Genetic manipulation of endothelial cells by viral vectors

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Summary
The need for uncovering molecular mechanisms in endothelial cell biology has tremendously increased in the last decades as it became more and more clear that the endothelium is an important target in nearly all diseases and treatments (drug delivery) and plays a central role in regeneration processes. One of the critical methods generally applied in cell biology research to uncover structural and functional aspects is the modulation of protein expression by over-expression, expression of mutant variants or gene silencing. This strategy, however, requires genetic manipulation of the respective cells. The classical gene transfer by chemical transfection techniques works pretty well in a large variety of cultured cells but fails for most endothelial cell types. Insufficient transfection rates and gene expression levels as well as the sensitivity of the endothelium against chemical transfection reagents limits utilisation of this technique for endothelial cell biology research. This holds true not only for primary endothelial cell cultures and endothelial cells in vivo but also for endothelial cell lines, e.g. endothelioma cells. The development of viral vectors originally designed for gene therapy approaches has significantly improved the methodological spectrum in endothelial cell research. Two viral vector systems, based on retroviruses and adenoviruses, deliver transgenic information highly efficient into both cultured endothelial cells and in endothelial cells in vivo, respectively. This review aims to give a comprehensive overview of these two vector systems that appear to be reliable and efficient tools for gene delivery into endothelial cell types.

Keywords
Endothelial cells, gene transfer, gene therapy, viral infection

Introduction
The vascular endothelium, covering the inner surface of the vasculature and the heart, is involved in a huge variety of diseases such as infections, arteriosclerosis, metabolic disorders, local and systemic inflammation, tumor growth, autoimmune diseases and graft versus host reaction. The endothelium functions as an interface between the interstitial tissue and the flowing blood and controls blood pressure and organ perfusion, coagulation/anticoagulation balance and angiogenesis; see “Endothelial Biomedicine” by Aird and references therein (1). Thus, it is a central player in the development of diseases as well as in tissue regeneration during healing processes. The central functions of the endothelium make it an attractive and important target for genetic manipulation related to both gene therapy and basic- or preclinical research. In cell biology classical gene transfection protocols or electroporation techniques are efficient tools for many cell lines (e.g. CHO cells-, MDCK or 293 fibroblasts). In contrast, research of endothelial cell biology suffered in the last decade from insufficient gene transfer by chemical treatment and in addition frequently caused cell impairment. Classical gene delivery techniques into endothelial cells have been successfully used in studies that did not require high numbers of gene-manipulated cells. However, for many techniques, such as immunoprecipitations, Western blot analyses, quantitative rtPCR or functional tests such as cell migration or barrier function measurements, high numbers of genetically modified cells are required to obtain reproducible and reliable results. Fortunately, development of viral gene transfer systems led to highly efficient gene delivery and protein expression. These viral vectors are of particular interest for gene delivery and vaccination in humans (gene therapy) as well as for preclinical- and basic research that require efficient gene delivery into animals, in vitro cultivated primary cell population or endothelial cell lines.

Most viral vector systems used for gene transduction in cell culture models are replication-deficient due to multiple deletions of sequences from the viral DNA or RNA genome. Deleted parts of the viral genome typically comprise coding sequences for regulatory and structural proteins such as capsid- or envelope proteins and enzymes (2). Those modified viral vector genomes are able to induce transcription and translation of inserted and co-transferred...
transgenic material upon arrival in target cells but fails to produce new viral particles due to no or minimal residual viral gene expression. For gene transduction or gene therapy, however, viral particles are required that can undergo a single-round of infection. Thus, production of viral vector particles requires a second component, the packaging system. This system can either be provided by co-transfection of multiple packaging plasmids into packaging cells (e.g. 293T cells) as is the case for retroviral gene transfer systems or helper cells (e.g. 293 cells) that constitutively express viral genes in trans to complement the replication deficiency of the introduced transfer vector genome as is the case for adenovirus vector systems (3). In this paper we focus on the applicability of replication-deficient retro- and adenoviral vectors used in endothelial cell research.

