We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Gene Delivery Systems: Tailoring Vectors to Reach Specific Tissues

Carlos J. Alméciga-Díaz, Rocio Cuaspa and Luis A. Barrera
*Institute for the Study of Inborn Errors of Metabolism
 Pontificia Universidad Javeriana, Bogotá D.C.
 Colombia*

1. Introduction

The gene therapy concept was developed more than forty years ago. Edward Tatum in a historic lecture affirmed: *"We can anticipate that viruses will be used effectively for man's benefit, in theoretical studies concerning somatic cell genetics and possibly in genetic therapy... We even can be somewhat optimistic about the long-range possibility of therapy based on the isolation or design, synthesis, and introduction of new genes into defective cells of particular organs"*. Two years later Marshall Nirenberg predicted that: *"in 25 years it would be possible to program the cells with synthetic messages"*, but called to attention that *"it should be postponed until having the sufficient wisdom to use this knowledge for the benefit of mankind"*.

Initial efforts to construct vectors were achieved by Rogers and Pfudere in 1968. However, it was not until 1990 that the first gene therapy clinical trial was carried-out in a patient with adenosine deaminase (ADA) deficiency (Friedmann, 1992). Within the last several years, significant advances have been attained in therapies for cancer, AIDS, Parkinson's disease, X-linked severe combined immunodeficiency (X-SCID), hemophilias, cystic fibrosis, Leber's congenital amaurosi, and β -thalassemia, among others. Nonetheless, an adolescent patient suffering from a urea cycle defect died of an anaphylactic reaction a few hours after being injected with an adenoviral vector. Soon after, five children participating in a clinical trial to correct a X-SCID developed a leukemia-like condition due to the activation of an oncogene (Edelstein, 2007). Recently, the tragic death of a volunteer in an clinical trial for rheumatoid arthritis using an adenoassociated virus vector was initially associated to a side effect of the vector (Williams, 2007). However, further studies showed that this was caused by an infection with *Histoplasma capsulatum*, which was produced by an immunosuppression condition induced for a simultaneous systemic anti-TNF-alpha therapy in the form of the drug adalimumab (Williams, 2007). All these failures halted the progress of gene therapy and seriously questioned the efficacy of the procedure. It also prompted the need for more basic studies on the immunogenic aspects and how to make safer, and more efficient vectors.

During the last 35 years a long list of viral and non-viral vectors have been developed to design a vector that allows gene delivery to specific cell types, has a high gene transfer efficiency, produces therapeutic levels of gene expression during long-term periods, and minimizes the generation of side effects (Verma & Weitzman, 2005). Although the field has made great strides in producing an ideal vector, one of the main challenges remains to aim for a cell-specific vector. Most probably a vector left in the general circulation without a specific targeting signal, will be sequestered by the liver or may end-up in cells with no need of the

transfected gene product. In addition, to date gene therapy still encounters two main problems: (i) gene delivery to the central nervous system (CNS), as the gene or its product are naturally blocked by the blood brain barrier; and (ii) gene delivery into poorly circulated tissues such as bone, where up until now, it has only been achieved with low transfection efficiencies.

In this chapter, we will explore the modifications carried out in viral and non-viral vectors to enhance their natural tropism. Regardless of the vector, modification objectives can be summarized in: (i) tropism expansion to permit gene delivery to cells not easily transfected with non-modified vector, (ii) cell-type specific transfection reducing side effects, and (iii) stealth vector improvement to reduce protein interactions or cells that limit its therapeutic activity, e.g. immune system (Yu & Schaffer, 2005).

2. Retroviral vectors

Retroviruses are a family of enveloped, diploid, and positive-stranded RNA virus. This family is formed by alpharetrovirus, betaretrovirus, deltaretrovirus, epsilon retrovirus, gammaretrovirus, lentivirus, and spumavirus (foamy virus), from which only the last three have been used as gene therapy vectors (Verma & Weitzman, 2005). The genome basically consists of three genes: (i) *gag*, which encodes for viral matrix, capsid and nucleocapsid proteins, (ii) *pol*, which encodes for protease, reverse transcriptase and integrase, and (iii) *env*, which encodes a bipartite membrane-anchored surface (SF) protein (Escors & Breckpot, 2010). In addition, complex retroviruses (e.g. lentivirus and foamy virus) have accessory genes for the regulation of gene expression, assembly and replication (Escors & Breckpot, 2010). In the viral life cycle, glycoproteins (GPs) play an important role in viral tropism, receptor recognition and cell entry (Liang et al., 2009). The binding of the viral GP to the cellular surface protein induces a conformational change into the viral GP and leads to fusion and insertion of the capsid into the cytoplasm (Yu & Schaffer, 2005). Most of the advantages and limitations of retrovirus targeting are related with this cell entry mechanism.

Retroviral vectors used in gene therapy have properties that allow the transduction of a broad array of cells (Cronin et al., 2005; Frecha et al., 2008). However, the use of targeted retroviral vectors allowing the delivery of the transgene to cells which are not normally transduced, or limiting the delivery to cells which should not be transduced would be advantageous (Cronin et al., 2005; Frecha et al., 2008; Lavillette et al., 2001). In general, retroviral targeting can be achieved by: (i) engineering the GP to produce a vector that recognizes a specific cell surface protein (direct targeting), (ii) pseudotyping with GPs derived from other retrovirus and that recognize specific receptors, (iii) using ligands that block the cell entry of the vector to the cells expressing the receptor for the ligand, while allow the transduction of others cell types (indirect targeting), or (iv) using biospecific antibodies, chemically crosslinking ligands and grafting polymers (Fig. 1) (Lavillette et al., 2001; Verhoeven & Cosset, 2004; Yu & Schaffer, 2005).

2.1 Retroviral targeting by engineering the glycoproteins

Direct targeting consists of the insertion of ligands or antibody fragments into the viral GP (Fig. 1), which allows recognition of cell surface proteins different from those naturally used by the vector (Lavillette et al., 2001). Direct modification of a GP was first reported in 1990, with the successful fusion of CD4 with the GP of a gammaretroviral vector (Young et al., 1990). Later, a functional murine leukemia virus (MLV) vector bearing a GP protein fused with an antibody fragment against a hapten (4-hidroxy-5-iodo-3-nitrophenacetyl caproate) demonstrated binding of the modified retroviral vector to hapten, and the ability to package a transgene and transduce culture cells (Russell et al., 1993).

After these pioneer works revealed the feasibility of GP engineering, different authors studied the real potential of this targeting approach. The fusion of the erythropoietin polypeptide with the GP of a MLV vector showed for the first time that a chimeric GP was not only correctly incorporated into the virus envelope but also allowed the specific transduction of cells expressing the EPO receptor (Kasahara et al., 1994). A similar result was reported with a gammaretrovirus bearing a GP fused with a single-chain antigen-binding site against a cell surface expressed on human carcinoma cells (Kasahara et al., 1994).

Nevertheless, different studies have exposed the limitations of this approach. The first evidence of this limitation was observed with vectors carrying the Ram-1 phosphate transporter or the epidermal growth factor (EFG) into the GP, which showed a reduced transduction efficiency although the chimeric GPs were correctly expressed, processed, and exposed in the viral envelope (Cosset et al., 1995). A similar observation was reported for a gammaretroviral vector with a GP bearing a peptide binding to the $\alpha_v\beta_3$ integrin (Wu et al., 2000a), or with the stromal cell derived factor 1-alpha (Katane et al., 2002). These low transductions efficiencies were associated with loss or impairment of viral and cell surface proteins binding and subsequent membrane fusion and penetration of the viral core into the cytosol (Frecha et al., 2008; Lavillette et al., 2001; Yu & Schaffer, 2005). In addition, these results strongly suggest that only few set of peptides sequences can be inserted into the GPs without altering the mechanism of retrovirus cell entry (Verhoeven & Cosset, 2004). The insertion of spacer sequences between the inserted peptide and the GP has proved to overcome this issue, allowing a significant improvement in the transduction efficiency of the modified vectors (Kayman et al., 1999).

2.2 Retroviral targeting by pseudotyping

Pseudotyping involves the production of viral particles bearing GP derived from other enveloped viruses (Fig. 1). This strategy was developed due to the narrow cellular transduction profile observed in vectors with native GPs (Cronin et al., 2005; Yu & Schaffer,

Fig. 1. Strategies for retroviral targeting. Retroviral vectors can be modified by ligand or antibody fraction insertion (engineered glycoproteins), use of glycoprotein (GP) from other retrovirus (pseudotyping), or the use of bi-specific antibodies (non-genetic targeting).

2005). Production of pseudotyped retroviral vectors is a well-established protocol (Bischof & Cornetta, 2010), which in most cases is used to allow virus purification, improve the cellular transduction, extend the range of transduced cells, and reduce cell toxicity (Cronin et al., 2005). However, it should be possible to employ natural tropism in GPs used for pseudotyping to target vectors bearing these proteins.

A large set of GPs has been evaluated for vector pseudotyping, among which vesicular stomatitis virus glycoprotein (VSV-G) is the most used [reviewed in (Bischof & Cornetta, 2010; Cronin et al., 2005)]. However, only a few GPs have allowed the transduction of different cell types compared to those observed VSV-G pseudotyped vectors. The use of a GP derived from rabies virus (RV) for pseudotyping an HIV-1 lentiviral vector allowed the transduction of neurons usually non-transduced with VSV-G vectors, since the RV glycoprotein induced a retrograde transport along axons (Mazarakis et al., 2001). The infusion of an animal model of familial amyotrophic lateral sclerosis (ALS) with a RV-HIV-1 vector carrying the gene of vascular endothelial growth factor (VEGF) delayed the onset of the disease and slowed progression in treated animals. Although treatment was initiated at the onset of paralysis CNS tropism of the vector was still significantly improved (Azzouz et al., 2004).

An interesting tropism was also observed for an HIV-1 vector pseudotyped with a GP from the lymphocytic choriomeningitis virus (LCMV) (Miletic et al., 2004). *In-vitro* and *in-vivo* evaluation of a vector pseudotyped with a LCMV GP showed that it transduced, almost exclusively, astrocytes; while VSV-G pseudotypes vectors infected neurons as well as astrocytes. In addition, LCMV-HIV-1 vector presented a specific transduction of infiltrating tumor cells, while VSV-G-HIV-1 vectors transduced mostly normal brain cells in infiltrating tumor areas. An HIV-1 vector pseudotyped with an Ebola Zaire virus-derived GP appeared useful in the treatment of airway diseases (e.g. cystic fibrosis). This vector allowed *in-vitro* and *in-vivo* transduction of airway epithelial cells, which was not observed with a VSV-G vector (Kobinger et al., 2001).

A recent modification of this approach used an HIV-1 vector pseudotyped with the GP and the fusion protein from a measles virus. The GP had an epidermal growth factor (EGF) or a single chain antibody against CD20 (Funke et al., 2008). The CD20-bearing vector was able to transduce primary and CD20-positive B cells both alone or within a cell mixture, while a VSV-G vector did not transduce these cell types.

2.3 Indirect retroviral targeting

This strategy is based on the finding that inclusion of certain peptides into the GP significantly impairs transduction of some cells. This prohibits gene delivering into cells that should not be transduced and extends viral tropism (Lavillette et al., 2001; Yu & Schaffer, 2005). First evidence of this approach was discovered while producing a MLV vector bearing a GP fused with epidermal growth factor (EGF). Although the chimeric GP was correctly expressed and processed, and the modified vector had the ability to bind to the EGF receptor, this manipulation completely inhibited vector transduction into cells expressing the EGF receptor (Cosset et al., 1995). This transduction inhibition was produced because of the modified vector was directed to the late endosome and destroyed by lysosomal enzymes.

