapies. Current research on the future of incretin-based therapy focuses on optimizing its place in diabetes treatment and examines its potential in type 1 diabetes, in subjects with obesity without type 2 diabetes and in cardiovascular and neurodegenerative disorders. Other studies aim at prolonging the duration of action of the GLP-1 receptor agonists to allow weekly administration, and to develop orally GLP-1 receptor agonists. Furthermore, other investigators focus on stimulation of GLP-1 secretion by activating GLP-1-producing L-cells or using gene therapy. Finally, also other gastro-entero-pancreatic bioactive peptides are potential targets for drug development as are synthetic peptides engineered as co-agonists stimulating more than one receptor. We can therefore expect a dynamic development within this field in the coming years.

PMID:
21841042

J Dent Res. 2011 Aug 12. [Epub ahead of print]

Single-Molecule Imaging of BMP4 Dimerization on Human Periodontal Ligament Cells.

Mi HW, Lee MC, Chiang YC, Chow LP, Lin CP.

We expressed bone morphogenetic protein 4 (BMP4) fused with enhanced green fluorescent protein (BMP4-EGFP) in the secretory pathways of producer cells. Fluorescent EGFP was acquired only after we interrupted the transport of BMP4-EGFP by culturing cells at a lower temperature (20°C), and the dynamics of BMP4-EGFP could be monitored by single-molecule microscopy. Western blotting analysis confirmed that exposure to low temperature helped the integrated formation of BMP4-EGFP fusion proteins. In this study, for the first time, we could image the fluorescently labeled BMP4 molecules localized on the plasma membrane of living hPDL cells. The one-step photobleaching with EGFP and the "blinking" behavior of quantum dots suggest that the fluorescent spots represent the events of single BMP4 molecules. Single-molecule tracking showed that the BMP receptors (BMPR) dimerize after BMP4 stimulation, or that a complex of one BMP4 molecule and a pre-formed BMPR dimer develops first, followed by the binding of the second BMP4 molecule. Furthermore, BMP4-EGFP enhanced the osteogenic differentiation of hPDL cells via signal transduction involving BMP receptors. This single-molecule imaging technique might be a valuable tool for the future development of BMP4 gene therapy and regenerative medicine mediated by hPDLs. Abbreviations: BMP4, bone morphogenetic protein 4; BMPR, BMP receptor; EGFP, enhanced green fluorescent protein; hPDL cells, human periodontal ligament cells; QDs, quantum dots; TIRFM, total internal reflection fluorescence microscopy; 293 cells, human embryonic kidney cells; oDM, osteogenic differentiation medium; Hcol, type I collagen; ALP, alkaline phosphatase; BSP, bone sialoprotein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

PMID:
21840595

Biomaterials. 2011 Aug 12. [Epub ahead of print]

Magnetically enhanced adeno-associated viral vector delivery for human neural stem cell infection.

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Gene therapy technology is a powerful tool to elucidate the molecular cues that precisely regulate stem cell fates, but developing safe vehicles or mechanisms that are capable of delivering genes to stem cells with high efficiency remains a challenge. In this study, we developed a magnetically guided adeno-associated virus (AAV) delivery system for gene delivery to human neural stem cells (hNSCs). Magnetically guided AAV delivery resulted in rapid accumulation of vectors on target cells followed by forced penetration of the vectors across the plasma membrane, ultimately leading to fast and efficient cellular transduction. To combine AAV vectors with the magnetically guided delivery, AAV was genetically modified to display hexa-histidine (6xHis) on the physically exposed loop of the AAV2 capsid (6xHis AAV), which interacted with nickel ions chelated on NTA-biotin conjugated to streptavidin-coated superparamagnetic iron oxide nanoparticles (NiStNPs). NiStNP-mediated 6xHis AAV delivery under magnetic fields led to significantly enhanced cellular transduction in a non-permissive cell type (i.e., hNSCs). In addition, this delivery method reduced the viral exposure times required to induce a high level of transduction by as much as to 2-10 min of hNSC infection, thus demonstrating the great potential of magnetically guided AAV delivery for numerous gene therapy and stem cell applications.

PMID:
21840374

Biochim Biophys Acta. 2011 Aug 5. [Epub ahead of print]

Potential efficacy of cell-penetrating peptides for nucleic acid and drug delivery in cancer.

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Cell penetrating peptides (CPPs) are short amphipathic and cationic peptides that are rapidly internalized across cell membranes. They can be used to deliver molecular cargo, such as imaging agents (fluorescent dyes and quantum dots), drugs, liposomes, peptide/protein, oligonucleotide/DNA/RNA, nanoparticles and bacteriophage into cells. The utilized CPP, attached cargo, concentration and cell type, all significantly affect the mechanism of internalization. The mechanism of cellular uptake and subsequent processing still remains controversial. It is now clear that CPP can mediate intracellular delivery via both endocytic and non-endocytic pathways. In addition, the orientation of the peptide and cargo and the type of linkage are likely important. In gene therapy, the designed cationic peptides must be able to 1) tightly condense DNA into small, compact particles; 2) target the condensate to specific cell surface receptors; 3) induce endosomal escape; and 4) target the DNA cargo to the nucleus for gene expression. The other studies have demonstrated that these small peptides can be conjugated to tumor homing peptides in order to achieve tumor-targeted delivery in vivo. On the other hand, one of the major aims in molecular cancer research is the development of new therapeutic strategies and compounds that target directly the genetic and biochemical agents of malignant transformation. For example, cell penetrating peptide aptamers might disrupt protein-protein interactions crucial for cancer cell growth or survival. In this review, we discuss potential functions of CPPs especially for drug and gene delivery in cancer and indicate their powerful promise for clinical efficacy.

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21840308

Exp Mol Pathol. 2011 Aug 7. [Epub ahead of print]

Combination gene therapy using VEGF-shRNA and fusion suicide gene yCDglyTK inhibits gastric carcinoma growth.

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Clinical trials of suicide gene therapy have achieved limited success, which suggests a need for improvement. Angiogenesis plays a crucial role in the progression of cancers, which is greatly regulated by vascular endothelial growth factor (VEGF). The current study was designed to evaluate the anti-tumor effects of VEGF siRNA in combination with fusion suicide gene yCDglyTK. Introduction of a VEGF-targeted small hairpin RNA (shVEGF) to CDTK/5-FC system could induce cell apoptosis more effectively and decrease micro vessel density in xenograft tissue, thus resulted in a significant tumor growth delay in SGC7901 xenografts. These findings for the first time suggest the potential of combination gene therapy using suicide gene therapy and anti-angiogenesis gene therapy.

PMID:
21839810

Neuroscience. 2011 Aug 2. [Epub ahead of print]

Dopamine signaling as a neural correlate of consciousness.

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The neural correlates of consciousness are largely unknown but many neural circuits are likely to be involved. Our experiments with mice that cannot synthesize dopamine suggest that dopamine signaling is a critical component necessary for the expression of consciousness. Although dopamine-deficient mice are awake and respond to many stimuli, they are unmotivated and have profound deficits in all but the simplest learning tasks. Dopamine-deficient mice are unable to attend to salient sensory information, integrate it with prior experience, store it in long-term memory, or choose appropriate actions. While clearly conscious from a general anesthetic point of view, dopamine-deficient mice have marginal arousal and appear to be virtually unconscious from a behavioral point of view. Restoration of dopamine signaling within the striatum by viral gene therapy strategies restores most behaviors. Therefore, I propose that dopaminergic modulation of glutamatergic inputs from the cortex and thalamus onto medium spiny neurons in the striatum contributes to cognition and the expression of consciousness.

PMID:
21839775

Pharmacol Ther. 2011 Aug 3. [Epub ahead of print]

Anthracyclines and ellipticines as DNA-damaging anticancer drugs: Recent advances.

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Over the past forty years, anthracyclines and ellipticines have attracted attention as promising cytostatics. In this review, we focus on their mechanisms of cytotoxicity, DNA-damaging effects and adverse side-effects. We also summarize ways to enhance the therapeutic effects of these drugs together with a decrease in their adverse effects. Current drug design strategies are focused on drug bioavailability and their tissue targeting, whereas drug delivery to specific intracellular compartments is rarely addressed. Therefore, therapies utilizing the antineoplastic activities of anthracyclines and ellipticines combined with novel strategies such as nanotechnologies for safer drug delivery, as well as strategies based on gene therapy, could significantly contribute to medical practice.

PMID:
21838677

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Current Options and Future Prospects for the Treatment of Dyskinesia and Motor Fluctuations in Parkinson's Disease.

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Dyskinesia and motor fluctuations affect up to 90% of patients with Parkinson's disease (PD) within ten years of L-DOPA pharmacotherapy, and represent a major challenge to a successful clinical management of this disorder. There are currently two main treatment options for these complications, namely, deep brain electrical stimulation or continuous dopaminergic agonist infusion. The latter is achieved using either subcutaneous apomorphine infusion or intrajejunal L-DOPA infusion. Some patients also benefit from the antidyskinetic effect of amantadine as an adjunct to L-DOPA treatment. Ongoing research in animal models of PD aims at discovering additional, novel treatment options that can either prevent or reverse dyskinesia and motor fluctuations. Alternative methods of continuous L-DOPA delivery (including gene therapy), and in pharmacological agents that target nondopaminergic receptor systems are currently under experimental scrutiny. Because clinical response profiles show large individual variation in PD, an increased number of treatment options for dyskinesia and motor fluctuations will eventually allow for antiparkinsonian and antidyskinetic therapies to be tailor-made to the needs of different patients and/or PD subtypes.