



CLINIGENE CURRENT GENE THERAPY WEEKLY

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Table of contents:

Knockdown of Efp by DNA-modified small interfering RNA inhibits breast cancer cell proliferation and in vivo tumor growth.	3
Antigen quality determines the efficiency of antitumor immune responses generated in the absence of regulatory T cells.	3
Enhanced efficiency of prodrug activation therapy by tumor-selective replicating retrovirus vectors armed with the Escherichia coli purine nucleoside phosphorylase gene.	4
MicroRNA and drug resistance.	4
Genomic integration of adenoviral gene transfer vectors following transduction of fertilized mouse oocytes.	5
Mg(2+) substituted calcium phosphate nano particles synthesis for non viral gene delivery application.	5
A combinatorial approach for targeted delivery using small molecules and reversible masking to bypass nonspecific uptake in vivo.	6
Gene doping detection: evaluation of approach for direct detection of gene transfer using erythropoietin as a model system.	6
BDNF regulation under GFAP promoter provides engineered astrocytes as a new approach for long-term protection in Huntington's disease.	7
Ectopic matrix metalloproteinase-9 expression in human brain tumor cells enhances oncolytic HSV vector infection.	7
Increased interstitial pressure improves nucleic acid delivery to skin enabling a comparative analysis of constitutive promoters.	8
Delivery of an EBV episome by a self-circularizing helper-dependent adenovirus: long-term transgene expression in immunocompetent mice.	8
Efficient inhibition of B-cell lymphoma xenografts with a novel recombinant fusion protein: anti-CD20Fab-LDM.	9
Self-complementary AAV mediates gene targeting and enhances endonuclease delivery for double-strand break repair.	9
Quantitative fine-tuning of photoreceptor cis-regulatory elements through affinity modulation of transcription factor binding sites.	10
Gene therapy for prostate cancer.	10
p53-based Cancer Therapy.	11
Corpus Callosum: a Favorable Target for rSFV-Mediated Gene Transfer to Rat Brain with Broad and Efficient Expression.	11
Vasoactive Intestinal Peptide Increases Hepatic Transduction and Reduces Innate Immune Response Following Administration of Helper-dependent Ad.	12
Suppression of Livin Gene Expression by siRNA Leads to Growth Inhibition and Apoptosis Induction in Human Bladder Cancer T24 Cells.	12
HSF1 overexpression enhances oncolytic effect of replicative adenovirus.	13
Development of Novel Cardiovascular Therapeutics From Small Regulatory RNA Molecules - An Outline of Key Requirements.	13
Transplantation of sendai viral angiopoietin-1-modified mesenchymal stem cells for ischemic limb disease.	14
Marek's disease virus VP22 enhances potentially the immune response of ESAT-6/CFP-10 against	

Mycobacterium bovis infection.	14
A self-inactivating lentiviral vector for SCID-X1 gene therapy that does not activate LMO2 expression in human T cells.....	15
Cellular endocytosis and gene delivery.	15
Alpha-galactosidase A-Tat fusion enhances storage reduction in hearts and kidneys of fabry mice.	16
Nanoparticles for retinal gene therapy.	16
Facile synthesis of carbon-11-labeled cholesterol-based cationic lipids as new potential PET probes for imaging of gene delivery in cancer.....	17
Current strategies to target p53 in cancer.	17
Selection of genetically modified hematopoietic cells in vitro and in vivo using alkylating agent lysomustine.....	18

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Knockdown of Efp by DNA-modified small interfering RNA inhibits breast cancer cell proliferation and in vivo tumor growth.

Ueyama K, Ikeda K, Sato W, Nakasato N, Horie-Inoue K, Takeda S, Inoue S.

[1] Division of Gene Regulation and Signal Transduction, Research Center for Genomic Medicine, Saitama Medical University, Saitama, Japan [2] Department of Obstetrics and Gynecology, Juntendo University School of Medicine, Tokyo, Japan.

The estrogen-responsive gene Efp promotes the growth of breast cancer cells by stimulating the degradation of a negative cell-cycle regulator, 14-3-3sigma, and is hence considered a suitable molecular target for breast cancer therapy. The use of small interfering RNA (siRNA) and its derivatives to silence cancer-related genes is being investigated with the aim of identifying clinical applications for these molecules. Recently, it has been shown that DNA-modified siRNA (chimeric siRNA) has good potential in clinical applications, because it induces fewer off-target effects or immune responses in mammalian cells. In the present study, we identified the most specific and effective siRNA (siEfp-1) for silencing Efp expression in MCF-7 breast cancer cells. For this purpose, we used an algorithm that primarily eliminates off-target effects. siEfp-1 considerably suppressed the in vitro proliferation and cell-cycle progression of MCF-7 cells, as well as the in vivo growth of MCF-7 tumors, in athymic mice. DNA-modified siEfp-1 (chimeric siEfp) significantly inhibited the expression of Efp, proliferation of cultured cells and the in vivo growth of MCF-7-derived tumors in athymic mice. In addition, the silencing of Efp expression by siEfp-1 and chimeric siEfp increased the expression of the 14-3-3sigma protein. These results suggest that siEfp-1 and chimeric siEfp could be useful in breast cancer therapy. Chimeric siEfp, in particular, has a high specificity and induces few side effects and is therefore expected to be used as a novel nucleic acid-based therapeutic agent.

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Antigen quality determines the efficiency of antitumor immune responses generated in the absence of regulatory T cells.

Bergot AS, Durgeau A, Levacher B, Colombo BM, Cohen JL, Klatzmann D.

[1] UPMC Univ Paris 06, UMR 7211, Immunology-Immunopathology-Immunotherapy (I3), Paris, France [2] CNRS, UMR 7211, Immunology-Immunopathology-Immunotherapy (I3), Paris, France.