A similar result was observed with a vector carrying the stem cell factor (SCF, a c-Kit receptor ligand), which selectively inhibited vector transduction on c-Kit-expressing cells (Fielding et al., 1998). When a mixture of EGF receptor-positive cancer cells and c-Kit-

positive hematopoietic cells were exposed to the above mentioned vectors, cancer cells were selectively transduced by the SCF-displaying vector, whereas hematopoietic cells were selectively transduced by the EGF-displaying vector. *In-vivo* proof-of-concept of this strategy was established after intravenous infusion of an EGF-displaying HIV-1 vector, showing preferable transduction of spleen with very low levels on EGF-receptor rich tissues (e.g. liver). Furthermore, the VSV-G vector transduced heart, skeletal muscle, lung, brain, kidney, ovaries and bone-marrow (Peng et al., 2001). Transduction inhibition of human and canine cells was also observed for a spleen necrosis virus bearing EFG, which was restored after cleavage via factor Xa at a site located between EFG and GP (Merten et al., 2003).

A variant of this strategy takes advantage of protease-activatable gene delivery vehicles. Matrix metalloproteinases (MMPs) are commonly overexpressed in angiogenesis, inflammation, and cancer invasion. The inclusion of a MMPs-cleavable peptide within an EGF-retroviral vector permitted that in the presence of exogenous MMPs, the infectivity of the MMP-EGF vector but not of the EGF-vector, could be restored. This MMPs-sensitive vector could efficiently discriminate between two different cell types, infecting only MMP-positive cells (Peng et al., 1997).

2.4 Non-genetic retroviral targeting

This strategy involves the use of antibodies or adapter molecules bound to the GP (Fig. 1) (Lavillette et al., 2001; Yu & Schaffer, 2005). This was the first approach used for cell targeting of a gammaretrovirus by using two biotinylated antibodies bridged by streptavidin: one against a GP and the other one against a specific cell membrane marker (Roux et al., 1989). This strategy allowed the specific transduction of cells expressing class I or II major histocompatibility complex (MHC) molecules by using monoclonal antibodies against these MHC molecules and against GP from the retroviral vector (Roux et al., 1989).

For targeting of a retroviral vector to folate receptor-expressing epidermoid carcinoma cells, a myeloproliferative sarcoma retrovirus was modified with folic acid (Reddy et al., 2001). Although receptor binding was observed, this vector was not able to induce gene expression. Similar results were observed for a MLV vector bearing a single chain anti-folate receptor antibody to produce vectors targeting ovarian cancer cells (Pizzato et al., 2001), manifesting the limitations of folate receptor targeting. A modification of this approach combines genetic modification of the GP by inclusion of an IgG-binding domain and the conjugation with an antibody that reacts with specific cell surface molecules expressing the antigen (Ohno et al., 1997). By changing the monoclonal antibody it was possible to transduce efficiently and specifically a variety of cell lines. The potential of this approach was confirmed by *in-vivo* evaluation of modified vectors conjugated with antibodies against surface proteins expressed in cancer cells, indicating that after intravenous infusion the vector preferentially transduced these cells (Liang et al., 2009). This strategy has been used both with gammaretroviral and lentiviral vectors. It represents a powerful tool that can be easily modified to obtain a site-specific gene expression for the treatment of cancers, genetic, infectious and immune diseases.

3. Adenoviral vectors

An adenovirus (AdV) is a non-enveloped virus formed by an icosahedral protein capsid surrounding a lineal double-stranded DNA of 36 kb (Douglas, 2007). The AdV capsid is characterized by the presence of 252 different capsomers and long fibers protruding from

each of the twelve vertices. Seven polypeptides form this complex capsid: hexon (II), penton base (III), fiber (IV), IIIa, VI, VIII, and IX (Campos & Barry, 2007; Sharma et al., 2009). The cell receptor depends on the virus subgroup: A, E and F subgroups use the cell surface coxsackievirus B and adenovirus receptor (CAR); B1 and B2 subgroups use CD46, CD80/86, receptor X, or heparan sulfate proteoglycan (HSPG); C subgroup uses CAR, HSPG, MHC-I, vascular cell adhesion molecule-I (VCAM-I), or integrins; and D subgroup uses CAR, sialic acid, or CD46 (Arnberg, 2009; Campos & Barry, 2007; Sharma et al., 2009).

Adenoviral vectors have been used as tools for gene therapy since the late 80s (Friedmann, 1992), and the first clinical trial was started in 1993 (Douglas, 2007). Since then, over 392 clinical trials using AdV vectors have been carried out. Currently AdV are the most used vectors for gene therapy and represent 24% of clinical trials total (Edelstein, 2007). In addition, the first commercially approved gene therapy product, Gendicine, is based on an AdV vector engineered to express p53 for treatment of patients with head and neck squamous cell carcinoma (Peng, 2005).

AdV vectors are mainly derived from serotypes 2 (AdV2) and 5 (AdV5). They have the advantage of inducing high levels of gene expression, are able to infect both dividing and non-dividing cells, and can be purified to high titers. Furthermore, AdV2 and AdV5 have high bloodstream stability with a reduced risk of insertional mutagenesis, since their genome remains extrachromosomal (Douglas, 2007; Edelstein, 2007; Volpers & Kochanek, 2004). Nonetheless, several drawbacks have been identified during *in-vivo* evaluation of these vectors: (i) presence of pre-existing antibodies that rapidly neutralize the vector, (ii) after intravenous administration the vector is mainly taken up by liver cells, limiting the vector to reach its target tissue in adequate concentrations, and (iii) use of high doses in an effort to overcome these problems has not proven to be an adequate and safe approach (Arnberg, 2009). Three strategies can be used to solve these issues: (i) use of vectors from other subgroups different from AdV2 and AdV5 (subgroup C), (ii) use of non-human serotypes, and (iii) retargeting of the vector to cells or tissues of interest (Arnberg, 2009). In this section we will explore different strategies for AdV retargeting that include genetic modification of the capsid, use of molecular adaptors and chemical modification of the capsid (Fig. 2) (Campos & Barry, 2007).

3.1 Genetic modification of the AdV capsid

Genetic modification of the viral capsid involves incorporation of foreign peptides into exposed regions of the capsid. In this manner the gene encoding for the peptide is inserted into the vector's genome (Fig. 2) (Campos & Barry, 2007). The possibility of altering the AdV vector capsid was first evaluated by the insertion of octapeptides into the hexon (Crompton et al., 1994) or the fiber knob (Krasnykh et al., 1998). This established the possibility of producing viable vectors with foreign proteins present on the vector capsid surface. Further experiments revealed the possibility of expanding the vector tropism by inserting a heparin-binding domain (Wickham et al., 1996), or an Arg-Gly-Asp (RGD)-containing peptide into the fiber knob (Dmitriev et al., 1998).

Although different studies indicated the feasibility to target vectors to specific cell types, genetic modification often resulted in failure to rescue viable viruses, or in an impaired virus packing, peptide exposure, and vector transduction (Leissner et al., 2001; Wu et al., 2005). Because the insertion of a pre-selected peptide into a fiber knob often fails to generate an adenovirus vector, use of random peptide libraries displayed directly on the AdV capsid allows isolation of viable vectors with high affinity for specific tissues or cells (Miura et al.,

Fig. 2. Strategies for Adenoviral vectors targeting. Adv vectors can be modified by ligand or antibody fraction insertion (genetic modification), use of bi-specific antibodies (molecular adaptors), or conjugation with polymers bearing or not a ligand (chemical modification).

2007). A similar approach involves the pre-selection of peptides by using a phage display peptide library, from which peptides with high affinity for a certain cell type are selected and then inserted into the AdV vector capsid. This has allowed the targeting of AdV vectors to epithelium cells (Nicklin et al., 2001b), tumor cells (Rittner et al., 2007), and neurons (Schmidt et al., 2007).

Most efforts to modify the AdV vector capsid have been on vector targeting to tumor cells (Bachtarzi et al., 2008). Some examples of this approach include: (i) production of an AdV vector bearing a CAR protein fused with EGF producing an enhanced gene transfer efficiency in pancreatic carcinoma cells (Wesseling et al., 2001); (ii) a vector with a polylysine motif in the fiber for a CAR-independent binding to HSPG for specific transduction of different breast cancer cells (Ranki et al., 2007); and (iii) vectors with high affinity for prostate adenocarcinoma (Wu et al., 2010), and human colon carcinoma cells (Rittner et al., 2007).

In addition to cancer cell, genetically modified AdV vectors have been used as tools for gene delivery to other cell types. The insertion of an RGD-modified AdV allowed the production of a vector that mediated cell entry via RGD binding to integrins. Administration into the synovial lining improved the outcome of gene therapy for arthritis (Bakker et al., 2001). Targeting to endothelial cells, an important target in vascular gene therapy, was possible after insertion of the SIGYPLP peptide into the fiber protein in combination with fiber mutations that block natural CAR binding (detargeting) (Nicklin et al., 2001b). For specific kidney gene delivery, the intravenous administration of HTTHREP- and HITSLLS-bearing AdV vectors resulted in selective renal targeting, specifically of tubular epithelium and glomeruli (Denby et al., 2007).

Although genetically modified AdV vectors have been widely studied, most of these modifications have been carried out only into fiber or hexo. Recently it was determined that protein IX in the capsid is a viable platform for the insertion of single-chain variable-fragment antibodies (scFv) or single-domain antibodies (sdAb) for AdV vector retargeting. Even so, only sbAb enhanced virus infection of cells expressing the targeted receptor. Thus, proving that the nature of the ligand can significantly affect vector targeting as already had been observed with fiber and hexo (Poulin et al., 2010).

3.2 Molecular adaptors for AdV targeting

Although genetic modification is the most direct form of vector targeting it has the disadvantage of not all peptides can be inserted into the vector capsid. An approach to overcome this difficulty is to use molecular adaptors consisting of a capsid-binding domain fused with a cell-binding domain (Fig. 2) (Campos & Barry, 2007). This strategy has the advantages of not requiring a correct processing of the cell-binding peptide, since it is not translated with the viral capsid proteins; and that virtually any cell-binding peptide can be fused with the capsid.

The primary adaptor molecules are bi-specific antibodies against a capsid protein and a cellular receptor. The use of a single-chain bi-specific antibody directed against human EGFR and fiber knob significantly increased gene transfer into primary glioma cells and organotypic glioma spheroids, while reducing natural tropism dramatically (Grill et al., 2001). The human epithelial cell adhesion molecule (EpCAM) is highly expressed in malignant lesions of the stomach and esophagus. By using bi-specific antibodies against adenovirus fiber knob protein and EpCAM a specific transduction of gastric and esophageal cancer cell lines was observed with reduced transduction in normal cells (Heideman et al., 2001). For other epithelial tumors, e.g. colon, lung and breast, it was possible to target vectors by using a bi-specific adapter protein, which fused the ectodomain of CAR with a single-chain anti-carcinoembryonic antigen (CEA) antibody. This adaptor molecule allowed the targeting of AdV vectors to CEA-positive epithelial tumor cells in cell culture, in subcutaneous tumor grafts, and in hepatic tumor grafts (Li et al., 2007). A similar strategy was used for vector targeting to pancreatic carcinoma cells by using a vector bearing an EGF peptide fused with CAR (Wesseling et al., 2001). The combination of this adaptor molecule with the insertion of an RGD-containing peptide into the fiber knob resulted in a significant enhancement of gene transfer.