**Retroviruses**

**Retrovirus particles**
The family of *Retroviridae* comprises two subfamilies, the *orthoretrovirinae* with six genera and the *spumaretrovirinae* with a single genus (4). The retroviral genome codes for at least three structural proteins, the group specific antigen (Gag), the polymerase (Pol) and the envelope (Env) (Fig. 1A). A typical lipid membrane enveloped retroviral particle has a diameter of 100–120 nm containing typical spike like structures on its surface (Fig. 1B). A retroviral virion harbours two copies of its linear RNA genome with + strand orientation packaged into a protein shell (capsid) consisting of various layers and subunits derived from the Gag pre-
cursor protein. In addition, processing products of the Pol precursor protein that contain proteolytic (PR), reverse transcriptase (RT) and integrase (IN) activities, all essential for retroviral replication, are located in the retroviral virion. Finally, this RNA-protein structure is surrounded by a lipid bilayer, acquired from the host cell during the viral budding process in the virus-producing cell, in which oligomeric complexes of viral envelope glycoprotein (Env) subunits are embedded. These Env subunits, which form typical spike structures at the retroviral particle surface, are involved in target cell receptor recognition, mediated by the surface (SU) subunit, thereby determining the tissue tropism of the virus, and fusion of viral- and cellular lipid membranes, induced by the transmembrane (TM) subunit during retroviral target cell entry. An interesting feature of retroviruses is their capability to convert their viral RNA genome upon target cell entry into double-stranded DNA that is stably integrated as provirus into the host cell genome, in a more or less randomly manner. This provides a long-term gene expression even in highly proliferative target tissues and is advantageous for gene transfer purposes.

Retrovirus replication

A characteristic orthoretroviral replication cycle starts with the Env-mediated attachment to a specific receptor in the host cell membrane, thereby determining the virus tropism (Fig. 1C). Viruses can be taken up by endocytosis and capsids are released into the cytoplasm by Env-mediated fusion of viral- and cellular lipid membranes. However, membrane fusion can also occur at the plasma membrane. Cytoplasmic capsids start to disassemble and simultaneously convert their RNA genome into a reverse transcriptase-dependent process into DNA. This viral DNA together with viral- and cellular proteins, also called preintegration complex (PIC), needs to get access to the nucleus to be stably inserted into the host cell genome by a viral integrase-mediated process. Nuclear access of the PIC can be dependent on cell division (murine leukemia virus, MLV) and is either associated with nuclear membrane breakdown or an active import process through the nuclear pore (human immunodeficiency virus, HIV). Retroviral transcription is controlled either exclusively by cellular transcription factors (MLV) recognising target sites in the retroviral LTR sequences or can also be dependent on viral transactivators (HIV). Unspliced viral genomic RNAs as well as single- or multiple spliced viral RNAs are exported to the cytoplasm, hijacking standard RNA export machineries, sometimes requiring the help of viral adaptor proteins (HIV). In the cytoplasm viral mRNAs are translated at free- (Gag, Pol) or endoplasmatic reticulum (ER)-associated ribosomes (Env). Viral particles are generated by assembly of capsid through the oligomerisation potential of the Gag precursor protein at the plasma membrane (C-type) or at intracellular sites (B/D-type). The polymerase subunits are incorporated into the virion mediated by Pol precursor expression as a Gag-Pol fusion protein translated by ribosomal frameshift- or termination suppression mechanisms. The membrane-resident viral glycoprotein (Env) complex is acquired during the budding process mediated by the Gag protein and cellular machineries releasing immature and non-infectious viral particles into the environment. During or shortly after particle release the viral protease (PR) is activated and leads to the processing of the Gag and Gag-Pol precursor proteins into their respective subunits converting the virion into its mature, infectious state.