The observation that depletion or inhibition of regulatory T cells (Tregs) unleashes efficient antitumor effector immune responses that can lead to tumor eradication in mice has opened new perspectives for the development of cancer immunotherapy. The quality and overall efficiency of the effector immune responses induced in the absence of Tregs seem to depend on multiple factors that determine the result of a battle involving effector T cells (Teffs), Tregs and tumor cells. In this study, we investigated the quality of tumor-associated antigens (TAAs) as one such factor. We show that the presence of a strong dominant antigen is required for the induction of effector responses capable of tumor eradication in the absence of Tregs. The sole addition of a dominant antigen on tumor cells does not change tumor growth in unmanipulated mice, but improves tumor eradication rate from a few to almost 100% in the absence of Tregs. This eradication can be shown to result from the recruitment and activation of specific Teffs recognizing this antigen. We also show that the presence of such dominant antigens has the side effect of restricting the breadth of the immune response to other TAAs, which could favor the generation of escape mutant by tumor editing. Taken together, our results highlight the potential, and some requirements for cancer immunotherapy based on Treg depletion. They also show that, ultimately, tumor fate depends on multiple factors that should all be taken into consideration for the design of more efficient immunotherapy.

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Enhanced efficiency of prodrug activation therapy by tumor-selective replicating retrovirus vectors armed with the Escherichia coli purine nucleoside phosphorylase gene.

Tai CK, Wang W, Lai YH, Logg CR, Parker WB, Li YF, Hong JS, Sorscher EJ, Chen TC, Kasahara N.

[1] Department of Life Science, National Chung Cheng University, Min-Hsiung, Chia-Yi, Taiwan [2] Department of Medicine, University of California, Los Angeles, CA, USA.

Gene transfer of the Escherichia coli purine nucleoside phosphorylase (PNP) results in potent cytotoxicity after administration of the prodrug fludarabine phosphate (F-araAMP). Here, we have tested whether application of this strategy in the context of replication-competent retrovirus (RCR) vectors, which can achieve highly efficient tumor-restricted transduction as well as persistent expression of transgenes, would result in effective tumor inhibition, or, alternatively, would adversely affect viral replication. We found that RCR vectors could achieve high levels of PNP expression concomitant with the efficiency of their replicative spread, with significant cell killing activity in vitro and potent therapeutic effects in vivo. In U-87 xenograft models, replicative spread of the vector resulted in progressive transmission of the PNP transgene, as evidenced by increasing PNP enzyme activity with time after vector inoculation. On F-araAMP administration, high efficiency gene transfer of PNP by the RCR vector resulted in significant suppression of tumor growth and extended survival time. As the RCR mediates stable integration of the PNP gene and continuous expression, an additional round of F-araAMP administration resulted in further survival benefit. RCR-mediated PNP suicide gene therapy thus represents a highly efficient form of intracellular chemotherapy, and may achieve effective antitumor activity with less systemic toxicity.

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MicroRNA and drug resistance.

Ma J, Dong C, Ji C.

Department of Hematology, Qilu Hospital, Shandong University, Jinan, Shandong, China.

Chemotherapy is the preferred treatment for malignancies. However, a successful long-term use of chemotherapy is often prevented by the development of drug resistance. Many mechanisms such as gene mutation, DNA methylation and histone modification have important roles in the resistance of cancer cells to chemotherapeutic agents. Climent suggested miR-125b was involved in the development of drug resistance by microRNA (miRNA) dysregulation. miRNAs are endogenously expressed small non-coding RNAs, which are evolutionarily conserved and function as regulators of gene expression. Much effort has been exerted in analyzing the role of miRNAs in the development of drug resistance in a variety of malignancies. Several research groups have shown that the expressions of miRNAs in chemoresistant cancer cells and their parental chemosensitive ones are different. The molecular targets and mechanisms of chemosensitivity and chemoresistance are also elucidated. This article reviews the functions of miRNAs in the development of drug resistance.

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Genomic integration of adenoviral gene transfer vectors following transduction of fertilized mouse oocytes.

Larochelle N, Stucka R, Rieger N, Schermelleh L, Schiedner G, Kochanek S, Wolf E, Lochmüller H.

Department of Neurology Friedrich-Baur-Institute, Ludwig-Maximilians-Universität München, Munich, Germany, nancy.larochelle@mcgill.ca.

Adenoviral vectors (AdV) are popular tools to deliver foreign genes into a wide range of cells. They have also been used in clinical gene therapy trials. Studies on AdV-mediated gene transfer to mammalian oocytes and transmission through the germ line have been reported controversially. In the present study we investigated whether AdV sequences integrate into the mouse genome by microinjecting AdV into the perivitelline space of fertilized oocytes. We applied a newly developed PCR technique (HiLo-PCR) for identification of chromosomal junctions next to the integrated AdV. We demonstrate that mouse oocytes can be transduced by different recombinant adenoviral vectors (first generation and gutless). In one transgenic mouse line using the first generation adenoviral vector, the genome has integrated into a highly repetitive cluster located on the Y chromosome. While the transgene (GFP) was expressed in early embryos, no expression was detected in adult transgenic mice. The use of gutless AdV resulted in expression of the transgene, albeit the vector was not transmitted to progeny. These results indicate that under optimized conditions fertilized mouse oocytes are transduced by AdV and give rise to transgenic founder animals. Therefore, adequate precautions should be taken in gene therapy protocols of reproductive patients since transduction of oocytes or early embryos and subsequent chromosomal integration cannot be ruled out entirely.

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Mg(2+) substituted calcium phosphate nano particles synthesis for non viral gene delivery application.

Hanifi A, Fathi MH, Mir Mohammad Sadeghi H, Varshosaz J.

Biomaterials Group, Materials Engineering Department, Isfahan University of Technology, 84156-83111, Isfahan, Iran, a.hanifi@gmail.com.

Gene therapy provides a unique approach to medicine as it can be adapted towards the treatment of both inherited and acquired diseases. Recently, calcium phosphate vectors as a new generation of the non viral gene delivery nano carriers have been studied because of their biocompatibility and DNA condensation and gene transfer ability. Substituting cations, like magnesium, affects physical and chemical properties of calcium phosphate nano particles. In this study, Mg(2+) substituted calcium phosphate nano particles have been prepared using the simple sol gel method. X-ray diffraction analysis, Fourier transform infra red spectroscopy, transmission electron microscopy, specific surface area analysis, zeta potential measurement and ion release evaluation were used for characterization of the samples. It was concluded that presence of Mg ions decrease particle size and crystallinity of the samples and increase positive surface charge as well as beta tricalcium phosphate fraction in chemical composition of calcium phosphate. These properties result in increasing the DNA condensation ability, specific surface area and dissolution rate of the samples which make them suitable particles for gene delivery application.

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A combinatorial approach for targeted delivery using small molecules and reversible masking to bypass nonspecific uptake in vivo.