3.3 Chemical modification of the AdV capsid

The third strategy for AdV targeting is the chemical modification of the viral capsid by using primary-amine reactive groups (Fig. 2) (Campos & Barry, 2007). The capsid modification with polyethylene glycol (PEG - PEGylation) is a promising strategy to diminish vector toxicity and immune response. Although other polymers have also been used PEGylation can be employed to modify tropism. Polymer-coated vectors can be targeted to specific cells by incorporation onto the polymer molecules that bind to specific cell-surface proteins (Campos & Barry, 2007; Volpers & Kochanek, 2004).

PEG-modified AdV vectors were conjugated with an anti-HER2/neu monoclonal antibody leading to an enhanced and specific transduction of HER2/neu over-expressed breast cancer cells. Furthermore, a significant reduction of the innate immune response against the vector was accomplished (Jung et al., 2007; Kim et al., 2011). Conjugation of PEG-AdV vectors with

an RGD peptide led to a specific $\alpha_v\beta_3$ integrin vector cell entry. This resulted in a significant improvement in transduction and specificity of gene delivery into endothelial cells. These events have implications on the treatment of rheumatoid arthritis, inflammatory bowel disease and epithelial tumors (Eto et al., 2005; Ogawara et al., 2006). Transduction of bone marrow derived human mesenchymal stem cells (MSC) can be significantly improved by using a PEGylated AdV conjugated with a blocked poly-L-lysine. However, modification on vector tropism was not clearly reported in this study (Park et al., 2010).

Although a great part of systemically administrated vector particles is scavenged by Kupffer cells in the liver, the use of a PEG- or a dextran-coated vectors allowed the specific transduction of hepatocytes independent of the presence of Kupffer cells, emphasizing the potential for therapeutic liver-directed gene transfer (Prill et al., 2010). Similar result can be observed by using a multivalent hydrophilic polymer based on poly-[N-(2-hydroxypropyl)methacrylamide] conjugated with EGF or VCAM, producing a CAR-independent binding and uptake into EGF- or VCAM-positive target cells selectively in mixed culture and also in xenografts *in-vivo* (Fisher et al., 2001).

4. Adeno-associated viral vectors

Adeno-associated virus (AAV) are non-enveloped virus belonging to the *Parvoviridae* family that need a helper virus, such as AdV or herpes simplex, for efficient infection and replication (Flotte, 2004). AAV are formed by a single-stranded DNA genome of 4.7 kb that contains two open reading frames (ORFs): (i) *rep*, which encodes for proteins Rep78, Rep68, Rep52 and Rep40, involved in virus genome replication, packing and integration; and (ii) *cap*, which encodes for the capsid proteins VP1, VP2 and VP3 (Wu et al., 2006). These ORFs are flanked by two Inverted Terminal Repeats (ITRs), involved in complementary DNA synthesis, Rep binding proteins, and site-specific genome integration in human chromosome 19 (Wu et al., 2006).

AAV capsid is formed by 60 subunits of VP1, VP2 and VP3 in a 1:1:10 ratio (Michelfelder & Trepel, 2009). These proteins share the C-terminal sequence, while the N-terminal sequences differ according to the start codon, being VP1>VP2>VP3 (Wu et al., 2006). Capsid structure has been elucidated for AAV2, AAV3, AAV4, AAV8 and AAV9 serotypes (Govindasamy et al., 2006; Lerch et al., 2010; Mitchell et al., 2009; Nam et al., 2007; Xie et al., 2002). The difference within capsid proteins allows for each serotype to use a specific receptor: (i) AAV2 and AAV3 use the HSPG, (ii) AAV1, 4 and 5 use glycans with sialic acid ends, and (iii) AAV8 uses the 37/67 kDa laminin receptor (Nam et al., 2007; Summerford & Samulski, 1998; Wu et al., 2006). AAV can infect a wide number of tissues including liver, lung, central nervous system, muscle, and heart; although as a result of the differences in receptor used, each serotype has a characteristic tropism (Flotte, 2004; Verma & Weitzman, 2005).

From the first clinical trial for cystic fibrosis patients with AAV vectors in 1995, over 75 clinical trials have been conducted. Pathologies such as α_1 -antitrypsin deficiency, Alzheimer's disease, Canavan's disease, hemophilia B, Leber congenital amaurosis, Parkinson's disease and muscular dystrophy; have attained promising results and no direct side effects have been associated with the vector (Warrington & Herzog, 2006). Although AAV vectors have shown to be promising tools for therapeutic gene delivery, they cannot transduce all cell types and could be useful to restrict its transduction to specific cell types. Strategies to modify the natural tropism of AAV vectors include: (i) the insertion of ligands into the viral capsid, (ii) use of chimeric or mosaic capsids, and (iii) conjugation with ligands

through non-genetic modifications (Fig. 3) (Choi et al., 2005; Kwon & Schaffer, 2008; Michelfelder & Trepel, 2009).

Fig. 3. Strategies for AAV vectors retargeting. AAV vectors can be modified by insertion of ligands into the capsid's proteins (ligand insertion), mixing capsid proteins from different serotypes (mosaic or chimeric capsids), or absorption of molecules onto the capsid using bi-specific antibodies or ligands fused with molecular bridges (non-genetic modification).

4.1 Targeting by ligand insertion

Mutagenesis analysis have permitted the identification of positions within the viral capsid that allow peptide insertion with little or no effect on DNA packaging and virus trafficking. For AAV2-derived vectors, peptides inserted in positions 1, 34, 138, 161, 459, 584, 587 and 588 (relative to VP1 start codon) are displayed on the vector surface, and allow production of vectors with similar viral titers to those observed for unmodified AAV2 vectors (Shi et al., 2001; Wu et al., 2000b). Most of the studies have used positions 138 (VP2 N-terminal), 587 and 588 (HSPG binding domain) to insert peptides ranging from 5 to 272 amino acids (Loiler et al., 2003; Michelfelder et al., 2009; Nicklin et al., 2001a; Perabo et al., 2006; Shi et al., 2001; White et al., 2007; White et al., 2004; Wu et al., 2000b; Yu et al., 2009). Capsid protein modifications have improved gene delivery to lung (Kwon & Schaffer, 2008), endothelial cells (Nicklin et al., 2001a), pancreatic islets (Loiler et al., 2003), vascular tissue (White et al., 2004), atherosclerotic lesions (White et al., 2007), muscle (Yu et al., 2009), myocardium (Yang et al., 2009), and cancer cells (Michelfelder et al., 2009).

Although the therapeutic effect of these vectors still remains to be seen, there is a long list of reports showing different advantages of peptide insertion within different positions of the viral capsid. Insertion after position 588 provided a complete AAV2 retargeting with inhibition

of binding to HSPG (Büning et al., 2003; Perabo et al., 2006). AAV vectors designed for vascular tissue targeting were constructed by insertion after position 587 of peptides MSLTTPPAVARP and MTPFPTSNEANL, which showed increased transduction and specificity for venous endothelial cells and reduction of hepatocytes transduction (White et al., 2004). *In-vivo* experiments with these modified AAV vectors demonstrated a reduction in liver and kidney transduction, while a significantly higher and specific targeting towards vena cava cells was observed (White et al., 2004). Recently, two new peptides (CAPGPSKSC and CNHRYMQMC) were evaluated for AAV retargeting to atherosclerotic lesions (White et al., 2007). The modified vectors showed higher levels of *in vitro* transduction than those observed for the untagged vector in human, murine, and rat endothelial cells. *In vivo* experiments showed that substantial higher levels of both peptide-modified AAV2 vectors were detected in the brachiocephalic artery (the site of advanced atherosclerotic plaques) and aorta, whereas reduced levels of modified vector were detected in all other organs examined (White et al., 2007). Serpin receptor ligand inserted after position 138 increased by 15-fold the transduction of vector on a lung epithelial cell line expressing the serpin receptor (Wu et al., 2000b). Insertion of a peptide that binds to the luteinizing hormone receptor after position 139 allowed production of a modified vector able to specifically transduce ovarian cancer cells (a luteinizing hormone receptor-bearing cell line) in an HSPG-independent manner (Shi et al., 2001). Insertion after position 138 of the 28-amino acid ApoE-derived ligand led to a 90-fold increase in pancreatic islet cells *in-vitro* transduction. Additionally, a four-fold increase of human antitrypsin expression was observed compared to unmodified AAV vector transduction (Loiler et al., 2003). Viral dose can be reduced 100-fold with similar transduction results as a consequence of ApoE-derived ligand insertion with a critical impact on side effect reduction. Functional AAV-modified vectors with long peptides up to 30 kDa have been reported, yet a laborious producing protocol is necessary to increase yield (Warrington et al., 2004).

Although these studies have demonstrated the feasibility of AAV retargeting, there is only one study evaluating the therapeutic effect of a modified AAV vector. In the study, AAV vectors were designed for brain-endothelial targeting, using peptides identified by phage display (Chen et al., 2009). After intravenous administration of modified vectors in MPS VII mice, there was extensive expression of enzyme in brain, leading to correction of lysosomal storage and reversal of established behavioral deficits (Chen et al., 2009).

During the last years we have worked in the development of a gene therapy strategy for the Mucopolysaccharidosis IV A (Morquio A disease) by using AAV vectors (Alméciga-Díaz et al., 2010; Alméciga-Díaz et al., 2009; Gutierrez et al., 2008). Morquio A disease, as well as other mucopolysaccharidosis, has a marked bone involvement, and an effective therapy should focus on the treatment in these manifestations. Recently, we designed a bone-targeting AAV vector bearing an aspartic acid octapeptide inserted immediately after the N-terminal of the VP2 capsid protein. The vector was designed to interact with hydroxyapatite (HA), a main component of bone (Tomatsu et al., 2010). We observed that the unmodified AAV vector had low bone affinity, while the bone-targeting vector had significantly higher HA affinity with up to 36.6-fold higher vector genome copies in cortical bone compared with the unmodified vector matrix (Alméciga-Díaz CJ, Montaña A, Barrera L, Tomatsu S., unpublished data).

4.2 Chimeric or mosaic capsids

A mosaic capsid AAV is a virion that is composed of a mixture of viral capsid proteins from different serotypes, which are mixed during viral assembly. On the other hand, a chimeric

capsid AAV is a vector produced by the insertion of a sequence from another wild-type AAV into the ORF of the capsid gene (Fig. 3) (Choi et al., 2005; Michelfelder & Trepel, 2009). AAV vectors produced by the mixture of capsid proteins of AAV serotypes 1 to 5 led to high-titer viral particles with mixtures of serotype 1, 2, or 3; whereas intermediate titers were observed from AAV5 mixtures (Rabinowitz et al., 2004). Transduction levels varied depending on capsid mixture, producing a synergistic effect in transduction when AAV1 capsids were combined with AAV2 or AAV3 (Rabinowitz et al., 2004). Similar results were observed for a vector produced by the mixture of AAV1 and AAV2 capsid proteins, which exhibited similar titers to those observed for native vectors. The biodistribution profile combined transduction characteristics of both parent vectors (i.e. muscle and liver transduction) (Hauck et al., 2003).

A variant of this strategy uses capsid proteins bearing foreign peptides mixed with wild-type capsids. An AAV2 vector containing wild-type and immunoglobulin-binding Z34C fragment of protein A-bearing capsid proteins; made possible the production of a vector with high virus particle and transduction titers, and the capacity to transduce selectively and efficiently MO7e (human megakaryoblastic leukemia cell line) and Jurkat cells (T lymphocyte cells line) (Gigout et al., 2005). A mosaic vector produced by the mixture of wild-type and integrin-targeted capsid proteins resulted in a vector with a 50 - 100-fold enhancement in endothelial cell gene transfer. This is higher compared to the vector produced only with integrin-targeted capsid (Stachler & Bartlett, 2006).