Retroviral vectors as gene transfer tools for endothelial cells

Retroviruses were the first viruses to be exploited for gene transfer purposes in therapeutic approaches and tested in clinical trials (5, 6). Currently, retroviral vector systems derived from proviral DNA genomes of three retroviral genera are most widely used for gene transfer into mammalian cells (Table 1).

Gammaparetrovirus vectors

The first is the orthoretrovirinae genus of gammaparetroviruses with MLV as its most prominent member. According to its genome organisation MLV belongs to the simple retroviruses, harbouring only open reading frames for the structural proteins Gag and Env as well as the viral enzymatic activities contained within the Pol protein (Fig. 1). MLV vector systems, often also referred to as “oncoretroviral” or “retroviral” vectors, were first developed in the early 1980s and were the gene transfer vehicle used for first gene therapy trials in humans in the early 1990s (6–8). First therapeutic successes using these systems were reported for the treatment of patients with severe combined immunodeficiency disease (SCID) (9). However, the initial success was soon followed by reports of leukemia in several of the treated patients as a result of the random integration, clonal selection and transfer vector associated proto-oncogene activation (10). MLV vectors require breakdown of the nuclear envelope associated with cell division for integration of their vector genome into the host-cell chromosomes and therefore can transduce only replicating cells efficiently (11, 12). In many cases transgene expression

<table>
<thead>
<tr>
<th>Retroviral genus</th>
<th>Most commonly used species</th>
<th>Transgene capacity</th>
<th>Vector titers (transducing units/ml)</th>
<th>Transduction of non-dividing cells</th>
<th>Pseudotyping capability</th>
<th>Concentration capability</th>
<th>Parental virus pathogenic potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-Retrovirus</td>
<td>Murine leukemia virus (MLV)</td>
<td>up to 8 kb</td>
<td>up to 10^2</td>
<td>no</td>
<td>yes</td>
<td>Env-dependent</td>
<td>oncogenic</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Human immunodeficiency virus type 1 (HIV-1)</td>
<td>up to 8 kb</td>
<td>up to 10^9</td>
<td>yes</td>
<td>yes</td>
<td>Env-dependent</td>
<td>immuno-destructive</td>
</tr>
<tr>
<td>Spumavirus</td>
<td>Human- or prototype foamy virus (HFV/PFV)</td>
<td>up to 9 kb</td>
<td>up to 10^7</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>none identified</td>
</tr>
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in MLV vectors is driven by viral promoter-enhancer elements located in the 5′ long terminal repeat (LTR). However, also so-called self-inactivating (SIN) vectors are frequently used, whose proviral vector genome integrated into the target cell genome is flanked by LTR elements with all viral transcription control elements being deleted. Internal heterologous enhancer-promoter elements are used to drive transgene expression in this vector type.

MLV vectors have been used for gene manipulation of endothelial cells. For example, murine and human endothelio ma cell lines were frequently generated by transduction of MLV vectors expressing the polyomavirus middle T antigen (13, 14). However, initial approaches employing MLV-based vectors utilising ecotropic- or amphotropic MLV envelope proteins for gene transfer in primary endothelial cells were inefficient (15–17). Transduction procedures had to be repeated on successive days or transduced cells had to be enriched by drug resistance selection, enabled by co-transferred resistance genes present in the retroviral vectors employed, or by flow cytometric sorting of marked cells (16, 17).