Shi Q, Nguyen AT, Angell Y, Deng D, Na CR, Burgess K, Roberts DD, Brunicardi FC, Templeton NS.

Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA.

We have developed a multi-disciplinary approach combining molecular biology, delivery technology, combinatorial chemistry and reversible masking to create improved systemic, targeted delivery of plasmid DNA while avoiding nonspecific uptake in vivo. We initially used a well-characterized model targeting the asialoglycoprotein receptor in the liver. Using our bilamellar invaginated vesicle (BIV) liposomal delivery system with reversible masking, we increased expression in the liver by 76-fold, nearly equaling expression in first-pass organs using non-targeted complexes, with no expression in other organs. The same technology was then applied to efficiently target delivery to a human tumor microenvironment model. We achieved efficient, targeted delivery by attachment of specific targeting ligands to the surface of our BIV complexes in conjunction with reversible masking to bypass nonspecific tissues and organs. We identified ligands that target a human tumor microenvironment created in vitro by co-culturing primary human endothelial cells with human lung or pancreatic cancer cells. The model was confirmed by increased expression of tumor endothelial phenotypes including CD31 and vascular endothelial growth factor-A, and prolonged survival of endothelial capillary-like structures. The co-cultures were used for high-throughput screening of a specialized small molecule library to identify ligands specific for human tumor-associated endothelial cells in vitro. We identified small molecules that enhanced the transfection efficiency of tumor-associated endothelial cells, but not normal human endothelial cells or cancer cells. Intravenous (i.v.) injection of our targeted, reversibly masked complexes into mice, bearing human pancreatic tumor and endothelial cells, specifically increased transfection to this tumor microenvironment approximately 200-fold. Efficacy studies using our optimized targeted delivery of a plasmid encoding thrombospondin-1 eliminated tumors completely after five i.v. injections administered once every week.

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20463760

Gene doping detection: evaluation of approach for direct detection of gene transfer using erythropoietin as a model system.

Baoutina A, Coldham T, Bains GS, Emslie KR.

Bioanalysis, National Measurement Institute, New South Wales, Australia.

As clinical gene therapy has progressed toward realizing its potential, concern over misuse of the technology to enhance performance in athletes is growing. Although 'gene doping' is banned by the World Anti-Doping Agency, its detection remains a major challenge. In this study, we developed a methodology for direct detection of the transferred genetic material and evaluated its feasibility for gene doping detection in blood samples from athletes. Using erythropoietin (EPO) as a model gene and a simple in vitro system, we developed real-time PCR assays that target sequences within the transgene complementary DNA corresponding to exon/exon junctions. As these junctions are absent in the endogenous gene due to their interruption by introns, the approach allows detection of trace amounts of a transgene in a large background of the endogenous gene. Two developed assays and one commercial gene expression assay for EPO were validated. On the basis of ability of these assays to selectively amplify transgenic DNA and analysis of literature on testing of gene transfer in preclinical and clinical gene therapy, it is concluded that the developed approach would potentially be suitable to detect gene doping through gene transfer by analysis of small volumes of blood using regular out-of-competition testing.

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BDNF regulation under GFAP promoter provides engineered astrocytes as a new approach for long-term protection in Huntington's disease.

Giralt A, Friedman HC, Caneda-Ferrón B, Urbán N, Moreno E, Rubio N, Blanco J, Peterson A, Canals JM, Alberch J.

[1] Facultat de Medicina, Departament de Biologia Cel·lular, Immunologia i Neurociències, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Universitat de Barcelona, Barcelona, Spain [2] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas, Barcelona, Spain.

Brain-derived neurotrophic factor (BDNF) is the main candidate for neuroprotective therapeutic strategies for Huntington's disease. However, the administration system and the control over the dosage are still important problems to be solved. Here we generated transgenic mice overexpressing BDNF under the promoter of the glial fibrillary acidic protein (GFAP) (pGFAP-BDNF mice). These mice are viable and have a normal phenotype. However, intrastriatal administration of quinolinate increased the number of reactive astrocytes and enhanced the release of BDNF in pGFAP-BDNF mice compared with wild-type mice. Coincidentally, pGFAP-BDNF mice are more resistant to quinolinate than wild-type mice, suggesting a protective effect of astrocyte-derived BDNF. To verify this, we next cultured astrocytes from pGFAP-BDNF and wild-type mice for grafting. Wild-type and pGFAP-BDNF-derived astrocytes behave similarly in nonlesioned mice. However, pGFAP-BDNF-derived astrocytes showed higher levels of BDNF and larger neuroprotective effects than the wild-type ones when quinolinate was injected 30 days after grafting. Interestingly, mice grafted with pGFAP-BDNF astrocytes showed important and sustained behavioral improvements over time after quinolinate administration as compared with mice grafted with wild-type astrocytes. These findings show that astrocytes engineered to release BDNF can constitute a therapeutic approach for Huntington's disease.

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Ectopic matrix metalloproteinase-9 expression in human brain tumor cells enhances oncolytic HSV vector infection.

Hong CS, Fellows W, Niranjan A, Alber S, Watkins S, Cohen JB, Glorioso JC, Grandi P. Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

Oncolytic herpes simplex virus (oHSV) vectors have shown promise in the treatment of patients with recurrent brain tumors although few complete responses have accrued. Impediments to effective therapy include limited vector distribution on delivery, a consequence of injected virion particle trapping in the tumor extracellular matrix (ECM). To enhance virus delivery and spread, we investigated the use of the matrix metalloproteinase-9 (MMP-9) as a means to degrade collagen type IV, a major component of the ECM and basement membranes of gliomas that is absent in normal brain tissue. SK-N-AS neuroblastoma cells were transduced for constitutive, elevated expression of MMP-9, which did not enhance tumor cell migration in vitro or tumor progression in a murine xenograft brain tumor model. MMP-9 expression improved the distribution and infection of oHSV vectors in spheroid model in vitro. Furthermore, MMP9 induced a vector infection over larger areas of brain tumors in vivo. These results suggest that vector delivery and distribution in vivo can be improved by compromising the ECM, potentially enhancing oncolytic efficacy.

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20463756

Increased interstitial pressure improves nucleic acid delivery to skin enabling a comparative analysis of constitutive promoters.

González-González E, Ra H, Spitler R, Hickerson RP, Contag CH, Kaspar RL.