4.3 Adsorption of receptor ligands

This strategy involves the binding of molecules onto the viral capsid to alter natural tropism of AAV vectors (Fig. 3). Initial approaches used bi-specific antibodies consisting of an anti-AAV antibody cross-linked with another antibody that binds specifically to a cellular receptor (Choi et al., 2005). In the first approach, an anti-AAV antibody was chemically cross-linked to Fab arms of the $\alpha_1\beta_3$ integrin binding monoclonal AP-2 antibody, allowing the specific transduction of megakaryocyte cells expressing the integrin (Ponnazhagan et al., 1996).

In a second approach, a high-affinity biotin-avidin interaction was used as a molecular bridge for the adsorption of streptavidin-fused ligands (Ponnazhagan et al., 2002). This strategy did not affect virus production and allowed the vector to be specifically targeted to EGFR- or fibroblast growth factor receptor (FGFR)-positive cells after conjugation of EGF or FGF onto the biotinylated capsid. Recently the exogenous glycation of the viral capsid was presented as a potential alternative to redirect vectors from liver to skeletal and cardiac muscle after systemic administration in mice (Horowitz et al., 2011).

5. Non-viral vectors

Non-viral vectors for gene delivery offer several advantages over viral vectors, since these include lack of an immune response, simple synthesis, and low scale-up production cost (Niidome & Huang, 2002). Non-viral vectors may include lipoplexes (DNA-liposome complex), polyplexes (DNA-polymer complex), lipopolyplexes (DNA-liposome-polymer complex) and peptide-based complexes (Douglas, 2008); which can be mixed with physical methods (e.g. electroporation, ultrasound, gene gun or hydrodynamic infusion) to improve the gene delivery (Niidome & Huang, 2002). In addition, most used non-viral vectors do not integrate into the host genome reducing the risk of mutagenic events in the transfected cells

(Kim et al., 2010). However, transduction efficiencies are significantly lower than that of viral vectors (Douglas, 2008). The reader is referred to other chapters in this book edition for more thorough treatises of non-viral vectors.

Although some physical methods allow gene delivery to a specific cell type, we will only consider some strategies developed to modify vector tropism. It is important to note that although most of these studies have been evaluated exclusively *in-vitro*, they constitute proof-of-concept.

5.1 Lipoplexes

Lipoplexes are the result of a complex formation between cationic liposomes (artificial closed vesicles of lipid bilayer membranes) and genetic material. They can be considered a synthetic means of encapsulating genetic material until it reaches its cellular target (Tros et al., 2010). The main strategies for targeting of lipoplexes involve use of chelator lipids that bind metal-bearing ligands, or the modification with polymers bearing a ligand (Fig. 4).

The insertion of the chelator lipid 3 (nitrilotriacetic acid)-ditetradecylamine (NTA(3)-DTDA) in a lipoplex preparation allowed the engraftment of a His-tagged vascular epidermal growth factor (VEGF) ligand and specific transfection of cells expressing the VEGF receptor (Fig. 4) (Herringson et al., 2009). A similar approach was used to target small iRNAs to B lymphocytes by using a lipoplex bearing the NTA(3)-DTDA chelator lipid engrafted with a His-tagged CD4 or to cells expressing the receptor for tumor necrosis factor alpha (TNF α) by engrafting with a His-tagged TNF α ligand (Herringson & Altin, 2009).

Fig. 4. Modification of lipoplexes and polyplexes. Ligands can be bound to non-viral vectors through direct binding, spacer (polymer)-mediated binding, or chelator lipid-mediated binding (ligand should be fused with a metal).

To improve hepatic specific delivery of a lipoplex after systemic administration, PEG molecules were added to the complex and galactose molecules were covalently affixed at the distal end of the PEG chain to allow an active targeting to the asialoglycoprotein-receptor present on hepatocytes. This resulted in an 18-fold increase in hepatic gene expression (Morille et al., 2009). Inclusion of a PEG conjugated with a nuclear localization signal (NLS) and added to a lipoplex preparation permitted transfection of endothelial cells in the

presence of serum. This method was used as a model for transfection of blood-brain barrier cells considered difficult targets for non-modified viral and non-viral vectors (Zhang et al., 2009). A similar strategy was used for targeting of a lipoplex to tumor cells expressing the tumor-associated glycoprotein (TAG)-72 (Kim et al., 2008). In this work, a Fab' fragment from an anti-TAG-72 monoclonal antibody was conjugated with PEG and included within the lipoplex, providing specific gene delivery to TAG-expressing tumor cells both *in-vitro* and *in-vivo*, while non-targeted lipoplexes did not produce the transfection of those cells.

Ligands can be also directly conjugated with liposome molecules (Fig. 4). A hyaluronic acid-liposome conjugate resulted in an increased gene delivery to breast cancer cells with high CD44 levels. Conversely, cells with low CD44 levels were not transfected (Surace et al., 2009). Conjugation of the peptide 4-fluorobenzoyl-RR-(L-3-(2-naphthyl)alanine)-CYEK-(L-citrulline)-PYR-(L-citrulline)-CR with a liposome permitted targeting of a lipoplex to CXCR4-expressing cells, with transfection efficiency depending upon CXCR4 expression levels (Driessen et al., 2008). Conjugation of a liposome with transferrin presented a significant increment in iRNA delivery to hepatocarcinoma cells, with reduced toxicity and enhanced specific gene silencing compared to non-modified lipoplexes (Cardoso et al., 2007). Transferrin-conjugated lipoplexes were also used to evaluate specific gene delivery to a metastatic mammary carcinoma cell line, although the results did not demonstrate a significant increase in cytotoxicity with modified lipoplexes in comparison with non-modified lipoplex (Lopez-Barcons et al., 2005).

Another strategy based on *ex-vivo* gene therapy assessed the challenge of nuclear entry by using plasmids transfected with a cationic lipid vector in MSC (Hoare et al., 2010). To enhance gene expression in MSC, they used NLS peptides to direct the plasmid to the nucleus. A significant increase was observed in reporter gene delivery compared to non-modified plasmid. They also noticed the lipid had a protective impact on the plasmid-NLS complex. Differentiation potential was not affected by NLS peptides.

5.2 Polyplexes

Polyplexes are non-viral vectors formed by genetic material coupled with cationic polymers including polylysine (PLL), polyarginine, PEG, polyethylenimine (PEI), polyamidoamine (PAMAM), dendrimers, and chitosan. The mechanism involves interaction between negatively charged DNA or iRNA molecules with positively charged molecules within the polymer (Tros et al., 2010). To target polyplexes to specific cells, they can be modified by incorporation of ligands as small molecules, vitamins, carbohydrates, peptides, or proteins (Wagner et al., 2005).

PEG-derived vectors are widely used as gene therapy tools as a result of their well-known capacity to enhance gene delivery and induce a longer DNA half-life time (Midoux et al., 2008). Due to the possibility to bind ligands in the terminal ends of the polymer (Fig. 4), PEG-derived vectors are commonly used for cell specific gene delivery using non-viral vectors (Hughes & Rao, 2005). PEG polyplexes have been conjugated with a transferrin peptide to target vectors to receptor-expressing cells in two prostate cancer cell lines (Nie et al., 2011). In addition they have been conjugated with EGFR ligand or lactose for specific transfection of human hepatocarcinoma cell lines (Klutze et al., 2011; Oishi et al., 2007).

PEG has also been used as a spacer to bind ligands to polyplexes (Fig. 4), although it has been associated in some cases with reduced transfection efficiencies. A complex of DNA and the HIV-derived TAT protein (which has important translocation abilities due to its strong

cell surface adherence) was conjugated with an ICAM-1 ligand by using a PEG spacer allowing an efficient and specific transfection of ICAM-1 expressing cells (Khondee et al., 2011). Similarly, a PEI-derived polyplex was engineered for enhanced and specific delivery of VEGF iRNA in prostate cancer cells. The conjugation with prostate cancer-binding peptide via a PEG spacer, showed a significantly higher VEGF inhibition than with the unmodified PEI polyplex in human prostate carcinoma cells (Kim et al., 2009). Anionic liposomes and cationic polymers do not display an efficient targeting to liver or tumors cells. Thus, a lipopolyplex was constructed with a liposome. A PEI polymer and a PEG polymer bearing a monoclonal antibody permitted an *in-vitro* and *in-vivo* transfection of tumor cells (Hu et al., 2010). A similar strategy was used to target an iRNA to tumor cells expressing the EGFR by using this lipopolyplex bearing the EGFR ligand (Hu et al., 2011).

Direct binding of ligands in other polyplexes has also been evaluated. Conjugation of folic acid with the aminomethacrylate-phosphoryl-choline based copolymer, allowed transfection of cells overexpressing the receptor for folic acid in some human tumors cells (Lam et al., 2009). A PEI-derived polyplex conjugated with an Arg-Gly-Asp peptide, which binds to the $\alpha_v\beta_3$ integrin receptor, resulted in efficient transfection of HeLa cells with low and high densities of $\alpha_v\beta_3$ integrin (Ng et al., 2009). As observed in viral vectors and lipoplexes, antibodies can be also conjugated with polyplexes to target genetic material to specific cell types. A successful overexpression of the human epidermal growth factor receptor-2 (HER2) by transfection with a polyplex conjugated with an anti-HER2 monoclonal antibody has been reported for a breast cancer cell line (Chiu et al., 2004). Another approach in adult MSCs used a modification of a PLL polymer with a palmitic acid substitution to target bone marrow stromal cells. A transfection efficiency similar to that of adenoviral vectors was obtained, opening the possibly to efficiently transfecting these cells without the side-effects and cell toxicity observed with viral vectors (Incarni et al., 2007).

In-vivo evaluations of these modified polyplexes include use of a PEI-derived complex bearing an FGFR ligand for transfection of tumor cell lines. It demonstrated *in-vitro* and *in-vivo* specific transduction of tumor cells overexpressing the FGFR (Li & Huang, 2006), and the use of a poly[N-(2-hydroxypropyl)methacrylamide]-derived polyplex modified with an anti-P-selectin antibody. *In-vivo* specific and enhanced gene delivery to inflamed cremasteric venules was observed (Newman et al., 2009). A significant retargeting to tumor cells was also observed with a PEI polyplex carrying an antibody against a tumor-specific protein via a PEG spacer, with low expression in non-tumor cells (Duan et al., 2010).

6. Conclusions

Modification of gene therapy vectors for specific cell type gene delivery can have a significant impact on vector tropism limitations, side effects, and consolidation of gene therapy as a viable treatment. Vector modifications can be grouped in three basic strategies regardless of vector type: genetic modification (ligand insertion within the capsid), chemical modification (polymers or ligand cross-linking), or the use of adaptor molecules (bi-specific antibodies). Most studies have focused on vector design and proof-of-concept of specific gene delivery, while only few of them have evaluated the therapeutic impact of these modifications. In that sense, future studies should focus on assessing the potential of these modified vectors to improve gene therapy trials in an effort to advance from the bench science to the clinic trials. Special attention should be placed in the development of modified vectors for gene delivery to CNS and bone. Since, under normal conditions they show a

limited transfection profile with the currently available vectors. Finally, parallel research should be carried out in tissue-specific promoters to design safer integrating vectors, reduce immune response, and improve the processes of vector production.

7. Acknowledgments

We are very grateful to Drs. Shunji Tomatsu and Adriana Montaña for a long time collaboration in research in this and other areas. This work was supported in part by Pontificia Universidad Javeriana (Projects ID 3763 and 4423). Rocío Cuaspa received a young researcher fellowship from the Administrative Department of Science, Technology and Innovation from Colombia (COLCIENCIAS). The authors thank María Lucía Gutiérrez and Dr. Bernd Stab for assistance with editing of the manuscript.