The incorporation of heterologous viral glycoproteins, termed pseudotyping, into retroviral vector particles enhances the target cell tropism of other viruses and thereby selectively alters the host range of recombinant vector particles and their transduction capacities for specific target cell types (reviewed in [18]). Introduction of vesicular-stomatitis-virus (VSV) glycoprotein G pseudotypes of retroviral vectors in particular, but also the development of retroviral packaging systems based on transient transfection of 293T cells or derivatives thereof, greatly improved MLV-based gene transfer into primary endothelial cells (15, 19–22). In addition, MLV vectors pseudotyped with the envelope protein of gibbon ape leukaemia virus (GALV) were shown to transduce primary human saphenous vein endothelial cells more efficiently than corresponding vectors harbouring the amphotropic MLV Env (23). Many reports on genetic modification of endothelial cells deal with approaches to manipulate tumour vascularisation as therapeutic approach. Endothelial cell-specific targeting of MLV-based vector specific was attempted by genetic modification of the MLV envelope protein. Liu et al. have incorporated NGR tumour vasculature targeting motifs (TVTMs) thereby allowing an increased endothelial specific transduction (24). Masood et al. replaced the receptor-binding-domain with an IgG-binding motif and coexpression with a wild-type Env protein and incubation of vector particles with antibodies to endothel-specific marker proteins led to a better gene transfer into endothelial cells (25). Nevertheless in both approaches an exclusive EC targeting was not achieved.

**Lentivirus vectors**

A second genus of retroviruses, the lentiviruses, has gained a lot of interest as gene transfer tool starting in the middle 1990s (26). Most lentiviral vectors developed and utilised are based on human immunodeficiency type 1 (HIV-1), but systems from related viruses, such as simian immunodeficiency virus (SIV) and equine infectious anaemia virus (EIAV), are also available (reviewed in [27]). The genome of lentiviruses encodes for several additional proteins besides the common retroviral Gag, Pol, and Env polyproteins. Therefore lentiviruses belong to the class of complex retroviruses according to their genome organisation. In general, lentiviral vectors are of SIN type due to safety issues and the viral transactivator (Tat) dependency of the HIV-1 LTR promoter. The major advantage of lentiviral vectors is their capability to also enable efficient gene transfer into non-dividing target cells (28, 29). Only a few reports are published on transduction of human endothelial cells by lentiviral vectors. A first study by Shichinohe et al. suggested that VSV-G pseudotyped lentiviral vectors displayed only a low relative transduction efficiency of human umbilical vein endothelial cells (HUVEC) and bovine aortic cells (30). However, the lentiviral vector used in this study was apparently lacking an important cis-acting genetic element of HIV, the central poly purine tract (cPPT) that is important for transduction of certain non-proliferating cell types (31). Further studies performed with cPPT containing lentiviral vector systems demonstrated an efficient gene transfer in vitro into proliferating- and growth-arrested HUVECs and in vivo (mice and rats) (32, 33). Cefai et al. (32) directly compared cPPT lacking and cPPT containing lentiviral vectors and demonstrated a cell type- and promoter-specific influence on transgene transduction. In HUVEC cPPT containing vectors showed a significantly higher transduction efficiency that was very pronounced for an EF1α promoter driving marker gene expression but less pronounced for an internal CMV promoter. In contrast, for human coronary artery endothelial cells (HCAEC) and human coronary artery smooth muscle cells (HCASMC) only a small advantage for the presence of the cPPT element in the lentiviral transfer vector was observed. De Palma et al. (33) compared a panel of endothelial-cell specific transcription control elements to ubiquitously expressed promoters. They demonstrated a good endothelial-cell specificity of some of the elements examined.