[1] Molecular Imaging Program at Stanford (MIPS), Stanford University School of Medicine, Stanford, CA, USA [2] Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA.

Nucleic acid-based therapies hold great promise for treatment of skin disorders if delivery challenges can be overcome. To investigate one mechanism of nucleic acid delivery to keratinocytes, a fixed mass of expression plasmid was intradermally injected into mouse footpads in different volumes, and reporter expression was monitored by intravital imaging or skin sectioning. Reporter gene expression increased with higher delivery volumes, suggesting that pressure drives nucleic acid uptake into cells after intradermal injections similar to previously published studies for muscle and liver. For spatiotemporal analysis of reporter gene expression, a dual-axis confocal (DAC) fluorescence microscope was used for intravital imaging following intradermal injections. Individual keratinocytes expressing hMGFP were readily visualized in vivo and initially appeared to preferentially express in the stratum granulosum and subsequently migrate to the stratum corneum over time. Fluorescence microscopy of frozen skin sections confirmed the patterns observed by intravital imaging. Intravital imaging with the DAC microscope is a noninvasive method for probing spatiotemporal control of gene expression and should facilitate development and testing of new nucleic acid delivery technologies.

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20463755

Delivery of an EBV episome by a self-circularizing helper-dependent adenovirus: long-term transgene expression in immunocompetent mice.

Gil JS, Gallaher SD, Berk AJ.

[1] Molecular Biology Institute, University of California, Los Angeles, CA, USA [2] Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, CA, USA.

Epstein-Barr virus (EBV) evolved an episomal system for maintaining life-long, latent infection of human B lymphocytes. Circular episomes engineered from EBV components required for this latent form of infection have the capacity to persist in most types of replicating mammalian cells without DNA integration and the pitfalls of insertional mutagenesis. EBV episomes are typically transduced using low-efficiency methods. Here we present a method for efficient delivery of EBV episomes to nuclei of hepatocytes in living mice using a helper-dependent adenoviral vector and Cre-mediated recombination in vivo to generate circular EBV episomes following infection. Cre is transiently expressed from a hepatocyte-specific promoter so that vector generation and transgene expression are tissue specific. We show long-term persistence of the circularized vector DNA and expression of a reporter gene in hepatocytes of immunocompetent mice.

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Efficient inhibition of B-cell lymphoma xenografts with a novel recombinant fusion protein: anti-CD20Fab-LDM.

Xin C, Ye S, Ming Y, Shenghua Z, Qingfang M, Hongxing G, Xu S, Yuanfu X, Yuan Z, Dongmei F, Juanni L, Yingdai G, Lianfang J, Rongguang S, Zhenping Z, Jianxiang W, Tao C, Chunzheng Y, Dongsheng X, Yongsu Z.

State Key Laboratory of Experimental Hematology, Department of Pharmacy, Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, PRC.

Lidamycin (LDM) is a new member of enediyne antitumor antibiotics family that can be separated and reconstituted. It consists of a labile active enediyne chromophore (AE) and a noncovalently bound apoprotein (LDP). LDM is now in phase II clinical trials. In this study, we described the antitumor features of a fusion protein of LDM, anti-CD20Fab-LDM, targeted to CD20 expressed by B-lymphoid malignancies. Especially, LDM was prepared by a novel two-step method including DNA recombination and molecular reconstitution. Anti-CD20Fab-LDM exerted potent cytotoxicity against CD20(+) B-cell lymphoma cell lines in vitro (IC₅₀: 10-30 pM) and in the Raji xenograft model. Two Raji xenografts were allowed to grow to an initial mass of 80 and 500 mm³, respectively, and then anti-CD20Fab-LDM was administered intravenously with the highest dose of 4 nmol kg⁻¹. The inhibition rates of tumor growth were 90.1 and 85%, which were saliently superior to those of nontargeted LDM. It is noteworthy that anti-CD20Fab-LDM can inhibit the growth of patient-derived cells, including rituximab-resistant patient-derived cells. Thus, CD20-targeted delivery of LDM is a specific and potent therapeutic strategy for B-lymphoid malignancies. In addition, the two-step approach could serve as a new technology platform for making a series of highly potent engineered antibody-based drugs.

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Self-complementary AAV mediates gene targeting and enhances endonuclease delivery for double-strand break repair.

Hirsch ML, Green L, Porteus MH, Samulski RJ.

UNC Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Adeno-associated virus (AAV) mediates gene targeting in humans by providing exogenous DNA for allelic replacement through homologous recombination. In comparison to other methods of DNA delivery or alternative DNA substrates, AAV gene targeting is reported to be very efficient, perhaps due to its single-stranded DNA genome, the inverted terminal repeats (ITRs), and/or the consequence of induced cellular signals on infection or uncoating. These viral attributes were investigated in the presence and absence of an I-Sce endonuclease-induced double-strand break (DSB) within a chromosomal defective reporter in human embryonic kidney cells. Gene correction was evaluated using self-complementary (sc) AAV, which forms a duplexed DNA molecule and results in earlier and robust transgene expression compared with conventional single-strand (ss) AAV genomes. An scAAV repair substrate was modestly enhanced for reporter correction showing no dependency on ssAAV genomes for this process. The AAV ITR sequences were also investigated in a plasmid repair context. No correction was noted in the absence of a DSB, however, a modest inhibitory effect correlated with the increasing presence of ITR sequences. Similarly, signaling cascades stimulated upon recombinant AAV transduction had no effect on plasmid-mediated DSB repair. Noteworthy, was the 20-fold additional enhancement in reporter correction using scAAV vectors, over ss versions, to deliver both the repair substrate and the endonuclease. In this case, homologous recombination repaired the defective reporter in 4% of cells without any selection. This report provides novel insights regarding the recombination substrates used by AAV vectors in promoting homologous recombination and points to the initial steps in vector optimization that could facilitate their use in gene correction of genetic disorders.

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20463752

Quantitative fine-tuning of photoreceptor cis-regulatory elements through affinity modulation of transcription factor binding sites.

Lee J, Myers CA, Williams N, Abdelaziz M, Corbo JC.
Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO, USA.

Given the remarkable recent progress in gene therapy-based treatments for retinal disease, there is an urgent need for the development of new approaches to quantitative design and analysis of photoreceptor-specific promoters. In this study, we determined the relative binding affinity of all single-nucleotide variants of the consensus binding site of the mammalian photoreceptor transcription factor, Crx. We then showed that it is possible to use these data to accurately predict the relative binding affinity of Crx for all possible 8 bp sequences. By rationally adjusting the binding affinity of three Crx sites, we were able to fine-tune the expression of the rod-specific Rhodopsin promoter over a 225-fold range in living retinas. In addition, we showed that it is possible to fine-tune the activity of the rod-specific Gnat1 promoter over approximately 275-fold range by modulating the affinity of a single Crx-binding site. We found that the action of individual binding sites depends on the precise promoter context of the site and that increasing binding affinity does not always equate with increased promoter output. Despite these caveats, this tuning approach permits quantitative engineering of photoreceptor-specific cis-regulatory elements, which can be used as drivers in gene therapy vectors for the treatment of blindness.

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20463426

Gene therapy for prostate cancer.

Tangney M, Ahmad S, Collins SA, O'Sullivan GC.
Cork Cancer Research Centre, Mercy University Hospital, Cork, Ireland. m.tangney@ucc.ie

Cancer remains a leading cause of morbidity and mortality. Despite advances in understanding, detection, and treatment, it accounts for almost one-fourth of all deaths per year in Western countries. Prostate cancer is currently the most commonly diagnosed noncutaneous cancer in men in Europe and the United States, accounting for 15% of all cancers in men. As life expectancy of individuals increases, it is expected that there will also be an increase in the incidence and mortality of prostate cancer. Prostate cancer may be inoperable at initial presentation, unresponsive to chemotherapy and radiotherapy, or recur following appropriate treatment. At the time of presentation, patients may already have metastases in their tissues. Preventing tumor recurrence requires systemic therapy; however, current modalities are limited by toxicity or lack of efficacy. For patients with such metastatic cancers, the development of alternative therapies is essential. Gene therapy is a realistic prospect for the treatment of prostate and other cancers, and involves the delivery of genetic information to the patient to facilitate the production of therapeutic proteins. Therapeutics can act directly (eg, by inducing tumor cells to produce cytotoxic agents) or indirectly by upregulating the immune system to efficiently target tumor cells or by destroying the tumor's vasculature. However, technological difficulties must be addressed before an efficient and safe gene medicine is achieved (primarily by developing a means of delivering genes to the target cells or tissue safely and efficiently). A wealth of research has been carried out over the past 20 years, involving various strategies for the treatment of prostate cancer at preclinical and clinical trial levels. The therapeutic efficacy observed with many of these approaches in patients indicates that these treatment modalities will serve as an important component of urological malignancy treatment in the clinic, either in isolation or in combination with current approaches.

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20463003

p53-based Cancer Therapy.

Lane DP, Cheok CF, Lain S.

p53 Laboratory (A-Star) 8A Biomedical Grove Immunos Singapore 138648.

Inactivation of p53 functions is an almost universal feature of human cancer cells. This has spurred a tremendous effort to develop p53 based cancer therapies. Gene therapy using wild-type p53, delivered by adenovirus vectors, is now in widespread use in China. Other biologic approaches include the development of oncolytic viruses designed to replicate and kill only p53 defective cells and also the development of siRNA and antisense RNA's that activate p53 by inhibiting the function of the negative regulators Mdm2, MdmX, and HPV E6. The altered processing of p53 that occurs in tumor cells can elicit T-cell and B-cell responses to p53 that could be effective in eliminating cancer cells and p53 based vaccines are now in clinical trial. A number of small molecules that directly or indirectly activate the p53 response have also reached the clinic, of which the most advanced are the p53 mdm2 interaction inhibitors. Increased understanding of the p53 response is also allowing the development of powerful drug combinations that may increase the selectivity and safety of chemotherapy, by selective protection of normal cells and tissues.

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20461495

Corpus Callosum: a Favorable Target for rSFV-Mediated Gene Transfer to Rat Brain with Broad and Efficient Expression.

Li ZJ, Sun P, Zhang HD, Li SF, Liu X, Wang RZ.

Department of Neurosurgery, the Affiliated Hospital of Medical College, Qingdao University, Qingdao, 266003, China.

Recombinant Semliki Forest virus (rSFV), as a new kind of neurotropic vector system, has great potential of gene therapy for stroke. However, very little is known about its transduction characteristics in cerebral cortex or corpus callosum (CC) in vivo, which are common targets for gene transfer in experimental stroke therapy. Here, we investigate and compare rSFV-mediated gene expression at above two brain regions in rat; 2.0×10^7 IU of rSFV encoding green fluorescent protein (rSFV-GFP) was locally injected into CC or cerebral cortex in two groups. At 36 h following injection, the number of GFP-positive cells, GFP distribution volume, and GFP expression level were examined in the rat brain of each group using continuous frozen sections and enzyme-linked immunosorbent assay. rSFV vector displayed noticeably different transduction patterns in CC and cerebral cortex in vivo. CC injection of vector increased GFP-positive cell number by 802%, GFP transduction volume by 958%, and GFP expression level by 508% compared with cortical injection (all $P < 0.01$). We concluded that rSFV CC delivery significantly enhances transduction efficiency in rat brain with its ability to achieve transgene extensive transduction and abundant expression, and CC may be a favorable target for improving rSFV-based gene delivery efficiency to brain.

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20461064

Vasoactive Intestinal Peptide Increases Hepatic Transduction and Reduces Innate Immune Response Following Administration of Helper-dependent Ad.

Vetrini F, Brunetti-Pierri N, Palmer DJ, Bertin T, Grove NC, Finegold MJ, Ng P.
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA.

Helper-dependent adenoviral vectors (HDAd) are effective tools for liver-directed gene therapy because they can mediate long-term transgene expression in the absence of chronic toxicity. However, high vector doses required for efficient hepatocyte transduction by intravascular delivery result in systemic vector dissemination and dose-dependent activation of the innate immunity. Therefore, strategies to achieve high-efficiency hepatocyte transduction using low vector doses and/or to reduce the acute elevations of proinflammatory cytokines and chemokines may have significant clinical potential. Vasoactive intestinal peptide (VIP) is an endogenous neuropeptide involved in the regulation of hepatic blood flow and plays an important role as modulator of immune functions. Here, we show that VIP pretreatment in mice is able to increase hepatocyte transduction by HDAd, decrease vector uptake by the spleen, reduce elevation of proinflammatory serum cytokines interleukin (IL)-6 and IL-12, and reduce serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) following intravenous HDAd injection. VIP pretreatment also resulted in a reduction in the expression of the chemokines macrophage-inflammatory protein 2 (MIP-2), monocyte chemoattractant protein 1 (MCP-1), and regulated on activation normal T-cell expressed and secreted (RANTES) in the livers of mice injected with HDAd. These results suggest that VIP can improve the therapeutic index of HDAd by increasing hepatocyte transduction efficiency while reducing cytokine and chemokine expression following intravascular delivery of HDAd.

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20460713

Suppression of Livin Gene Expression by siRNA Leads to Growth Inhibition and Apoptosis Induction in Human Bladder Cancer T24 Cells.

Yang D, Song X, Zhagn J, Ye L, Wang S, Che X, Wang J, Zhang Z, Wang L.
Department of Urology, First Affiliated Hospital of Dalian Medical University.

Apoptosis deficiency is a hallmark of many cancer cells. Functional suppression of specific antiapoptotic factors might provide a feasible strategy in cancer gene therapy. Livin, the latest found inhibitor of apoptosis protein (IAP) family member, plays important role in cell growth and apoptosis. It has been reported that Livin is highly expressed in bladder cancer tissues. In this study, we found that, unlike other cancer cell lines, there was only Livin-alpha not Livin-beta expression in bladder cancer cell lines. We further investigated the effects of Livin knockdown on human bladder cancer T24 cell growth and apoptosis. We found that small interfering RNA (siRNA) mediated Livin suppression significantly inhibited T24 cell proliferation and colony formation ability. Livin knockdown dramatically increased the T24 cell apoptotic rate in response to different proapoptotic stimuli, such as Mitomycin and TNF-alpha, and this was associated with caspase-3 and caspase-9 activation. These results suggest that Livin knockdown can inhibit cell growth and increase sensitivity to apoptotic stimuli, and might serve as a potent target in bladder cancer gene therapy.

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HSF1 overexpression enhances oncolytic effect of replicative adenovirus.

Wang C, Dai Z, Fan R, Deng Y, Lv G, Lu G.

ABSTRACT: **BACKGROUND:** E1B55kD deleted oncolytic adenovirus was designed to achieve cancer-specific cytotoxicity, but showed limitations in clinical study. To find a method to increase its efficacy, we investigated the correlation between oncolytic effect of such oncolytic adenovirus Adel55 and intracellular heat shock transcription factor 1 (HSF1) activity. **METHODS:** In the present study, human breast cancer cell line Bcap37 was stably transfected with constitutively active HSF1 (cHSF1) or HSF1 specific siRNA (HSF1i) to establish increased or decreased HSF1 expression levels. Cytotoxicity of Adel55 was analyzed in these cell lines in vitro and in vivo. Furthermore, Adel55 incorporated with cHSF1 (Adel55-cHSF1) was used to treat various tumor xenografts. **RESULTS:** Adel55 could achieve more efficient oncolysis in cHSF1 transfected Bcap37 cells, both in vitro and in vivo. However, inhibition of HSF1 expression by HSF1i could rescue Bcap37 cell line from oncolysis by Adel55. A time course study of viral replication established a correlation between higher replication of Adel55 and cytolysis or tumor growth inhibition. Then, we constructed Adel55-cHSF1 for tumor gene therapy and demonstrated that it is more potent than Adel55 itself in oncolysis and replication in both Bcap37 and SW620 xenografts. **CONCLUSIONS:** cHSF1 enhances the Adel55 cell-killing potential through increasing the viral replication and is a potential therapeutic implication to augment the potential of E1B55kD deleted oncolytic adenovirus by increasing its burst.

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Development of Novel Cardiovascular Therapeutics From Small Regulatory RNA Molecules - An Outline of Key Requirements.

Poller W, Fechner H.

Department of Cardiology & Pneumology, Charité Centrum 11 (Cardiovascular Medicine), Charité, Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, D-12200 Berlin, Germany. wolfgang.poller@charite.de.

Understanding of the roles of RNAs within the cell has changed and expanded dramatically during the past few years. Based on fundamentally new insights it is now increasingly possible to employ RNAs as highly valuable tools in molecular biology and medicine. At present, the most important therapeutic strategies are based on non-coding regulatory RNAs inducing RNA interference (RNAi) to silence single genes, and on modulation of cellular microRNAs (miRNAs) to alter complex gene expression patterns in diseased organs. Only recently it became possible to target therapeutic RNAi to specific organs via organotropic viral vector systems and we discuss the most recent strategies in this field, e.g. heart failure treatment by cardiac-targeted RNAi. Due to the peculiar biochemical properties of small RNA molecules, true therapeutic translation of results in vitro is more demanding than with small molecule drugs or proteins. Specifically, there is a critical requirement for extensive studies in animal models of human disease after pre-testing of the RNAi tools in vitro. This requirement likewise applies for miRNA modulations which have complex consequences in the recipient dependent on biochemical stability and distribution of the therapeutic RNA. Problems not yet fully solved are the prediction of targets and specificity of the RNA tools. However, major progress has been made to achieve their tissue-specific and regulatable expression, and breakthroughs in vector technologies from the gene therapy field have fundamentally improved safety and efficacy of RNA-based therapeutic approaches, too. In summary, insight into the molecular mechanisms of action of regulatory RNAs in combination with new delivery tools for RNA therapeutics will significantly expand our cardiovascular therapeutic repertoire beyond classical pharmacology.

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20458615

Transplantation of sendai viral angiopoietin-1-modified mesenchymal stem cells for ischemic limb disease.

Piao W, Wang H, Inoue M, Hasegawa M, Hamada H, Huang J.
Department of Laboratory Medicine, Ningxia People's Hospital, YinChuan, China.

Sendai viral vector (SeV) is emerging as a promising vector for gene therapy. However, little information is available regarding the combination of SeV-mediated gene and mesenchymal stem cell (MSC) therapy in dealing with ischemic diseases. In this study, we infected SeV to the MSCs in vitro; and injected MSCs modified with SeV harboring human angiopoietin-1 gene (SeVhAng-1) into the ischemic limb of rats in vivo. We found SeV had high transductive efficiency to the MSCs. Both MSCs and SeVhAng-1-modified MSCs improved the blood flow recovery and increased the capillary density of the ischemic limb, compared with the control. However, in contrast to MSCs, SeVhAng-1-modified MSCs had a better improvement of blood flow recovery in the ischemic limb. We further found the ischemic limb injected with SeVhAng-1-modified MSCs had strong expression of p-Akt, which improved survival of MSCs injected into the ischemic limb. This indicated SeVhAng-1 modification enhanced angiogenetic effect of MSCs by both angiogenesis and cell protection. We conclude that SeVhAng-1-modified MSCs may serve as a more effective tool in dealing with ischemic limb disease.

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20458447

Marek's disease virus VP22 enhances potentially the immune response of ESAT-6/CFP-10 against Mycobacterium bovis infection.

Chen H, Wang W, Song C, Yu S, Ding C.
Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai 200241, China.

Currently available vaccines against *Mycobacterium bovis*, the causative agent of tuberculosis, do not provide reliable efficacy and there is therefore a need for a novel vaccine with improved efficacy. Here, we use protein transduction technology to deliver DNA vaccines expressing mycobacterial antigens directly to target cells. We used various protein transduction domain (PTD) proteins including the VP22 conjugate from Marek's disease virus serotype 1 (MDV-1), as delivery systems for DNA constructs encoding the antigens early secretory antigenic target-6 kDa (ESAT-6) protein and culture filtrate protein 10 (CFP-10) of *M. bovis*. The eukaryotic expression plasmid pZ106, encoding antigens ESAT-6 and CFP-10, conjugated to various PTDs, was used to construct experimental preparations. Our findings demonstrated that VP22 alone or in combination with CFP-10:ESAT-6 fusion protein could spread into all the nuclei of the cell monolayer surrounding the transfected cells. Whereas trans-activating transcriptional PTD showed limited delivery of the fusion protein and 8R peptide was unable to deliver the fusion protein into any untransfected cells. We have demonstrated that immunization with a preparation fused to VP22 leads to a higher antibody and interferon-gamma titer ($P < 0.05$). Taken together, our results demonstrated that MDV-1 VP22 serves as a potential immune enhancer in gene therapy and immunization using DNA vaccines, offering a novel approach for the prevention of *M. bovis* infection.

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20457870

A self-inactivating lentiviral vector for SCID-X1 gene therapy that does not activate LMO2 expression in human T cells.

Zhou S, Mody D, Deravin SS, Hauer J, Lu T, Ma Z, Hacein-Bey Abina S, Gray JT, Greene MR, Cavazzana-Calvo M, Malech HL, Sorrentino BP.

Division of Experimental Hematology, Department of Hematology, St. Jude Children's Research Hospital, Memphis, TN;

In order to develop safer and more effective vectors for gene therapy of X-linked severe combined immunodeficiency (SCID-X1), we have evaluated new self-inactivating (SIN) lentiviral vectors based on the HIV virus. The CL20i4-hgamma(c)-Revgen vector contains the entire human common gamma chain (gammac) genomic sequence driven by the gammac promoter. The CL20i4-EF1alpha-hgamma(c)OPT vector utilizes a promoter fragment from the eukaryotic elongation factor alpha (EF1alpha) gene to express a codon-optimized human gamma(c) cDNA. Both vectors contain a 400bp insulator fragment from the chicken beta-globin locus within the SIN LTR. Transduction of bone marrow cells using either of these vectors restored T, B, and NK lymphocyte development and function in a mouse SCID-X1 transplant model. Transduction of human CD34(+) bone marrow cells from SCID-X1 patients with either vector restored T cell development in an in vitro assay. In safety studies using a Jurkat LMO2 activation assay, only the CL20i4-EF1alpha-hgamma(c)OPT vector lacked the ability to transactivate LMO2 protein expression, while the CL20i4-hgamma(c)-Revgen vector significantly activated LMO2 protein expression. Also the CL20i4-EF1alpha-hgamma(c)OPT vector has not caused any tumors in transplanted mice. We conclude that the CL20i4-EF1alpha-hgamma(c)OPT vector is suitable for testing in a clinical trial based on these preclinical demonstrations of efficacy and safety.

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20454523

Cellular endocytosis and gene delivery.

Ziello JE, Huang Y, Jovin IS.

Boyer Center for Molecular Medicine, Yale University, New Haven, Connecticut, United States of America.

Endocytosis is the process by which cells take up macromolecules from the surrounding medium. The best-characterized process is the so-called clathrin-dependent endocytosis, although much is also currently known about clathrin-independent endocytic processes such as those involving caveolae and lipid rafts. An understanding of endocytosis and the cellular trafficking that occurs thereafter has a great deal of relevance to current molecular medicine. Gene therapy, which is presently being investigated for its therapeutic potential in treating immunodeficiency and metabolic diseases, cancer and heart disease, employs a variety of viral and nonviral vectors, which can be delivered to the target cells of the body and are subsequently endocytosed and disassembled. A variety of vectors can be used to deliver genes to organs in vivo or cells ex vivo. Various routes of vector delivery have been investigated. The mechanisms by which vectors such as adenoviruses, adeno-associated viruses, retroviruses and liposomes enter the cell are increasingly being investigated as the effort to increase the efficiency of gene therapy continues. This review focuses on mechanisms of endocytosis and how they relate to the internal trafficking of viral and nonviral vectors in gene therapy.

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20454522

Alpha-galactosidase A-Tat fusion enhances storage reduction in hearts and kidneys of fabry mice.

Higuchi K, Yoshimitsu M, Fan X, Guo X, Rasaiah VI, Yen J, Tei C, Takenaka T, Medin JA.
University Health Network (UHN), Toronto, Ontario, Canada.

The protein transduction domain from human immunodeficiency virus (HIV) Tat allows proteins to penetrate the cell membrane. Enhanced cellular uptake of therapeutic proteins could benefit a number of disorders. This is especially true for lysosomal storage disorders (LSDs) where enzyme replacement therapy (ERT) and gene therapy have been developed. We developed a novel recombinant lentiviral vector (LV) that engineers expression of alpha-galactosidase A (alpha-gal A)-Tat fusion protein for correction of Fabry disease, the second-most prevalent LSD with manifestations in the brain, kidney and heart. In vitro experiments confirmed mannose-6-phosphate independent uptake of the fusion factor. Next, concentrated therapeutic LV was injected into neonatal Fabry mice. Analysis of tissues at 26 wks demonstrated similar alpha-gal A enzyme activities but enhanced globotriaosylceramide (Gb3) reduction in hearts and kidneys compared with the alpha-gal A LV control. This strategy might advance not only gene therapy for Fabry disease and other LSDs, but also ERT, especially for cardiac Fabry disease.

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20452457

Nanoparticles for retinal gene therapy.

Conley SM, Naash MI.

University of Oklahoma Health Sciences Center, Department of Cell Biology, BMSB 781, 940 Stanton L. Young Blvd, Oklahoma City, OK 73104, USA.

Ocular gene therapy is becoming a well-established field. Viral gene therapies for the treatment of Leber's congenital amaurosis (LCA) are in clinical trials, and many other gene therapy approaches are being rapidly developed for application to diverse ophthalmic pathologies. Of late, development of non-viral gene therapies has been an area of intense focus and one technology, polymer-compacted DNA nanoparticles, is especially promising. However, development of pharmaceutically and clinically viable therapeutics depends not only on having an effective and safe vector but also on a practical treatment strategy. Inherited retinal pathologies are caused by mutations in over 220 genes, some of which contain over 200 individual disease-causing mutations, which are individually very rare. This review will focus on both the progress and future of nanoparticles and also on what will be required to make them relevant ocular pharmaceuticals.

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20451540

Facile synthesis of carbon-11-labeled cholesterol-based cationic lipids as new potential PET probes for imaging of gene delivery in cancer.

Gao M, Wang M, Miller KD, Sledge GW, Hutchins GD, Zheng QH.
Department of Radiology and Imaging Sciences, Indiana University School of Medicine, 1345 West 16(th) Street, L3-208, Indianapolis, IN 46202, USA.

Gene therapy based on gene delivery is a promising strategy for the treatment of various human diseases such as cancer. Cationic lipids represent one of the important synthetic gene delivery systems. There is a great interest in imaging of gene therapy using the biomedical imaging technique positron emission tomography (PET). Carbon-11-labeled cholesterol-based cationic lipids were first designed and synthesized as new potential PET probes for imaging of gene delivery in cancer. The [(11)C-methyl]quaternary amine target tracers, N-[(11)C]methyl-N-[4-(cholest-5-en-3beta-yloxy-carbonyl)butyl]pyrrolidinium iodide ([[(11)C]4a), N-[(11)C]methyl-N'-[4-(cholest-5-en-3beta-yloxy-carbonyl)butyl]imidazolium iodide ([[(11)C]4b), N-[(11)C]methyl-N-[4-(cholest-5-en-3beta-yloxy-carbonyl)butyl]piperidinium iodide ([[(11)C]4c), N-[(11)C]methyl-N-[4-(cholest-5-en-3beta-yloxy-carbonyl)butyl]-4-methylpiperidinium iodide ([[(11)C]4d), and N-[(11)C]methyl-N-[4-(cholest-5-en-3beta-yloxy-carbonyl)butyl]morpholinium iodide ([[(11)C]4e), were prepared from their corresponding tertiary amine precursors with [(11)C]methyl iodide ([[(11)C]CH₃I) through N-[(11)C]methylation and isolated by a simplified solid-phase extraction (SPE) method using a Silica Sep-Pak cartridge in 50-60% radiochemical yields decay corrected to end-of-bombardment (EOB), based on [(11)C]CO₂, and 111-185GBq/mumol specific activity at the end of synthesis (EOS).

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20450892

Current strategies to target p53 in cancer.

Chen F, Wang W, El-Deiry WS.
Laboratory of Molecular Oncology and Cell Cycle Regulation, Department of Medicine (Hematology/Oncology), The Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, Philadelphia, PA 19103, United States; The Abramson Comprehensive Cancer Center, University of Pennsylvania School of Medicine, Philadelphia, PA 19103, United States.

Tumor suppressor p53 is a transcription factor that guards the genome stability and normal cell growth. Stresses like DNA damage, oncogenic assault will turn on p53 function which leads to cell cycle arrest for DNA repair, senescence for permanent growth arrest or apoptosis for programmed cell death. At the late stage of cancer progression, p53 is hijacked in all forms of tumors either trapped in the negative regulator such as MDM2/viral proteins or directly mutated/deleted. Re-introduction of a functional p53 alone has been proven to induce tumor regression robustly. Also, an active p53 pathway is essential for effective chemo- or radio-therapy. The emerging cyclotherapy in which p53 acts as a chemoprotectant of normal tissues further expands the utility of p53 activators. Functionally, it is unquestionable that drugging p53 will render tumor-specific intervention. One direct method is to deliver the functional wild-type (wt) p53 to tumors via gene therapy. The small molecule strategies consist of activation of p53 family member such as p73, manipulating p53 posttranslational modulators to increase wt p53 protein levels, protein-protein interaction inhibitors to free wt p53 from MDM2 or viral protein, and restoring p53 function to mutant p53 by direct modulation of its conformation. Although most of the current pre-clinical leads are in muM range and need further optimization, the success in proving that small molecules can reactivate p53 marks the beginning of the clinical development of p53-based cancer therapy. Copyright © 2010 Elsevier Inc.

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Selection of genetically modified hematopoietic cells in vitro and in vivo using alkylating agent lysomustine.

Rozov FN, Grinenko TS, Levit GL, Krasnov VP, Belyavsky AV.

Engelhard Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia.

Efficient gene transfer into hematopoietic stem cells is vital for success of gene therapy of hematopoietic and immune system disorders. In vivo selection system based on a mutant form of O6-methylguanine-DNA-methyltransferase gene (MGMTm) is considered as one of the most promising strategies for expansion of hematopoietic cells transduced with viral vectors. Here we demonstrate that MGMTm-expressing cells can be efficiently selected using lysomustine, a nitrosourea derivative of lysine. K562 and murine bone marrow cells expressing MGMTm are protected from cytotoxic action of lysomustine in vitro. We also show in a murine model that MGMTm-transduced hematopoietic cells can be expanded in vivo upon transplantation into sublethally irradiated recipients followed by lysomustine treatment. These results indicate that lysomustine can be used as a potent novel chemoselection drug applicable for gene therapy of hematopoietic and immune system disorders.