8. References

- Alméciga-Díaz, C., Montano, A.M., Tomatsu, S. & Barrera, L. (2010). Adeno-associated virus gene transfer on Morquio A: effect of promoters and sulfatase-modifying Factor 1. *FEBS Journal*. Vol. 277, No. 17, (September 2010), pp. 3608-3619, ISSN 1742-464X.
- Alméciga-Díaz, C., Rueda-Paramo, M., Espejo, A., Echeverri, O., Montano, A., Tomatsu, S. & Barrera, L. (2009). Effect of Elongation Factor 1 α promoter and SUMF1 over in-vitro expression of N-acetylgalactosamine-6-sulfate sulfatase. *Molecular Biology Reports*. Vol. 36, No. 7, (September 2009), pp. 1863-1870, ISSN 0301-4851.
- Arnberg, N. (2009). Adenovirus receptors: implications for tropism, treatment and targeting. *Reviews in Medical Virology*. Vol. 19, No. 3, (May 2009), pp. 165-178, ISSN 1052-9276.
- Azzouz, M., Ralph, G.S., Storkebaum, E., Walmsley, L.E., Mitrophanous, K.A., Kingsman, S.M., Carmeliet, P. & Mazarakis, N.D. (2004). VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature*. Vol. 429, No. 6990, (May 2004), pp. 413-417, ISSN 0028-0836.
- Bachtarzi, H., Stevenson, M. & Fisher, K. (2008). Cancer gene therapy with targeted adenoviruses. *Expert Opinion in Drug Delivery*. Vol. 5, No. 11, (November 2008), pp. 1231-1240, ISSN 1742-5247.
- Bakker, A.C., Van de Loo, F.A., Joosten, L.A., Bennink, M.B., Arntz, O.J., Dmitriev, I.P., Kashentsera, E.A., Curiel, D.T. & van den Berg, W.B. (2001). A tropism-modified adenoviral vector increased the effectiveness of gene therapy for arthritis. *Gene Therapy*. Vol. 8, No. 23, (December 2001), pp. 1785-1793, ISSN 0969-7128.
- Bischof, D. & Cornetta, K. (2010). Flexibility in cell targeting by pseudotyping lentiviral vectors. *Methods in Molecular Biology*. Vol. 614, (March 2010), pp. 53-68, ISSN 1940-6029.
- Büning, H., Ried, M., Perabo, L., Gerner, F., Huttner, N., Enssle, J. & Hallek, M. (2003). Receptor targeting of adeno-associated virus vectors. *Gene Therapy*. Vol. 10, (July 2003), pp. 1142-1151, ISSN 0969-7128.
- Campos, S.K. & Barry, M.A. (2007). Current advances and future challenges in Adenoviral vector biology and targeting. *Current Gene Therapy*. Vol. 7, No. 3, (Jun 2007), pp. 189-204, ISSN 1566-5232.
- Cardoso, A.L., Simoes, S., de, A.L.P., Pelisek, J., Culmsee, C., Wagner, E. & Pedrosa, d.L.M.C. (2007). siRNA delivery by a transferrin-associated lipid-based vector: a

- non-viral strategy to mediate gene silencing. *Journal of Gene Medicine*. Vol. 9, No. 3, (March 2007), pp. 170-183, ISSN 1099-498X.
- Chen, Y.H., Chang, M. & Davidson, B.L. (2009). Molecular signatures of disease brain endothelia provide new sites for CNS-directed enzyme therapy. *Nature Medicine*. Vol. 15, No. 10, (October 2009), pp. 1215-1218, ISSN 1546-170X.
- Chiu, S.J., Ueno, N.T. & Lee, R.J. (2004). Tumor-targeted gene delivery via anti-HER2 antibody (trastuzumab, Herceptin) conjugated polyethylenimine. *Journal of Controlled Release*. Vol. 97, No. 2, (June 2004), pp. 357-369, ISSN 0168-3659.
- Choi, V., McCarty, D. & Samulski, R.J. (2005). AAV Hybrid Serotypes: Improved Vectors for Gene Delivery. *Current Gene Therapy*. Vol. 5, (June 2005), pp. 299-310, ISSN 1566-5232.
- Cosset, F.L., Morling, F.J., Takeuchi, Y., Weiss, R.A., Collins, M.K. & Russell, S.J. (1995). Retroviral retargeting by envelopes expressing an N-terminal binding domain. *Journal of Virology*. Vol. 69, No. 10, (October 1995), pp. 6314-6322, ISSN 0022-538X.
- Crompton, J., Toogood, C.I., Wallis, N. & Hay, R.T. (1994). Expression of a foreign epitope on the surface of the adenovirus hexon. *Journal of Gene Medicine*. Vol. 75 (Pt 1), (January 1994), pp. 133-139, ISSN 0022-1317.
- Cronin, J., Zhang, X.Y. & Reiser, J. (2005). Altering the tropism of lentiviral vectors through pseudotyping. *Current Gene Therapy*. Vol. 5, No. 4, (August 2005), pp. 387-398, ISSN 1566-5232.
- Denby, L., Work, L.M., Von Seggern, D.J., Wu, E., Mcvey, J.H., Nicklin, S.A. & Baker, A.H. (2007). Development of renal-targeted vectors through combined in vivo phage display and capsid engineering of adenoviral fibers from serotype 19p. *Molecular Therapy*. Vol. 15, No. 9, (September 2007), pp. 1647-1654, ISSN 1525-0016.
- Dmitriev, I., Krasnykh, V., Miller, C.R., Wang, M., Kashentseva, E., Mikheeva, G., Belousova, N. & Curiel, D.T. (1998). An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. *Journal of Virology*. Vol. 72, No. 12, (December 1998), pp. 9706-9713, ISSN 0022-538X.
- Douglas, J.T. (2007). Adenoviral vectors for gene therapy. *Molecular Biotechnology*. Vol. 36, No. 1, (May 2007), pp. 71-80, ISSN 1073-6085.
- Douglas, K.L. (2008). Toward development of artificial viruses for gene therapy: a comparative evaluation of viral and non-viral transfection. *Biotechnology Progress*. Vol. 24, No. 4, (July 2008), pp. 871-883, ISSN 1520-6033.
- Driessen, W.H., Fujii, N., Tamamura, H. & Sullivan, S.M. (2008). Development of peptide-targeted lipoplexes to CXCR4-expressing rat glioma cells and rat proliferating endothelial cells. *Molecular Therapy*. Vol. 16, No. 3, (March 2008), pp. 516-524, ISSN 1525-0024.
- Duan, Y., Yang, C., Zhang, Z., Liu, J., Zheng, J. & Kong, D. (2010). Poly(ethylene glycol)-grafted polyethylenimine modified with G250 monoclonal antibody for tumor gene therapy. *Human Gene Therapy*. Vol. 21, No. 2, (February 2010), pp. 191-198, ISSN 1557-7422.
- Edelstein, M. (June 2010). Vectors used in gene therapy clinical trials, In: *Gene Therapy Clinical Trials Worldwide*, February 2011, Available from: <http://www.wiley.com.uk/genetherapy/clinical/>.

- Escors, D. & Breckpot, K. (2010). Lentiviral Vectors in Gene Therapy: Their Current Status and Future Potential. *Archivum Immunologiae et Therapiae Experimentalis*, (Feb 2010), ISSN 1661-4917.
- Eto, Y., Gao, J.Q., Sekiguchi, F., Kurachi, S., Katayama, K., Maeda, M., Kawasaki, K., Mizuguchi, H., Hayakawa, T., Tsutsumi, Y., Mayumi, T. & Nakagawa, S. (2005). PEGylated adenovirus vectors containing RGD peptides on the tip of PEG show high transduction efficiency and antibody evasion ability. *Journal of Gene Medicine*. Vol. 7, No. 5, (May 2005), pp. 604-612, ISSN 1099-498X.
- Fielding, A.K., Maurice, M., Morling, F.J., Cosset, F.L. & Russell, S.J. (1998). Inverse targeting of retroviral vectors: selective gene transfer in a mixed population of hematopoietic and nonhematopoietic cells. *Blood*. Vol. 91, No. 5, (March 1998), pp. 1802-1809, ISSN 0006-4971.
- Fisher, K.D., Stallwood, Y., Green, N.K., Ulbrich, K., Mautner, V. & Seymour, L.W. (2001). Polymer-coated adenovirus permits efficient retargeting and evades neutralising antibodies. *Gene Therapy*. Vol. 8, No. 5, (March 2001), pp. 341-348, ISSN 0969-7128.
- Flotte, T. (2004). Gene Therapy Progress and Prospects: Recombinant adeno-associated virus (rAAV) vectors. *Gene Therapy*. Vol. 11, (May 2004), pp. 805-810, ISSN 0969-7128.
- Frecha, C., Szecsi, J., Cosset, F.L. & Verhoeyen, E. (2008). Strategies for targeting lentiviral vectors. *Current Gene Therapy*. Vol. 8, No. 6, (December 2008), pp. 449-460, ISSN 1566-5232.
- Friedmann, T. (1992). A brief history of gene therapy. *Nature Genetics*. Vol. 2, (October 1992), pp. 93-98, ISSN 1061-4036.
- Funke, S., Maisner, A., Muhlebach, M.D., Koehl, U., Grez, M., Cattaneo, R., Cichutek, K. & Buchholz, C.J. (2008). Targeted cell entry of lentiviral vectors. *Molecular Therapy*. Vol. 16, No. 8, (August 2008), pp. 1427-1436, ISSN 1525-0016.
- Gigout, L., Rebollo, P., Clement, N., Jr., K.H.W., Muzyczka, N., Linden, R.M. & Weber, T. (2005). Altering AAV Tropism with Mosaic Viral Capsids. *Molecular Therapy*. Vol. 11, No. 6, (June 2005), pp. 856-865, ISSN 1525-0016.
- Govindasamy, L., Padron, E., McKenna, R., Muzyczka, N., Kaludov, N., Chiorini, J.A. & Agbandje-McKenna, M. (2006). Structurally mapping the diverse phenotype of adeno-associated virus serotype 4. *Journal of Virology*. Vol. 80, No. 23, (December 2006), pp. 11556-11570, ISSN 0022-538X.
- Grill, J., Van Beusechem, V.W., Van Der Valk, P., Dirven, C.M., Leonhart, A., Pherai, D.S., Haisma, H.J., Pinedo, H.M., Curiel, D.T. & Gerritsen, W.R. (2001). Combined targeting of adenoviruses to integrins and epidermal growth factor receptors increases gene transfer into primary glioma cells and spheroids. *Clinical Cancer Research*. Vol. 7, No. 3, (March 2001), pp. 641-650, ISSN 1078-0432.
- Gutierrez, M., Garcia-Vallejo, F., Tomatsu, S., Ceron, F., Alméciga-Díaz, C., Domínguez, M. & Barrera, L. (2008). Construction of an Adenoassociated virus-derived vector for the treatment of Morquio A disease. *Biomedica*. Vol. 28, No. 3, (September 2008), pp. 448-459, ISSN 0120-5147.
- Hauck, B., Chen, L. & Xiao, W. (2003). Generation and characterization of chimeric recombinant AAV vectors. *Molecular Therapy*. Vol. 7, No. 3, (March 2003), pp. 419-425, ISSN 1525-0016.
- Heideman, D.A., Snijders, P.J., Craanen, M.E., Bloemena, E., Meijer, C.J., Meuwissen, S.G., van Beusechem, V.W., Pinedo, H.M., Curiel, D.T., Haisma, H.J. & Gerritsen, W.R.