**Foamy virus vectors**

A third genus of retroviruses, spumaviruses, was only recently added to the repertoire of retroviral-based gene transfer systems. Spumaviruses, better known as foamy viruses (FVs), like LV belong to the class of complex type of retroviruses (reviewed in [34, 35]). FVs comprise the only genus in the retroviral subfamily of *spumaretrovirinae* and deviate in many aspects of their replication strategy from orthoretroviruses. Interestingly, several of their unique features bear homology to the replication cycle of hepatitis B virus, another virus undergoing a reverse transcription step in its life cycle, although at a different time point than found for orthoretroviruses. FVs assemble their viral particle by a special mechanism involving an essential unique interaction between the FV Gag and Env protein. This unique interaction currently prevents pseudotyping of FV vectors by heterologous viral glycoproteins (reviewed in [36]). Features that make FVs interesting candidates as gene transfer vehicle for clinical applications are their apparent apathogenicity in their natural non-human primate host as well as cattle, horses, cats and humans, and their natural broad host range. FV vector systems were only developed in the late 1990s but have recently been shown to efficiently transduce hematopoietic stem cells in various animal model systems (35). Currently there are no published studies about gene transfer efficiencies of FV for endothelial cells; however, preliminary experiments of the authors indicate that it might be not as efficient as observed for VSV-G pseudotyped LV (Lindemann & Schnittler, unpublished). Furthermore, although
pseudotyping of FVs by heterologous glycoproteins is currently impossible, FV Env mutants have been identified that allow efficient pseudotyping of HIV- and MLV-based retroviral vectors (Lindemann, unpublished). Comparison of LV vectors pseudotyped either with VSV-G or FV Env in respect to their transduction efficiency on different endothelial cell lines and primary cultures suggest that pseudotyping of LV by FV Env results in an improved gene transfer in certain endothelial cell types (Lindemann & Schnittler, unpublished).

Adenoviruses

General aspects

Adenoviruses (Ads) have gained much interest as gene delivery vectors in research and gene therapy. Adenoviruses (Ads) are members of the family Adenoviridae, which harbours about 100 distinct serotypes infecting different mammalian species, including humans. Particles are of about 90–100 nm icosahedral shape, non-enveloped and carry a double stranded linear DNA

![Diagram of Adenovirus Organization and Receptor Binding Sites](image)

Figure 2: Structural and genomic vector organisation of adenoviruses. A) Scheme of adenovirus organisation and receptor binding sites. B) Genomic organisation. C) Generations of adenoviral vectors. For further details please see text.
About 54 serotypes are found in humans (hAd) and belong to different species A-G (37). Human Ads cause acute diseases such as pneumonia, conjunctivitis and pharyngitis. Ads entry into host cells involves a specific interaction between the viral capsid and cell surface receptors. The Ads capsid consists of about 240 homotrimeric hexons and 12 pentameric pentons located at the icosahedral capsid. A homotrimeric fiber (fiber shaft) with a globular carboxyterminal domain (fiber knob) mediates in addition to the 12 pentons virus entry (37, 38) (Fig. 2). The broad spectrum of target cells and diseases indicates different Ad receptors on the host cells. Indeed, currently there are at least 11 known different receptors that exhibit binding specificity to the human species A to G mostly by binding to sequences displayed on either the penton base, the fiber shaft or the globular fiber knob of the capsid (Fig. 2) (37, 38). Host cell receptors include sialic acids, CD80/CD86, heparan sulfate, VCAM-1, MHCI complexes, scavenger receptor, integrins and the coxsackievirus-adenovirus receptor (CAR) (Fig. 2). Most of those receptors are found on endothelial cells (Fig. 2), but the CA-receptor, localised at endothelial cell junctions, appears to be the most effective one for Ad serotype 2/5 (39). The presence of certain receptors on endothelial cells makes them suitable target cells for Ad-gene transfer. In addition to direct virus-receptor interaction there are three described indirect interactions via mediator molecules which are dipalmitoyl phosphatidylcholine (DPPC), a phospholipid found in high concentrations in the surfactant of the lung, coagulation factor X and IX, the complement factor C 4 binding protein, and the low-density lipoprotein receptor-related protein (C4PR) (37). The most frequently used Ad serotypes for gene transduction are Ad2 and Ad5 of species C that use a wide variety of receptors for infection (37, 38). Ad-virus uptake and release of the viral DNA into the cytosol and subsequent nuclear translocation requires orchestrated processes and signals consistent with clathrin mediated endocytosis and macropinocytosis (Fig. 3). Immediately after receptor binding (e.g. CA-receptor, integrins) Ads are endocytosed, a process that requires activation of dynamin II, PKC, sodium proton exchanger, PI3-kinase, the small GTPases Rac and cdc42 and actin dynamics (40, 41). The membrane of Ad containing endosomes disrupts and Ad particles are transported via dynein-mediated transport along the microtubules to microtubule organisation centre (MTOC) and subsequently bind to the nuclear pore complexes (42). Here the particles finally disassemble and viral DNA is transferred to the nucleus where viral transcription starts (43).

Adenoviral vectors

The development of adenoviral vectors for gene transduction has taken much attention particularly in gene therapy of humans. Currently Ad-virus vector are the most frequently used viral vector systems in gene- and cancer therapy. These vectors are also extremely useful tools in cell biology research, particularly of endothelial cells that are hardly transfectable by conventional chemical based transfection methods. Ads infect, as outlined above, a wide variety of cells including endothelial cells and efficiently transfer their genome into the host. This holds also true for genes of interest that were artificially introduced into the adenoviral genome. However, cells infected with wild-type adenoviruses undergo cytolysis due to virus replication. This makes replication competent Ads an insufficient tool for gene transduc-
tion. Deletions of certain DNA sequences from the Ad genome blocks Ad-replication. However, those recombinant replication deficient Ads successfully deliver genes into target cells. The Ad genome is composed of the early transcription units E1A, E1B, E2, E3, and E4, three delayed early transcription units pIX, Iva2 and E2-late and the major late transcription units L1 to L5 that code for structural proteins (Fig. 2). Packaging requires a signal sequence that is located between the left inverted terminal repeat (ITR) and the E1A region. Deletion of the E1 and E3 regions led to the first generation of usable adenovirus vectors as the deletion of the E1 region causes replication deficiency and the deletion of the E3 region decreases the immune-suppression. Since the E1 region is required for virus replication, producer cells have been established, such as the well-known 293 cell line, that was generated by transfection with sheared adenovirus DNA (3). Replication-deficient Ads still lead to efficient expression of foreign genes of interest but there are also viral proteins expressed that might cause cell toxicity (see below). Due to overlapping sequences of the E1 domain expressed in 293 helper cells and the remaining Ad vectors, recombination might take place leading to propagation of replication competent viruses. This is a safety-relevant problem (44). To reduce the immune response in vivo and to further reduce side effects and cell toxicity, a number of further deletions were performed. Those second-generation Ads vectors allow inserts of up to about 14 kb (45). In the third generation of Ad vectors almost the entire genome was removed, comprising all coding sequences, except viral sequences harbouring the flanking ITRs and the encapsidation signal. This type of "gutless" Ad vectors might carry up to 35 kb of foreign DNA and are well suited for long-term expression in quiescent cells (Fig. 2C). The "gutless" vectors are non-toxic as no viral proteins are produced in the target cells. Propagation of replication deficient Ad viruses using "gutless" vectors, however, needs sufficient helper cells that trans-complement the replication machinery of the virus to generate replication-deficient viruses (46).

Applications of adenoviral vectors in endothelial cell biology

Ads vectors and gene transduction systems are meanwhile commercially available and have been successfully used for a wide variety of genes to be expressed in endothelial cells in cell culture models and in animal models (47, 48). This includes certain types of genes such as transcription factors and their modulators, such as NFκB, IkB and AP-1 (49–52); modulators of reactive oxygen species (ROS) signalling, such as catalase, uncoupling protein 2, and sodium dismutase (SOD) (53–56); nitric oxide synthase (NOS) (57–60); Rho-GTPase (96, 97) growth factors (61–64); cytokines, such as viral or non-viral IL-10 (65, 66), enzymes (50); and tissue factor pathway inhibitor (67). More recently Ads vectors have also been used to deliver shRNA to lung endothelial cells (68) but the experience with this technique is currently limited.

Current attempts are directed to use Ad vectors to target defined tissues via endothelial cells to interfere with pathological processes (tumour development) or for treatment of organ-specific diseases (e.g. primary pulmonary hypertension) (69, 70). A central problem that clearly limits tissue-specific gene delivery is the high expression of CAR in the liver (37, 38) and the discontinuous liver sinus endothelium that allows direct access of any virus to hepatocytes (71). Unspecific gene transduction in the liver occurs after venous administration of Ads and causes severe side effects due to this ectopic gene expression (72). Other tissues do not express high CAR-levels and therefore are less well transduced by adenoviruses. Reynolds et al. (70) described an elegant approach, the "combined transductional and transcriptional transgene expression", in 2001. They combined transductional specific targeting of pulmonary lung endothelial cells using angiotensin converting enzyme (ACE) and the endothelial cell-specific promoter vascular growth factor receptor type 1, flt. Using this method a 300,000-fold increase in specific gene expression was achieved in lung endothelium (70). In analogy to this promising approach transcriptional targeting was applied in a mouse model to investigate angiogenesis related to tumour vascularisation and ischaemia (73). The authors used an Ad-vector system of the first generation and introduced constitutive active hypoxia-inducible factor-1α (mAIF-1α) under the control of the endothelial specific pre-proendothelin promoter. This approach resulted in a significant increase of angiogenesis in the altered tissues but not in the liver. Conversely liver expression was high when used the CMV promoter (73). These examples show that there are promising approaches for tissue-specific targeting of endothelial cells in vivo using tissue-specific promoters delivered by Ads vectors. Transcriptional targeting might also be a useful tool to specifically immortalise endothelial cells from a mixture of cells obtained from enzyme-digested tissue. Furthermore, Ad-virus tropism can be enhanced by using genetically modified fiber proteins that mediate specific receptor binding. First approaches included the use of chimeric fibers of different serotypes (74–77). However, the specificities of those Ads chimeras are limited by the specific fiber. Therefore, novel trials were undertaken to modify the carboxyterminal end of the fiber. Crystal structure analyses revealed a flexible loop that is potentially suited for positioning of an artificial ligand (78, 79). A suitable technique for those modifications is the "trans supply strategy" (80). A fiber-deleted adenovirus genome is used and the modified fiber protein is supplied in trans by a plasmid (containing the respective modified gene) that is expressed in a helper cell line (81–86). Based on this technique an easy cloning strategy has been described recently for endothelial-specific targeting by inserting certain cell specific peptide sequences (87).

Although Ads-mediated gene delivery has sufficient transduction efficiency in most cases there are still attempts for further optimisation. Poor transduction efficiency in some cell types, less specific tissue targeting, neutralising anti-Ad-virus antibodies and immunoreactivity are challenging problems (for review see [88, 89]). It was shown that certain chemicals were able to increase gene transduction efficiency and reduce endothelial cell activation and site effects. For example antioxidants as N-acetyl cysteine (NAC) poly-cations or adenovirus protein-cationic liposome complexes enhances Ad-virus entry and increase gene transcription (90–93). Polyethylene glycol (PEG) also has been used successfully not only to increase viral entry and protein expression but also decreased the sometimes-observable endothelial and platelet activation and thus reduces virus-induced thrombocytopenia (94, 95).
Conclusions and outlook

Viral vectors are effective tools for transfer of nucleic acids into endothelial cells. In comparison to classical transfection protocols, their use is associated with lower toxicity and higher efficiency. The adenoviral and retroviral vector systems discussed in this review are extremely useful tools for in vitro and in vivo endothelial cell research. Due to their high gene transduction efficiency these viral vector systems enable investigation of genetically altered endothelial cells using common biochemical and immunological techniques and functional assays without prior need for enrichment of modified cells. While adenovirus-based vector are well suited for transient gene transfer retroviral-based vectors can also be used for the establishment of stable genetically modified endothelial cell lines.

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