- (2001). Selective gene delivery toward gastric and esophageal adenocarcinoma cells via EpCAM-targeted adenoviral vectors. *Cancer Gene Therapy*. Vol. 8, No. 5, (May 2001), pp. 342-351, ISSN 0929-1903.
- Herrington, T.P. & Altin, J.G. (2009). Convenient targeting of stealth siRNA-lipoplexes to cells with chelator lipid-anchored molecules. *Journal of Controlled Release*. Vol. 139, No. 3, (November 2009), pp. 229-238, ISSN 1873-4995.
- Herrington, T.P., Patlolla, R.R. & Altin, J.G. (2009). Targeting of plasmid DNA-lipoplexes to cells with molecules anchored via a metal chelator lipid. *The Journal of Gene Medicine*. Vol. 11, No. 11, (November 2009), pp. 1048-1063, ISSN 1521-2254.
- Hoare, M., Greiser, U., Schu, S., Mashayekhi, K., Aydogan, E., Murphy, M., Barry, F., Ritter, T. & O'Brien, T. (2010). Enhanced lipoplex-mediated gene expression in mesenchymal stem cells using reiterated nuclear localization sequence peptides. *Journal of Gene Medicine*. Vol. 12, No. 2, (February 2010), pp. 207-218, ISSN 1521-2254.
- Horowitz, E.D., Weinberg, M.S. & Asokan, A. (2011). Glycated AAV Vectors: Chemical Redirection of Viral Tissue Tropism. *Bioconjugate Chemistry*, (March 2011), ISSN 1520-4812.
- Hu, Y., Li, K., Wang, L., Yin, S., Zhang, Z. & Zhang, Y. (2010). Pegylated immuno-lipopolyplexes: A novel non-viral gene delivery system for liver cancer therapy. *Journal of Controlled Release*. Vol. 144, No. 1, (May 2010), pp. 75-81, ISSN 1873-4995.
- Hu, Y., Shen, Y., Ji, B., Wang, L., Zhang, Z. & Zhang, Y. (2011). Combinational RNAi gene therapy of hepatocellular carcinoma by targeting human EGFR and TERT. *European Journal of Pharmaceutical Sciences*. Vol. 42, No. 4, (March 2011), pp. 387-391, ISSN 1879-0720.
- Hughes, J.A. & Rao, G.A. (2005). Targeted polymers for gene delivery. *Expert opinion on drug delivery*. Vol. 2, No. 1, (January 2005), pp. 145-157, ISSN 1742-5247.
- Incani, V., Tunis, E., Clements, B.A., Olson, C., Kucharski, C., Lavasanifar, A. & Uludag, H. (2007). Palmitic acid substitution on cationic polymers for effective delivery of plasmid DNA to bone marrow stromal cells. *Journal of Biomedical Materials Research A*. Vol. 81, No. 2, (May 2007), pp. 493-504, ISSN 1549-3296.
- Jung, Y., Park, H.J., Kim, P.H., Lee, J., Hyung, W., Yang, J., Ko, H., Sohn, J.H., Kim, J.H., Huh, Y.M., Yun, C.O. & Haam, S. (2007). Retargeting of adenoviral gene delivery via Herceptin-PEG-adenovirus conjugates to breast cancer cells. *Journal of Controlled Release*. Vol. 123, No. 2, (November 2007), pp. 164-171, ISSN 1873-4995.
- Kasahara, N., Dozy, A.M. & Kan, Y.W. (1994). Tissue-specific targeting of retroviral vectors through ligand-receptor interactions. *Science*. Vol. 266, No. 5189, (November 1994), pp. 1373-1376, ISSN 0036-8075.
- Katane, M., Takao, E., Kubo, Y., Fujita, R. & Amanuma, H. (2002). Factors affecting the direct targeting of murine leukemia virus vectors containing peptide ligands in the envelope protein. *EMBO Reports*. Vol. 3, No. 9, (September 2002), pp. 899-904, ISSN 1469-221X.
- Kayman, S.C., Park, H., Saxon, M. & Pinter, A. (1999). The hypervariable domain of the murine leukemia virus surface protein tolerates large insertions and deletions, enabling development of a retroviral particle display system. *Journal of Virology*. Vol. 73, No. 3, (March 1999), pp. 1802-1808, ISSN 0022-538X.

- Khondee, S., Baoum, A., Siahaan, T.J. & Berkland, C.J. (2011). Calcium-crosslinked LABL-TAT complexes effectively target gene delivery to ICAM-1 expressing cells. *Molecular Pharmaceutics*. Vol. In Press, (April 2011), ISSN 1543-8392.
- Kim, K.S., Lee, Y.K., Kim, J.S., Koo, K.H., Hong, H.J. & Park, Y.S. (2008). Targeted gene therapy of LS174 T human colon carcinoma by anti-TAG-72 immunoliposomes. *Cancer Gene Therapy*. Vol. 15, No. 5, (May 2008), pp. 331-340, ISSN 1476-5500.
- Kim, N.Y., Choi, Y.B., Kang, C.I., Kim, H.H., Yang, J.M. & Shin, S. (2010). An hydrophobically modified arginine peptide vector system effectively delivers DNA into human mesenchymal stem cells and maintains transgene expression with differentiation. *Journal of Gene Medicine*. Vol. 12, No. 9, (September 2010), pp. 779-789, ISSN 1521-2254.
- Kim, P.H., Sohn, J.H., Choi, J.W., Jung, Y., Kim, S.W., Haam, S. & Yun, C.O. (2011). Active targeting and safety profile of PEG-modified adenovirus conjugated with herceptin. *Biomaterials*. Vol. 32, No. 9, (March 2011), pp. 2314-2326, ISSN 1878-5905
- Kim, S.H., Lee, S.H., Tian, H., Chen, X. & Park, T.G. (2009). Prostate cancer cell-specific VEGF siRNA delivery system using cell targeting peptide conjugated polyplexes. *Journal of Drug Targeting*. Vol. 17, No. 4, (May 2009), pp. 311-317, ISSN 1029-2330.
- Klutzn, K., Schaffert, D., Willhauck, M.J., Grunwald, G.K., Haase, R., Wunderlich, N., Zach, C., Gildehaus, F.J., Senekowitsch-Schmidtke, R., Goke, B., Wagner, E., Ogris, M. & Spitzweg, C. (2011). Epidermal Growth Factor Receptor-targeted (131)I-therapy of Liver Cancer Following Systemic Delivery of the Sodium Iodide Symporter Gene. *Molecular Therapy*. Vol. 19, No. 4, (April 2011), pp. 676-685, ISSN 1525-0024.
- Kobinger, G.P., Weiner, D.J., Yu, Q.C. & Wilson, J.M. (2001). Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia in vivo. *Nature Biotechnology*. Vol. 19, No. 3, (March 2001), pp. 225-230, ISSN 1087-0156.
- Krasnykh, V., Dmitriev, I., Mikheeva, G., Miller, C.R., Belousova, N. & Curiel, D.T. (1998). Characterization of an adenovirus vector containing a heterologous peptide epitope in the HI loop of the fiber knob. *Journal of Virology*. Vol. 72, No. 3, (March 1998), pp. 1844-1852, ISSN 0022-538X.
- Kwon, I. & Schaffer, D.V. (2008). Designer gene delivery vectors: molecular engineering and evolution of adeno-associated viral vectors for enhanced gene transfer. *Pharmaceutical Research*. Vol. 25, No. 3, (March 2008), pp. 489-499, ISSN 0724-8741.
- Lam, J.K., Armes, S.P., Lewis, A.L. & Stolnik, S. (2009). Folate conjugated phosphorylcholine-based polycations for specific targeting in nucleic acids delivery. *Journal of Drug Targeting*. Vol. 17, No. 7, (August 2009), pp. 512-523, ISSN 1029-2330.
- Lavillette, D., Russell, S.J. & Cosset, F.L. (2001). Retargeting gene delivery using surface-engineered retroviral vector particles. *Current Opinion in Biotechnology*. Vol. 12, No. 5, (October 2001), pp. 461-466, ISSN 0958-1669.
- Leissner, P., Legrand, V., Schlesinger, Y., Hadji, D.A., van Raaij, M., Cusack, S., Pavirani, A. & Mehtali, M. (2001). Influence of adenoviral fiber mutations on viral encapsidation, infectivity and in vivo tropism. *Gene Therapy*. Vol. 8, No. 1, (January 2001), pp. 49-57, ISSN 0969-7128.
- Lerch, T.F., Xie, Q. & Chapman, M.S. (2010). The structure of adeno-associated virus serotype 3B (AAV-3B): Insights into receptor binding and immune evasion. *Virology*. Vol. 03, No. 1, (July 2010), pp. 26-36, ISSN 1096-0341.

- Li, H.J., Everts, M., Pereboeva, L., Komarova, S., Idan, A., Curiel, D.T. & Herschman, H.R. (2007). Adenovirus tumor targeting and hepatic untargeting by a coxsackie/adenovirus receptor ectodomain anti-carcinoembryonic antigen bispecific adapter. *Cancer Research*. Vol. 67, No. 11, (Jun 2007), pp. 5354-5361, ISSN 0008-5472.
- Li, S.D. & Huang, L. (2006). Gene therapy progress and prospects: non-viral gene therapy by systemic delivery. *Gene Therapy*. Vol. 13, No. 18, (September 2006), pp. 1313-1319, ISSN 0969-7128.
- Liang, M., Morizono, K., Pariente, N., Kamata, M., Lee, B. & Chen, I.S. (2009). Targeted transduction via CD4 by a lentiviral vector uses a clathrin-mediated entry pathway. *Journal of Virology*. Vol. 83, No. 24, (December 2009), pp. 13026-13031, ISSN 1098-5514.
- Loiler, S., Conlon, T., Song, S., Tang, Q., Jr, K.W., Agarwal, A., Kapturczak, M., Li, C., Ricordi, C., Atkinson, M., Muzyczka, N. & Flotte, T. (2003). Targeting recombinant adeno-associated virus vectors to enhance gene transfer to pancreatic islets and liver. *Gene Therapy*. Vol. 10, (September 2003), pp. 1551-1558, ISSN 0969-7128.
- Lopez-Barcons, L.A., Polo, D., Llorens, A., Reig, F. & Fabra, A. (2005). Targeted adriamycin delivery to MXT-B2 metastatic mammary carcinoma cells by transferrin liposomes: effect of adriamycin ADR-to-lipid ratio. *Oncology Reports*. Vol. 14, No. 5, (November 2005), pp. 1337-1343, ISSN 1021-335X.
- Mazarakis, N.D., Azzouz, M., Rohll, J.B., Ellard, F.M., Wilkes, F.J., Olsen, A.L., Carter, E.E., Barber, R.D., Baban, D.F., Kingsman, S.M., Kingsman, A.J., O'Malley, K. & Mitrophanous, K.A. (2001). Rabies virus glycoprotein pseudotyping of lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery. *Human Molecular Genetics*. Vol. 10, No. 19, (September 2001), pp. 2109-2121, ISSN 0964-6906.
- Merten, C.A., Engelstaedter, M., Buchholz, C.J. & Cichutek, K. (2003). Displaying epidermal growth factor on spleen necrosis virus-derived targeting vectors. *Virology*. Vol. 305, No. 1, (January 2003), pp. 106-114, ISSN 0042-6822.
- Michelfelder, S., Kohlschutter, J., Skorupa, A., Pfennings, S., Muller, O., Kleinschmidt, J.A. & Trepel, M. (2009). Successful expansion but not complete restriction of tropism of adeno-associated virus by in vivo biopanning of random virus display peptide libraries. *PLoS One*. Vol. 4, No. 4, (April 2009), pp. e5122, ISSN 1932-6203.
- Michelfelder, S. & Trepel, M. (2009). Adeno-associated viral vectors and their redirection to cell-type specific receptors. *Advances in Genetics*. Vol. 67, No. Pt A, (November 2009), pp. 29-60, ISSN 0065-2660.
- Midoux, P., Breuzard, G., Gomez, J.P. & Pichon, C. (2008). Polymer-based gene delivery: a current review on the uptake and intracellular trafficking of polyplexes. *Current Gene Therapy*. Vol. 8, No. 5, (Oct), pp. 335-352, ISSN 1566-5232.
- Miletic, H., Fischer, Y.H., Neumann, H., Hans, V., Stenzel, W., Giroglou, T., Hermann, M., Deckert, M. & Von Laer, D. (2004). Selective transduction of malignant glioma by lentiviral vectors pseudotyped with lymphocytic choriomeningitis virus glycoproteins. *Human Gene Therapy*. Vol. 15, No. 11, (November 2004), pp. 1091-1100, ISSN 1043-0342.
- Mitchell, M., Nam, H.J., Carter, A., McCall, A., Rence, C., Bennett, A., Gurda, B., McKenna, R., Porter, M., Sakai, Y., Byrne, B.J., Muzyczka, N., Aslanidi, G., Zolotukhin, S. &

- Agbandje-McKenna, M. (2009). Production, purification and preliminary X-ray crystallographic studies of adeno-associated virus serotype 9. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications*. Vol. 65, No. Pt 7, (July 2009), pp. 715-718, ISSN 1744-3091.
- Miura, Y., Yoshida, K., Nishimoto, T., Hatanaka, K., Ohnami, S., Asaka, M., Douglas, J.T., Curiel, D.T., Yoshida, T. & Aoki, K. (2007). Direct selection of targeted adenovirus vectors by random peptide display on the fiber knob. *Gene Therapy*. Vol. 14, No. 20, (October 2007), pp. 1448-1460, ISSN 0969-7128.
- Morille, M., Passirani, C., Letrou-Bonneval, E., Benoit, J.P. & Pitard, B. (2009). Galactosylated DNA lipid nanocapsules for efficient hepatocyte targeting. *International Journal of Pharmaceutics*. Vol. 379, No. 2, (September 2009), pp. 293-300, ISSN 1873-3476.
- Nam, H.-J., Douglas, M., Padron, E., Gurda, B., McKenna, R., Kohlbrenner, E., Aslanidi, G., Byrne, B., Muzyczka, N., Zolotukhin, S. & Agbandje-McKenna, M. (2007). Structure of Adeno-Associated virus serotype 8, a gene therapy vector. *Journal of Virology*. Vol. 81, No. 22, (August 2007), pp. 12260-12271, ISSN 0022-538X.
- Newman, C.M., Crosdale, D.J., Fisher, K.D., Briggs, S.S., Norman, K.E., Seymour, L.W. & Hellewell, P.G. (2009). P-selectin dependent targeting to inflamed endothelium of recombinant P-selectin glycoprotein ligand-1 immunoglobulin chimera-coated poly[N-(2-hydroxypropyl) methacrylamide]-DNA polyplexes in vivo visualised by intravital microscopy. *Journal of Gene Medicine*. Vol. 11, No. 4, (April 2009), pp. 326-334, ISSN 1521-2254.
- Ng, Q.K., Sutton, M.K., Soonsawad, P., Xing, L., Cheng, H. & Segura, T. (2009). Engineering clustered ligand binding into nonviral vectors: alphavbeta3 targeting as an example. *Molecular Therapy*. Vol. 17, No. 5, (May 2009), pp. 828-836, ISSN 1525-0024.
- Nicklin, S.A., Buening, H., Dishart, K.L., Alwis, M.d., Girod, A., Hacker, U., Thrasher, A.J., Ali, R.R., Hallek, M. & Baker, A.H. (2001a). Efficient and Selective AAV2-Mediated Gene Transfer Directed to Human Vascular Endothelial Cells. *Molecular Therapy*. Vol. 4, (September 2001), pp. 174-181, ISSN 1525-0016.
- Nicklin, S.A., Von Seggern, D.J., Work, L.M., Pek, D.C., Dominiczak, A.F., Nemerow, G.R. & Baker, A.H. (2001b). Ablating adenovirus type 5 fiber-CAR binding and HI loop insertion of the SIGYPLP peptide generate an endothelial cell-selective adenovirus. *Molecular Therapy*. Vol. 4, No. 6, (December 2001), pp. 534-542, ISSN 1525-0016.
- Nie, Y., Schaffert, D., Rodl, W., Ogris, M., Wagner, E. & Gunther, M. (2011). Dual-targeted polyplexes: One step towards a synthetic virus for cancer gene therapy. *Journal of Controlled Release*. Vol. In press, (March 2011), ISSN 1873-4995.
- Niidome, T. & Huang, L. (2002). Gene therapy progress and prospects: nonviral vectors. *Gene Therapy*. Vol. 9, No. 24, (December 2002), pp. 1647-1652, ISSN 0969-7128.
- Ogawara, K., Kuldo, J.M., Oosterhuis, K., Kroesen, B.J., Rots, M.G., Trautwein, C., Kimura, T., Haisma, H.J. & Molema, G. (2006). Functional inhibition of NF-kappaB signal transduction in alphavbeta3 integrin expressing endothelial cells by using RGD-PEG-modified adenovirus with a mutant IkappaB gene. *Arthritis Research & Therapy*. Vol. 8, No. 1, (January 2006), pp. R32, ISSN 1478-6362.
- Ohno, K., Sawai, K., Iijima, Y., Levin, B. & Meruelo, D. (1997). Cell-specific targeting of Sindbis virus vectors displaying IgG-binding domains of protein A. *Nature Biotechnology*. Vol. 15, No. 8, (August 1997), pp. 763-767, ISSN 1087-0156.

- Oishi, M., Nagasaki, Y., Nishiyama, N., Itaka, K., Takagi, M., Shimamoto, A., Furuichi, Y. & Kataoka, K. (2007). Enhanced growth inhibition of hepatic multicellular tumor spheroids by lactosylated poly(ethylene glycol)-siRNA conjugate formulated in PEGylated polyplexes. *ChemMedChem*. Vol. 2, No. 9, (September 2007), pp. 1290-1297, ISSN 1860-7187.
- Park, J.W., Mok, H. & Park, T.G. (2010). Physical adsorption of PEG grafted and blocked poly-L-lysine copolymers on adenovirus surface for enhanced gene transduction. *Journal of Controlled Release*. Vol. 142, No. 2, (March 2010), pp. 238-244, ISSN 1873-4995.
- Peng, K.W., Morling, F.J., Cosset, F.L., Murphy, G. & Russell, S.J. (1997). A gene delivery system activatable by disease-associated matrix metalloproteinases. *Human Gene Therapy*. Vol. 8, No. 6, (April 1997), pp. 729-738, ISSN 1043-0342.
- Peng, K.W., Pham, L., Ye, H., Zufferey, R., Trono, D., Cosset, F.L. & Russell, S.J. (2001). Organ distribution of gene expression after intravenous infusion of targeted and untargeted lentiviral vectors. *Gene Therapy*. Vol. 8, No. 19, (October 2001), pp. 1456-1463, ISSN 0969-7128.
- Peng, Z. (2005). Current status of gendicine in China: recombinant human Ad-p53 agent for treatment of cancers. *Human Gene Therapy*. Vol. 16, No. 9, (September 2005), pp. 1016-1027, ISSN 1043-0342.
- Perabo, L., Goldnau, D., White, K., Endell, J., Boucas, J., Humme, S., Work, L.M., Janicki, H., Hallek, M., Baker, A.H. & Büning, H. (2006). Heparan Sulfate Proteoglycan Binding Properties of Adeno-Associated Virus Retargeting Mutants and Consequences for Their In Vivo Tropism. *Journal of Virology*. Vol. 80, No. 14, (July 2006), pp. 7265-7269, ISSN 0022-538X.
- Pizzato, M., Blair, E.D., Fling, M., Kopf, J., Tomassetti, A., Weiss, R.A. & Takeuchi, Y. (2001). Evidence for nonspecific adsorption of targeted retrovirus vector particles to cells. *Gene Therapy*. Vol. 8, No. 14, (July 2001), pp. 1088-1096, ISSN 0969-7128.
- Ponnazhagan, S., Mahendra, G., Kumar, S., Thompson, J.A. & Castillas, M. (2002). Conjugate-based targeting of recombinant adeno-associated virus type 2 vectors by using avidin-linked ligands. *Journal of Virology*. Vol. 76, No. 24, (December 2002), pp. 12900-12907, ISSN 0022-538X.
- Ponnazhagan, S., Wang, X.S., Woody, M.J., Luo, F., Kang, L.Y., Nallari, M.L., Munshi, N.C., Zhou, S.Z. & Srivastava, A. (1996). Differential expression in human cells from the p6 promoter of human parvovirus B19 following plasmid transfection and recombinant adeno-associated virus 2 (AAV) infection: human megakaryocytic leukaemia cells are non-permissive for AAV infection. *The Journal of general virology*. Vol. 77 (Pt 6), (Jun 1996), pp. 1111-1122, ISSN 0022-1317.
- Poulin, K.L., Lanthier, R.M., Smith, A.C., Christou, C., Risco Quiroz, M., Powell, K.L., O'Meara, R.W., Kothary, R., Lorimer, I.A. & Parks, R.J. (2010). Retargeting of adenovirus vectors through genetic fusion of a single-chain or single-domain antibody to capsid protein IX. *Journal of Virology*. Vol. 84, No. 19, (October 2010), pp. 10074-10086, ISSN 1098-5514.
- Prill, J.M., Espenlaub, S., Samen, U., Engler, T., Schmidt, E., Vetrini, F., Rosewell, A., Grove, N., Palmer, D., Ng, P., Kochanek, S. & Kreppel, F. (2010). Modifications of Adenovirus Hexon Allow for Either Hepatocyte Detargeting or Targeting With

- Potential Evasion From Kupffer Cells. *Molecular Therapy*, (October 2010), ISSN 1525-0024.
- Rabinowitz, J.E., Bowles, D.E., Faust, S.M., Ledford, J.G., Cunningham, S.E. & Samulski, R.J. (2004). Cross-dressing the virion: the transcapsidation of adeno-associated virus serotypes functionally defines subgroups. *Journal of Virology*. Vol. 78, No. 9, (May 2004), pp. 4421-4432, ISSN 0022-538X.
- Ranki, T., Kanerva, A., Ristimäki, A., Hakkarainen, T., Sarkioja, M., Kangasniemi, L., Raki, M., Laakkonen, P., Goodison, S. & Hemminki, A. (2007). A heparan sulfate-targeted conditionally replicative adenovirus, Ad5.pk7-Delta24, for the treatment of advanced breast cancer. *Gene Therapy*. Vol. 14, No. 1, (January 2006), pp. 58-67, ISSN 0969-7128.
- Reddy, J.A., Clapp, D.W. & Low, P.S. (2001). Retargeting of viral vectors to the folate receptor endocytic pathway. *Journal of Controlled Release*. Vol. 74, No. 1-3, (July 2001), pp. 77-82, ISSN 0168-3659.
- Rittner, K., Schreiber, V., Erbs, P. & Lusky, M. (2007). Targeting of adenovirus vectors carrying a tumor cell-specific peptide: in vitro and in vivo studies. *Cancer Gene Therapy*. Vol. 14, No. 5, (May 2007), pp. 509-518, ISSN 0929-1903.
- Roux, P., Jeanteur, P. & Piechaczyk, M. (1989). A versatile and potentially general approach to the targeting of specific cell types by retroviruses: application to the infection of human cells by means of major histocompatibility complex class I and class II antigens by mouse ecotropic murine leukemia virus-derived viruses. *Proceedings of the National Academy of Sciences of the United States of America*. Vol. 86, No. 23, (December 1989), pp. 9079-9083, ISSN 0027-8424.
- Russell, S.J., Hawkins, R.E. & Winter, G. (1993). Retroviral vectors displaying functional antibody fragments. *Nucleic Acids Research*. Vol. 21, No. 5, (March 1993), pp. 1081-1085, ISSN 0305-1048.
- Schmidt, A., Haas, S.J., Hildebrandt, S., Scheibe, J., Eckhoff, B., Racek, T., Kempermann, G., Wree, A. & Putzer, B.M. (2007). Selective targeting of adenoviral vectors to neural precursor cells in the hippocampus of adult mice: new prospects for in situ gene therapy. *Stem Cells*. Vol. 25, No. 11, (November 2007), pp. 2910-2918, ISSN 1549-4918.
- Sharma, A., Li, X.X., Bangari, D.S. & Mittal, S.K. (2009). Adenovirus receptors and their implications in gene delivery. *Virus Research*. Vol. 143, No. 2, (August 2009), pp. 184-194, ISSN 0168-1702.
- Shi, W., Arnold, G. & Bartlett, J. (2001). Insertional Mutagenesis of the Adeno-Associated Virus Type 2 (AAV2) Capsid Gene and Generation of AAV2 Vectors Targeted to Alternative Cell-Surface Receptors. *Human Gene Therapy*. Vol. 12, (September 2001), pp. 1697-1711, ISSN 1043-0342.
- Stachler, M.D. & Bartlett, J.S. (2006). Mosaic vectors comprised of modified AAV1 capsid proteins for efficient vector purification and targeting to vascular endothelial cells. *Gene Therapy*. Vol. 13, No. 11, (June 2006), pp. 926-931, ISSN 0969-7128.
- Summerford, C. & Samulski, R.J. (1998). Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. *Journal of Virology*. Vol. 72, No. 2, (February 1998), pp. 1438-1445, ISSN 0022-538X.
- Surace, C., Arpicco, S., Dufay-Wojcicki, A., Marsaud, V., Bouclier, C., Clay, D., Cattel, L., Renoir, J.M. & Fattal, E. (2009). Lipoplexes targeting the CD44 hyaluronic acid

- receptor for efficient transfection of breast cancer cells. *Molecular Pharmaceutics*. Vol. 6, No. 4, (July 2009), pp. 1062-1073, ISSN 1543-8384.
- Tomatsu, S., Montano, A.M., Alméciga-Díaz, C. & Barrera, L. (2010). Delivery of therapeutic agents to the bone. US20100008979. USA
- Tros, d.I.C., Sun, Y. & Duzgunes, N. (2010). Gene delivery by lipoplexes and polyplexes. *European Journal of Pharmaceutical Sciences*. Vol. 40, No. 3, (June 2010), pp. 159-170, ISSN 1879-0720.
- Verhoeven, E. & Cosset, F.L. (2004). Surface-engineering of lentiviral vectors. *Journal of Gene Medicine*. Vol. 6 (Suppl 1), (February 2004), pp. S83-94, ISSN 1099-498X.
- Verma, I.M. & Weitzman, M.D. (2005). Gene Therapy: Twenty-First Century Medicine. *Annual Reviews in Biochemistry*. Vol. 74, (July 2005), pp. 711-738, ISSN 1545-4509.
- Volpers, C. & Kochanek, S. (2004). Adenoviral vectors for gene transfer and therapy. *Journal of Gene Medicine*. Vol. 6 (Suppl 1), (February 2004), pp. S164-171, ISSN 1099-498X.
- Wagner, E., Culmsee, C. & Boeckle, S. (2005). Targeting of polyplexes: toward synthetic virus vector systems. *Advances in Genetics*. Vol. 53, pp. 333-354, ISSN 0065-2660.
- Warrington, K. & Herzog, R. (2006). Treatment of human disease by adeno-associated viral gene transfer. *Human Genetics*. Vol. 119, (April 2006), pp. 571-603, ISSN 0340-6717.
- Warrington, K.H., Gorbatyuk, O.S., Harrison, J.K., Opie, S.R., Zolotukhin, S. & Muzyczka, N. (2004). Adeno-Associated Virus Type 2 VP2 Capsid Protein Is Nonessential and Can Tolerate Large Peptide Insertions at Its N Terminus. *Journal of Virology*. Vol. 78, No. 12, (January 2004), pp. 6595-6609, ISSN 0022-538X.
- Wesseling, J.G., Bosma, P.J., Krasnykh, V., Kashentseva, E.A., Blackwell, J.L., Reynolds, P.N., Li, H., Parameshwar, M., Vickers, S.M., Jaffee, E.M., Huibregtse, K., Curiel, D.T. & Dmitriev, I. (2001). Improved gene transfer efficiency to primary and established human pancreatic carcinoma target cells via epidermal growth factor receptor and integrin-targeted adenoviral vectors. *Gene Therapy*. Vol. 8, No. 13, (July 2001), pp. 969-976, ISSN 0969-7128.
- White, K., Büning, H., Kritz, A., Janicki, H., McVey, J., Perabo, L., Murphy, G., Odenthal, M., Work, L., Hallek, M., Nicklin, S. & Baker, A. (2007). Engineering adeno-associated virus 2 vectors for targeted gene delivery to atherosclerotic lesions. *Gene Therapy*. Vol. 15, No. 6, (April 2007), pp. 443-451, ISSN 0969-7128.
- White, S.J., Nicklin, S.A., Büning, H., Brosnan, M.J., Leike, K., Papadakis, E.D., Hallek, M. & Baker, A.H. (2004). Targeted Gene Delivery to Vascular Tissue In Vivo by Tropism-Modified Adeno-Associated Virus Vectors. *Circulation*. Vol. 109, (January 2004), pp. 513-519, ISSN 0009-7322.
- Wickham, T.J., Roelvink, P.W., Brough, D.E. & Kovesdi, I. (1996). Adenovirus targeted to heparan-containing receptors increases its gene delivery efficiency to multiple cell types. *Nature Biotechnology*. Vol. 14, No. 11, (November 1996), pp. 1570-1573, ISSN 1087-0156.
- Wu, B.W., Lu, J., Gallaher, T.K., Anderson, W.F. & Cannon, P.M. (2000a). Identification of regions in the Moloney murine leukemia virus SU protein that tolerate the insertion of an integrin-binding peptide. *Virology*. Vol. 269, No. 1, (March 2000), pp. 7-17, ISSN 0042-6822.
- Wu, H., Han, T., Belousova, N., Krasnykh, V., Kashentseva, E., Dmitriev, I., Kataram, M., Mahasreshti, P.J. & Curiel, D.T. (2005). Identification of sites in adenovirus hexon

- for foreign peptide incorporation. *Journal of Virology*. Vol. 79, No. 6, (March 2005), pp. 3382-3390, ISSN 0022-538X.
- Wu, P., Kudrolli, T.A., Chowdhury, W.H., Liu, M.M., Rodriguez, R. & Lupold, S.E. (2010). Adenovirus Targeting to Prostate-specific Membrane Antigen through Virus-displayed Semi-random Peptide Library Screening. *Cancer Research*, (July 2010), ISSN 1538-7445.
- Wu, P., Xiao, W., Conlon, T., Hughes, J., Agbandje-McKenna, M., Ferkol, T., Flotte, T. & Muzyczka, N. (2000b). Mutational Analysis of the Adeno-Associated Virus Type 2 (AAV2) Capsid Gene and Construction of AAV2 Vectors with Altered Tropism. *Journal of Virology*. Vol. 74, No. 18, (September 2000), pp. 8635-8647, ISSN 0022-538X.
- Wu, Z., Asokan, A. & Samulski, R. (2006). Adeno-associated virus serotypes: vector toolkit for human gene therapy. *Molecular Therapy*. Vol. 14, No. 3, (September 2006), pp. 316-327, ISSN 1525-0016.
- Xie, Q., Bu, W., Bhatia, S., Hare, J., Somasundaram, T., Azzi, A. & Chapman, M.S. (2002). The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. *Proceedings of the National Academy of Sciences of the United States of America*. Vol. 99, No. 16, (August 2002), pp. 10405-10410, ISSN 0027-8424.
- Yang, L., Jiang, J., Drouin, L.M., Agbandje-McKenna, M., Chen, C., Qiao, C., Pu, D., Hu, X., Wang, D.Z., Li, J. & Xiao, X. (2009). A myocardium tropic adeno-associated virus (AAV) evolved by DNA shuffling and in vivo selection. *Proceedings of the National Academy of Sciences of the United States of America*. Vol. 106, No. 10, (March 2009), pp. 3946-3951, ISSN 1091-6490.
- Young, J.A., Bates, P., Willert, K. & Varmus, H.E. (1990). Efficient incorporation of human CD4 protein into avian leukosis virus particles. *Science*. Vol. 250, No. 4986, (December 1990), pp. 1421-1423, ISSN 0036-8075.
- Yu, C.Y., Yuan, Z., Cao, Z., Wang, B., Qiao, C., Li, J. & Xiao, X. (2009). A muscle-targeting peptide displayed on AAV2 improves muscle tropism on systemic delivery. *Gene Therapy*. Vol. 16, No. 8, (August 2009), pp. 953-962, ISSN 1476-5462.
- Yu, J.H. & Schaffer, D.V. (2005). Advanced targeting strategies for murine retroviral and adeno-associated viral vectors, In: *Advances in Biochemical Engineering/Biotechnology*, Scheper, T. (ed). 147-167, Springer, ISBN 103-540-28404-4, Berlin.
- Williams, D. (2007). RAC Reviews Serious Adverse Event Associated with AAV Therapy Trial. *Molecular Therapy*, Vol. 15, No. 12, (December 2007), pp. 2053-2054, ISSN 1525-0016.
- Zhang, H., Mitin, A. & Vinogradov, S.V. (2009). Efficient transfection of blood-brain barrier endothelial cells by lipoplexes and polyplexes in the presence of nuclear targeting NLS-PEG-acridine conjugates. *Bioconjugate Chemistry*. Vol. 20, No. 1, (January 2009), pp. 120-128, ISSN 1520-4812.

Non-Viral Gene Therapy

Edited by Prof. Xubo Yuan

ISBN 978-953-307-538-9

Hard cover, 696 pages

Publisher InTech

Published online 07, November, 2011

Published in print edition November, 2011

This book focuses on recent advancement of gene delivery systems research. With the multidisciplinary contribution in gene delivery, the book covers several aspects in the gene therapy development: various gene delivery systems, methods to enhance delivery, materials with modification and multifunction for the tumor or tissue targeting. This book will help molecular biologists gain a basic knowledge of gene delivery vehicles, while drug delivery scientist will better understand DNA, molecular biology, and DNA manipulation.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Carlos J. Alméciga-Díaz, Rocio Cuaspa and Luis A. Barrera (2011). Gene Delivery Systems: Tailoring Vectors to Reach Specific Tissues, Non-Viral Gene Therapy, Prof. Xubo Yuan (Ed.), ISBN: 978-953-307-538-9, InTech, Available from: <http://www.intechopen.com/books/non-viral-gene-therapy/gene-delivery-systems-tailoring-vectors-to-reach-specific-tissues>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen