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Retroviral Vectors

Abstract

A large number of viruses including retroviruses have been used as gene transfer vectors in gene therapy. The family of *retroviridae* contains seven genera of which the mammalian and avian type C viruses and the lentiviruses have been studied in detail. From all three groups gene transfer vectors have been derived. The advantages of these vectors are the relatively simple genome organisation, the possibility to generate vector stocks free of replicating virus, and their ability to integrate the therapeutic gene into the host's chromosomes. Retroviruses were the first viral vectors used in gene therapy clinical trials about ten years ago. Only recently, clinical benefit for patients suffering from an inherited disease was achieved following transfer of retrovirally transduced cells [1]. It is now widely accepted that efficient gene delivery is crucial for the development of successful gene therapy protocols. This review will describe the generation of retroviral vector particles, it will focus on the different types of retroviral vectors and discuss recent developments in retroviral vector optimisation.

Keywords

Gene therapy · Retrovirus · Lentivirus · Cell Targeting

Development and safety of vectors derived from C-type retroviruses

The retroviral replication cycle

Like all retroviruses, C-type retroviruses are RNA viruses that replicate through a DNA intermediate (for review see [2]). The viral particle (about 100 nm in diameter) consists of a protein core, which contains viral replication enzymes and two copies of a linear, positive-sense, single-stranded RNA molecule 7 to 11 kilobases in size and an envelope (env), composed of host cell membrane and viral glycoproteins. The latter comprise the surface envelope glycoprotein (SU) and the transmembrane glycoprotein (TM). The infection cycle is initiated by specific interactions between SU and a cellular receptor on the surface of a target cell, resulting in the fusion of the viral and the cellular membrane mediated by TM, and the release of the viral core into the cytoplasm (Fig. 1, step 1). After cell entry, the viral RNA is reverse transcribed into linear double-stranded DNA (step 2), uncoated, transported into the nucleus and integrated into the genome of the host cell (step 3). Subsequently, it is replicated and expressed during cell cycle like a cellular gene and passed on to daughter cells (steps 4, 5). In the cytoplasm, viral core proteins assemble with viral RNA (step 6), then the viral glycoproteins will be incorporated and budding from the cellular membrane occurs. After particle release, final processing of core proteins mediates the formation of mature infectious virions (step 7).

The retroviral genome

The integrated retroviral DNA genome, the so-called provirus, encompasses at its 5´ and 3´ end two identical long terminal repeats (LTRs). The LTRs carry all cis-acting sequences necessary for viral gene expression, i.e. the promoter and the transcription termination and polyadenylation signals. The attachment sites (att) at the ends of the LTRs are essential for viral integration, and additional cis-acting signals necessary for reverse transcription, the primer binding site (PBS) and a polypurine tract (PPT), as well as an encapsidation signal psi (Ψ) necessary for packaging of viral RNA into particles are located next to the LTRs. Usually, the viral genes *gag/pro* (encoding the core proteins and the viral protease), *pol* (encoding viral enzymes necessary for replication and integration), and *env* (encoding the viral envelope glycoproteins) are located between the LTRs (Fig. 2). Following transcription, the retroviral RNA genome structure differs from the provirus by lacking the termini of the LTRs, which are restored during reverse transcription.

Design of retroviral vectors

In contrast to complex retroviruses, the relatively simple genome structure of C-type retroviruses enables an easy design of replication defective vector systems

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Retrovirale Vektoren

Zusammenfassung

In der humanen Gentherapie wird eine Vielzahl unterschiedlicher Viren als Gentransfer-Vektoren angewendet, darunter retrovirale Vektoren. Die Familie der Retroviren umfasst sieben Genera, wobei die C-Typ-Retroviren der Säuger und Vögel und die Lentiviren am besten untersucht sind. Retrovirale Vektoren wurden von allen drei Gruppen abgeleitet. Die Vorteile retroviraler Vektoren liegen in ihrer relativ einfachen Genomorganisation, in der Möglichkeit, Vektorstocks frei von replikationskompetenten Viren herzustellen sowie in ihrer Eigenschaft, das therapeutische Gen in das Wirtsgenom zu integrieren. Retroviren wurden bereits vor über zehn Jahren als erste Vektoren in klinischen Studien eingesetzt. Erst kürzlich wurde eine deutliche gesundheitliche Verbesserung durch Übertragung retroviral transduzierter Zellen bei Patienten mit einer monogenetischen Erbkrankheit erzielt [1]. Es wird gegenwärtig allgemein angenommen, dass die Effizienz des Gentransfers der entscheidende Punkt bei der Etablierung erfolgreicher Behandlungsstrategien ist. Dieser Übersichtsartikel beschreibt die Herstellung retroviraler Vektorpartikel, konzentriert sich auf die unterschiedlichen Typen retroviraler Vektoren und diskutiert neue Entwicklungen bezüglich der Optimierung retroviraler Vektoren.

Schlüsselwörter

Gentherapie · Retrovirus · Lentivirus · Zell-Targeting

[3]. A typical transfer vector genome consists of the LTRs and neighbouring regions, whereas all viral coding regions are replaced by one or more foreign gene(s) (transgen(s)) (Fig. 2).

In modern vector systems, all viral proteins necessary for vector particle formation are provided in trans from separate viral constructs missing encapsidation signals to prevent their packaging and the subsequent generation of replication competent retroviruses (RCRs). By using the resulting vector particles for infection (termed transduction) of target cells, only the transfer vector RNA genome, which is psi-positive and contains foreign but no viral genes, is transferred. Furthermore, viral enzymes necessary for protein processing and reverse transcription of the transfer vector RNA genome as well as proteins necessary for integration of the corresponding transfer vector DNA genome are incorporated into the vector particles. This design is important for the biosafety of vectors, since the undesired formation of replication competent retroviruses (RCRs) will require several recombination events during reverse transcription between a number of constructs exhibiting only minimal sequence overlap.

Usually, the retroviral proteins are encoded by two separate constructs: by a gag/pol-plasmid and by an env-plasmid. Gene expression of the viral genes on both plasmids may be controlled by heterologous promoter and termination elements. Both plasmids are lacking the psi-encapsidation signal. Vector particles are generated by transient triple-cotransfection of cell lines with these two plasmid constructs and the transfer vector DNA. The resulting vector particles will be secreted into the cell culture supernatant and can easily be harvested. However, for large-scale production of vector particles, stable cell lines (packaging cell lines) which constitutively express some or even all of the components necessary for vector packaging are being used (Fig. 3). A number of ready-to-use packaging cell lines are available, which express either constitutively or upon induction Gag/Pol proteins and Env proteins from different retroviruses and which are feasible for the packaging and transfer of vectors carrying the foreign genes of choice [3].

Transgene expression

Current vectors derived from C-type retroviruses allow for packaging of up to 8 kilobases (RNA) and usually encompass coding regions of two different foreign genes (transgenes). These are either therapeutic genes or marker genes as lacZ or EGFP, which are useful for the evaluation of vector potency. Transcription occurs either from the 5´LTR or from a heterologous promoter, and ex-

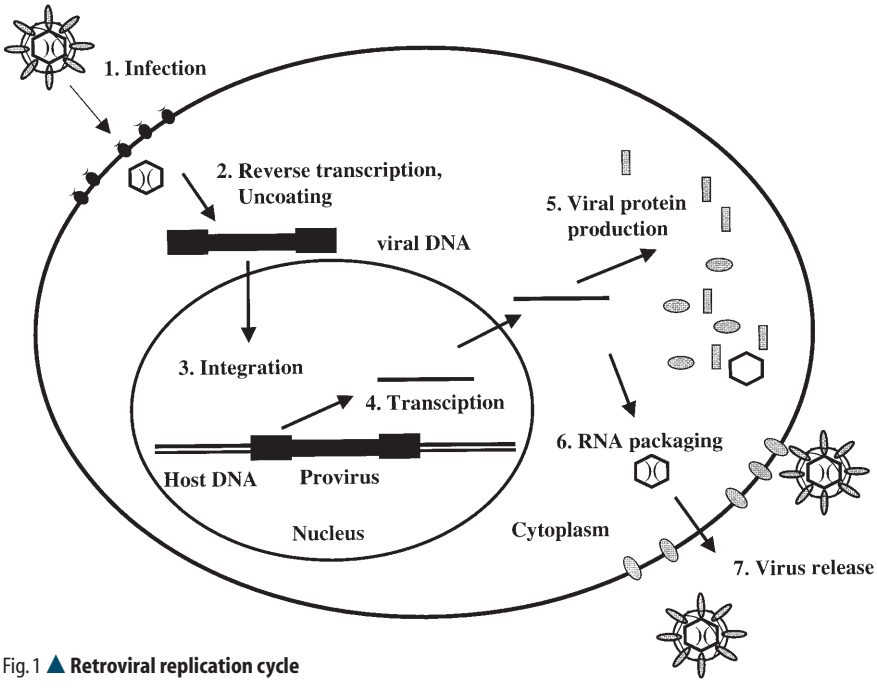

Fig. 1 ▲ **Retroviral replication cycle**

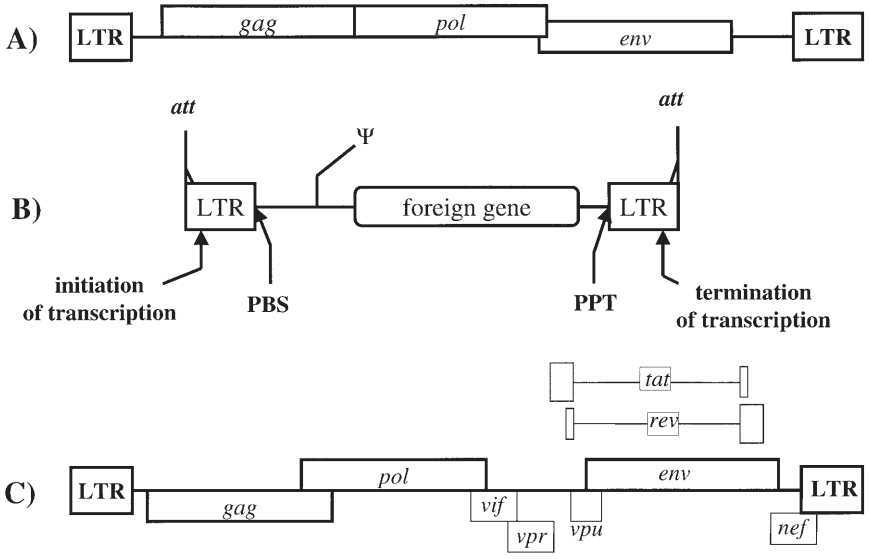

Fig. 2a–c ▲ **Retroviral genomes and cis-acting elements of a typical retroviral vector.** a **C-type retrovirus (MLV) genome structure.** b **Retroviral transfer vector. Sequences necessary for propagation of a retroviral construct are indicated.** c **Lentivirus (HIV-1) genome structure. Att: attachment sites; PBS: Primer binding site; Ψ: packaging signal; PPT: Polypurine tract; LTR: Long Terminal Repeat**

pression of each individual gene is enabled by splicing of the viral RNA or by an internal ribosomal entry site located between the two coding regions. Alternatively, the second gene may be expressed via an additional heterologous promoter. All promoters should mediate sustained expression of the transgene in the respective target cells. However, transgene expression is often hampered by silencing of foreign promoter sequences by host cell mechanisms, e.g. by CpG methylation [4]. Therefore, the development of optimal regulatory sequences specific for each target tissue is desirable. For example, a retroviral transfer vector has been developed using regulatory elements from Spleen Focus-forming Virus as well as from Murine Embryonic Stem Cell Virus to improve the expression of transgenes in CD34-positive human hematopoietic stem cells [5, 6].

Limitations

At present, the best charactarized and most frequently used retroviral vectors are derivatives of Murine Leukemia Viruses (MLVs). Vectors based on other C-type retroviruses, e.g. avian viruses like Spleen Necrosis Virus (SNV), Rous Sarcoma Virus (RSV), or Avian Leucosis Virus (ALV) still play a minor role. Due to their ability to infect a wide range of human cells, clinical application of amphotropic MLV-derived vectors is generally limited to ex vivo transduction followed by re-infusion or inoculation of the transduced autologous or heterologous cells. A major challenge for the in vivo application of retroviral vectors is the development of targeting vectors displaying a cell tropism restricted to specific target cells (see below). It will also be necessary to further enhance vector titers. At present, the highest titers that can be reached using available packaging cell lines are about 10^7 infectious units /ml. Considering the dilution of vector particles in body fluids this titer will not allow efficient in vivo application of MLV based retroviral vectors. Other problems that may complicate the in vivo application of MLV based retroviral vectors, like for example vector inactivation by human complement, can largely be avoided by the use of human packaging cell lines [7].

Another major restriction of vectors based on C-type retroviruses is their inability to infect non-dividing cells [8]. For these viruses the breakdown of the nuclear membrane at mitosis is necessary to allow the retroviral genome to enter the nucleus and to integrate into the cellular DNA [9]. Accordingly, many tissues such as brain, lung, and pancreas as well as non-replicating stem cells are not accessible to gene transfer using retroviral vectors derived from C-type viruses without prior stimulation of mitosis. The requirement for cell division thus limits the use of retroviral vectors for in vivo therapeutic gene transfer. On the other hand, the inability to infect non-dividing cells has also been regarded as an intrinsic targeting property steering transduction exclusively to replicating cells. For example, clinical studies aiming at the in vivo therapy of brain tumours by intratumoural injection of packaging cells producing MLV-derived vectors have been undertaken [10, 11, 12]. In these studies the MLV vectors were used to transfer a suicide gene specifically into tumour cells thereby mediating tumour cell death by the suicide gene mediated conversion of the substance ganciclovir to a toxic drug.

Safety aspects

A major issue of vectors derived from C-type retroviruses is their oncogenic potential. Integration of the retroviral vector DNA into the cellular genome occurs randomly. The random integration of the transfer vector DNA into the host chromosome may lead to the modification of cellular genes resulting in the activation of oncogenes or inactivation of tumour suppressor genes [2]. However, such an event has not yet been observed in humans. So far, tumour induction following retroviral vector application has only been described in murine models and non-human primates and was clearly due to the contamination of the vector batch by replication competent retroviruses [13].

A particularly attractive approach to enhance biosafety of retroviral vectors is the development of self inactivating vectors derived from a C-type retrovirus (SIN vectors). SIN transfer vectors encompass an intact 5´LTR, whereas the promoter in the 3´LTR is inactivated by an internal deletion [14]. As the 3´LTR is used as the template for reverse transcription [2], the resulting viral DNA will encompass the inactive 3´LTR on both ends. Therefore, the retroviral LTR-promoter is not integrated into the host cell DNA following gene transfer and cannot enhance the transcription of cellular genes like oncogenes. Due to the self-inactivation of the viral promoter a heterologous promoter is used to express the transgene transferred by the SIN vector.

Another type of SIN vectors use the cre/lox system to remove viral sequences after gene transfer [15]. In these vectors the viral sequences are inserted between loxP sites, which will allow the Cre recombinase, to excise all sequences inserted between these sites. Using SIN vectors and modern split-packaging cell lines as described above, the risk of generating RCRs by recombination is reduced. Nevertheless, regular testing of packaging cells, vector stocks, and the cells transduced ex vivo is still required. RCR testing may be done by polymerase chain reaction (PCR) or may involve virus detection assays in cell culture. Actually, RCR testing of vector batches is demanded before the application of a retroviral vector in a clinical trial is allowed [16, 17].

Development of lentiviral vectors

HIV-1 vector development

The basic science and concept of lentiviral vectors has been covered extensively in a number of reviews over the last few years [18, 19]. Lentiviruses, such as the Human Immunodeficiency Virus (HIV), have a much more complex genome structure as compared to C-type retroviruses and, consequently, display a complex replication cycle [20]. HIV-1 has been the best studied lentivirus. Its genome structure is illustrated in Fig. 2. The basic genome organisation is in principle the same as for C-type retroviruses. HIV-1 has the additional accessory genes tat, rev, nef, vif, vpu and vpr, which play a crucial role in the viral replication cycle. However, the precise involvement of these accessory genes in the aetiology of AIDS is still a matter of debate and none of them, besides tat and rev, is obligatory for virus propagation in vitro. The HIV-1 Tat protein activates the HIV-1 Long Terminal Repeat (LTR) in a way that viral RNA is produced very efficiently. The HIV-1 Rev protein interacts with a region of the viral RNA known as the Rev-responsive element (RRE) and promotes the transport of unspliced viral RNA from the cell nucleus to cytoplasm.

More relevant for lentiviral vector development are viral components that enable HIV-1 to productively infect non-dividing and terminally differentiated cells, a capability that is not known for C-type retroviruses [21]. Although the precise mechanism by which lentiviruses infect non-dividing cells is unknown, for HIV it appears to be facilitated by several viral proteins: the integrase protein (Int), the matrix protein (MA-Gag) and the accessory protein Vpr [22, 23]. Both integrase and matrix protein contain nuclear localisation signal sequences (NLS). In contrast, Vpr appears to bind directly to the nuclear pore complex. However, details about how these elements direct the viral genome through the nuclear envelope and into the nucleus of non-dividing cells still remains unknown. In addition, cells arrested in the Go phase of the cell cycle are also resistant to transduction by HIV-1-derived vectors.

C-type retrovirus-derived vectors cannot efficiently be used for the transduction of non-dividing cells such as hematopoietic stem cells or terminally differentiated neurons. Since lentiviruses are able infect non-dividing cells, there has been great interest in the development of lentiviral vectors for use in clinical gene therapy trials. However, the complex genome and replication cycle of lentiviruses made the development of vectors and packaging cell lines difficult. For example, whereas C-type retrovirus vectors could be propagated simply by providing Gag, Pol and Env proteins, HIV replication also requires the Tat and Rev proteins. Moreover, there have been a lot of difficulties in generating stable packaging cell lines, most likely due to the toxicity of some HIV proteins. Therefore, many of the first generation HIV vectors were nearly intact viral genomes containing only disruptions or deletions of the HIV env gene. In addition, some of these vectors carried a marker gene expressed from an internal heterologous promoter. Since these constructs expressed all HIV genes except env, the viral envelope was provided in trans for vector propagation. The use of the homologous HIV envelope glycoprotein did allow vector targeting to CD4-positive cells but, on the other hand, gene transfer into other cell types was limited. Therefore, other viral envelope proteins were used to substitute for the HIV envelope. For example, the Env protein of amphotropic MLV, the G protein of Vesicular Stomatitis Virus (VSV-G), and the modified Env protein of Gibbon Ape Leukemia Virus (GaLV) were effectively used to pseudotype lentiviral vectors and allowed broadening of the cell types that could be infected [24, 25]. Further details addressing these issues are described in the vector targeting section below.

The second generation of HIV-1 vectors more closely resembled the C-type retrovirus-derived vectors because all viral genes were deleted from the psi-positive transfer vector, whereas the essential cis-acting sequences were retained. Marker genes were expressed either directly from the HIV-LTR or from an internal promoter [26]. The new generation of HIV-1 vectors were thoroughly characterised and appeared to be safer than the previous generation. High ti-

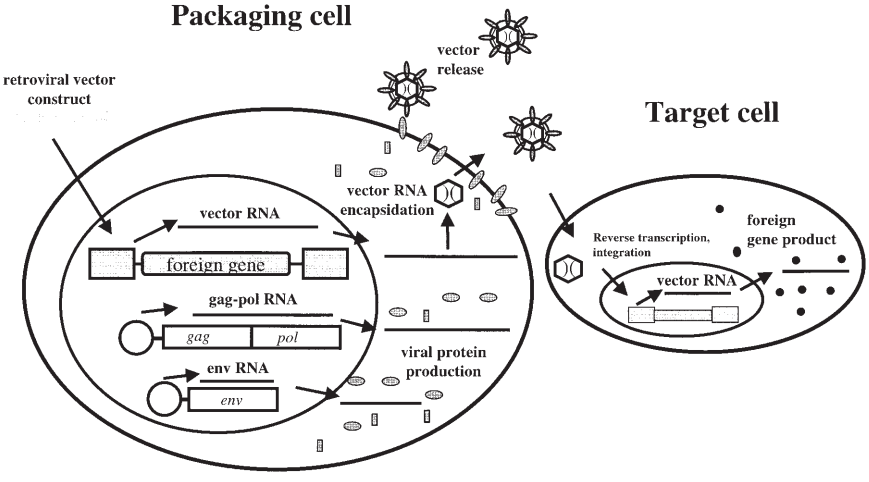

Fig. 3 ▲ **Retroviral packaging cell line and vector production. Viral genes expressed from non-retroviral promoters are stably introduced into cells, which produce viral structural and enzymatic proteins. When a retroviral vector is transfected into the cell, vector RNA can be encapsidated, resulting in the production of virus particles. These can be harvested from the supernatant and used to infect/transduce cells, thereby transferring and expressing the foreign gene in the target cells**

ter vector stocks could readily be produced, especially when VSV-G protein was used for vector pseudotyping. HIV-1 vectors expressing an internal marker gene were shown to be retain the capability of transducing resting cells [27]. Subsequent animal studies demonstrated that HIV-1 vectors could transduce terminally differentiated neuronal cells and in many cases the marker gene expression was stable for several months. Unlike C-type retrovirus-derived vectors, HIV vectors have been shown to efficiently infect hepatic, retinal and muscle cells [27, 28]. Moreover, HIV-1 vectors are capable of transducing human CD34-positive cells. The transduced cells were subsequently transplanted into mice and shown to engraft and differentiate into multiple hematopoietic cell lineages in NOD/SCID mice [29]. Most interestingly, neurodegeneration was prevented by lentiviral vector delivery of glial cell line-derived neurotrophic factor (GDNF) in a non-human primate model of Parkinson's disease [30].

Safety of lentiviral vectors

In order to be considered for clinical applications, lentiviral vectors must comply with established safety standards, taking into account the pathogenicity of the parental virus in humans. It is theoretically possible that multiple recombination events lead to the generation of replication competent recombinants between the vector constructs within the packaging cells. Although the use of a gene encoding a heterologous envelope protein reduces the probability of generating a replication competent virus (RCR), the contamination of any vector batch by such a RCR is unacceptable. Several factors determine the likelihood of a recombination between the constructs expressed by vector packaging cells. Among them, the extent of sequence overlaps between the constructs is important. Thus, advanced lentiviral vector development aims at minimising or abolishing sequence overlaps between the different constructs by deletion of unnecessary sequences or by mismatching the viral source of packaging and transfer constructs. Another important strategy to improve lentiviral vector safety involves the elimination of those viral genes from the packaging constructs that are not essential for gene transfer. Along this strategy, so-called multiply attenuated systems, constituting the third generation of delivery system, were an important step in the development of HIV vectors. Such vectors allow efficient gene transfer in the absence of any HIV accessory genes [18]. However, the lack of stable HIV packaging cell lines producing high vector titers is still a major problem, especially with regard to future clinical applications requiring GMP production.

Other lentiviral vectors

Although the development of HIV-1 vectors is currently most advanced, other lentiviral vectors derived from human as well as non-human primate lentiviruses have also been developed. The design of vectors based on Human Immunodeficiency Virus type 2 (HIV-2) and Simian Immunodeficiency Virus (SIV) follows the pathway already outlined for the HIV-1 vector development. HIV-2 is genetically more closely related to SIVmac from rhesus macaques than to HIV-1. It seems to be less pathogenic for humans and it can be studied in a primate model. Therefore, SIVmac and HIV-2 vector systems may offer some advantages for future therapeutic applications. However, more information regarding the biology and the pathogenesis of human and non-human primate lentiviruses and further characterisations of vector systems derived from these lentiviruses are needed before these vectors can be deemed superior for human clinical use.

Self-inactivating lentiviral vectors

One of the latest safety improvements in lentiviral vector design has been the development of self-inactivating transfer vectors (SINs) [31]. Such vectors have a number of advantages. The deletion in the 3´LTR, which is copied during reverse transcription and forms the 5´LTR in the integrated transfer vector, further reduces sequence homologies between the transfer vector and the packaging constructs thus minimising the chances of recombination. In addition, the potential competition between the 5´LTR promoter and the additional internal promoter driving transgene expression is avoided, thus improving the efficiency of transgene expression [31]. The combination of a SIN transfer vector with a third generation packaging system may for the first time allow achievement of a high level of vector safety. As lentiviral vectors are often superior C-type retroviruses-derived vectors, which are currently in clinical use, they may be acceptable for clinical application [18].

Vector targeting

Retroviral cell targeting vectors

As described above, amphotropic MLV vectors are currently used in many clinical gene therapy trials. Usually, they are used in ex vivo approaches which are tedious and often cost-intensive. In addition, the ex vivo gene therapy strategy can only be applied to a restricted number of tissues or cell types, i.e. those which are readily accessible such as blood cells. In contrast, in an in vivo gene transfer approach retroviral particles containing the gene to be delivered could be administered intravenously or otherwise directly into the organ/tissue to be genetically modified. This in vivo approach would impose less physical strain on the patient and would allow repetitive application of the vector. Such an approach would require the development of cell targeting vectors able to transduce only selected cell types or tissues. By use of cell targeting vectors the transduction of non-target cells could be avoided, thereby reducing the likelihood of undesired side effects.

Over the last years the number of laboratories working on basic vector targeting problems has grown substantially. Currently, scientists are focusing on the modification of the retroviral envelope proteins (Env proteins) to pseudotype retroviral vector particles. Overall, three different strategies of Env protein engineering can be distinguished, i) substitution of the complete Env protein against a heterologous Env protein which mediates a different host range, ii) substitution of the surface subunit of the Env protein (SU) or parts of it by single chain antibody variable region fragments (scFv) derived from immunoglobulins or by growth factor fragments, iii) protease-activatable vectors (Fig. 4).

Envelope substitution

Env protein substitution is usually achieved by transfecting env-negative packag-

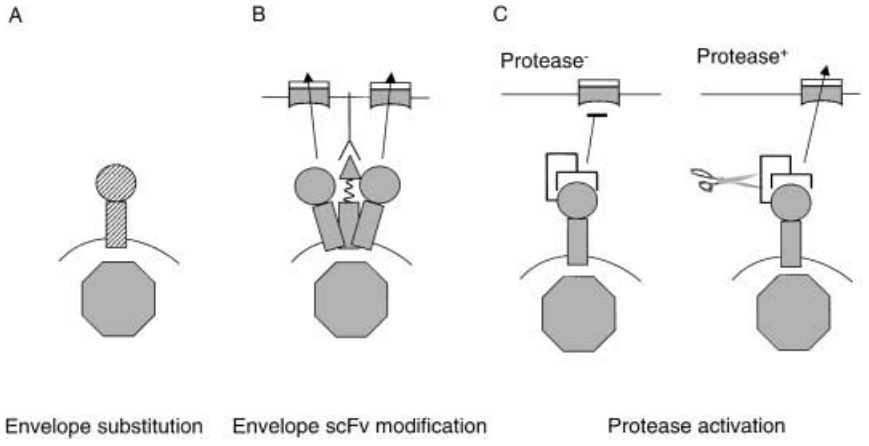

Fig. 4a–c ▲ **Targeting strategies for retroviral vectors.** a **Envelope substitution. Heterologous Env proteins (hatched rectangle and circle) that determine a restricted cell tropism are incorporated into MLV particles.** b **Envelope scFv modification. ScFv molecules (filled triangle) are displayed on the TM protein (grey rectangle in the vector envelope). These molecules form heterotrimers with the wt-Env (grey rectangle and grey circle). The vector particles enter their natural host cells via the yet unidentified SNV receptor (filled rectangle in the cell membrane) which mediates cell entry of those particles attached to the surface antigen (Y-shaped symbol) recognised by the displayed scFv fragment.** c **Protease activation. Blocking domains are displayed on the ampho-MLV Env protein via a proteolytically cleavable linker peptide. Upon proteolytic cleavage of the linker peptide the vector particles selectively enter protease (scissors) positive cells via their natural receptor (grey rectangle). Env: envelope protein; MLV: Murine Leukemia Virus; ScFv: single chain antibody variable region fragment; TM: transmembrane protein; wt: wildtype; SNV: Spleen Necrosis Virus)**

ing cell lines with a heterologous env expression construct. Efficient incorporation provided, pseudotype particles can be obtained which will reflect the host range of the virus from which the heterologous Env protein has been derived. Although the mechanism of Env protein incorporation into the viral capsid are not fully understood yet, a high expression level of the heterologous Env protein seems critical for the generation of functional vector particles. In addition, interactions between the cytoplasmic tail at the C-terminal domain of the transmembrane protein (TM) and the capsid proteins may influence the efficiency of Env incorporation [32].

A number of MLV-derived pseudotype vector particles have been developed to enhance the gene transfer efficiency of MLV vectors. For example, the use of MLV-derived pseudotype vectors containing the Gibbon Ape Leukemia Virus (GaLV) Env proteins or the Vesicular Stomatitis Virus (VSV) G protein have considerably improved the transduction efficiencies of hematopoietic stem cells [33], while human complement resistant vector stocks were obtained by pseudotyping MLV with the feline endogenous RD114 virus Env protein [34]. These vectors are, however, not suitable to target specific human cell types in vivo because the Env proteins used allow infection of a large number of human cells and tissues.

In contrast, pseudotype vectors carrying the HIV-1 Env protein are able to target gene transfer to human CD4-positive cells. Initially, attempts to generate pseudotypes with the unmodified HIV Env protein failed [35]. However, the deletion of 144 amino acids from the HIV-1 TM cytoplasmic tail, which left a short C-tail comprising 7 amino acids, allowed the generation of infectious [MLV(HIV-1)] pseudotype particles [36]. The modified Env protein was efficiently incorporated into MLV vector particles and the particles feature the $CD4^+$-cell restricted tropism typical of HIV. More recently, efficient transduction of airway epithelial cells was observed by pseudotyping HIV-based vector with a filovirus envelope protein, offering a novel approach for the treatment of cystic fibrosis [37].

SU substitution

Envelope engineering aims at the modification of Env proteins by using polypeptides derived from growth factors, chemokines or antibodies. Generally, two types of retroviral vectors modified by envelope engineering can be distinguished: vectors which solely carry the engineered Env protein and vectors which carry the engineered Env protein in addition to the unmodified Env protein.

The list of polypeptides displayed on the Moloney MLV SU protein has substantially grown during the past years and includes scFv's directed against various cell surface antigens, several chemokines and growth factors (reviewed in [38, 39]). Still, very low targeting efficiencies ($<10^2$ cfu/ml on target cells) were observed in the absence of unmodified (wildtype (wt)) Env protein. Yet, efficient binding of the modified vector particles to the corresponding receptor molecules has been demonstrated in most cases.

In contrast to vector particles containing the modified Env protein only, the co-incorporation of unmodified Env protein has allowed the generation of more efficient vector types. This is especially the case for vectors derived from the avian Spleen Necrosis Virus (SNV), which is unable to infect human cells due to the lack of a suitable human cell surface receptor [40]. In the SNV vector targeting system, a scFv molecule has been covalently linked to the N-terminus of TM protein via a glycine-serine linker peptide. In the first generation SNV vectors the scFv was directed against di-nitrophenol [41]. Since then, successful extension of the host range of SNV vectors to human cells has been described by using four different scFv molecules. The scFv B6.2 molecule directed against a human breast and colon cancer cell surface marker allowed transduction of human colon carcinoma cells with titers of up to 10^4 efu/ml [42, 43]. Other scFvs molecules were directed either against the human CD34 antigen, the transferrin receptor, or the Her2neu antigen (overexpressed in many human breast cancer cases), all of which mediated transduction of human cells, in part with considerable efficiency (2×10^5 efu/ml) [44]. More recently, Engelstaedter et al. [45] demonstrated that scFv molecules suitable for cell targeting can be directly selected from phage display and SNV vector libraries. The scFv termed 7A5 was particularly able to mediate selective transduction of human T-cell lines with high efficiency. In addition, stimulated primary human peripheral blood cells were efficiently transduced. The 7A5-vector has an about five-

fold preference for T-cell versus B-cell transduction. Moreover, the T-cell specific 7A5-SNV envelope has also been used to pseudotype MLV vector particles generated in human packaging cells. Thus, the resulting T-cell specific MLV particles will be resistant to inactivation by human serum mediated by human complement. This is an important improvement of the system making the in vivo application of this vector type in humans possible [46].

Protease-activatable envelope proteins

As mentioned before, the display of ligands or growth factors on the MLV SU protein results in efficient binding of the vectors to cells expressing the corresponding receptor, however, the vectors do not enter or transduce the targeted cells. For some types of polypeptides displayed on the retroviral envelope, complete inhibition of virus entry via the natural receptor has been observed. Among these are polypeptides derived from high affinity growth factors which block gene transfer into cells over-expressing the respective growth factor receptor by sequestering the virus onto the targeted receptor and preventing it from interacting with its natural viral receptor. This type of blocking has been demonstrated for epidermal growth factor (EGF) [34], stem cell factor (SCF) [47] and insulin-like growth factor I (IGF-I) [48]. The efficiencies of blocking as revealed by titer reduction can vary between 100- to 1000-fold depending on the type of ligand displayed and the densities of targeted and natural receptors on the cell surfaces.

Based on the blocking domains, protease-activatable vectors have been generated by inserting protease cleavable linker peptides between the blocking domains and the N-terminus of the SU protein. Exposure of such vector particles to the relevant protease leads to cleavage of the blocking domain and thereby to restoration of cell entry and thus infectivity (Fig. 4). Thus, the specificity of cell targeting can be determined by the sequence of the linker peptide (encompassing the protease cleavage site) that anchors the blocking polypeptide to the viral envelope, provided that the cell type of choice expresses the receptor recognised by the blocking peptide. Initially, a Factor-Xa (protease) cleavage site has been used. The system has then been extended to vectors activatable by membrane-type matrix metalloproteinase (MT-MMP) and plasmin. The latter two proteases are naturally associated with the cellular membrane and/or the extracellular matrix and play significant roles in pathological processes [49, 50, 51]. Especially, the specific expression of activated proteases on the surface of tumour cells, which often express the epidermal growth factor receptor (EGFR), provides a unique opportunity to deliver therapeutic genes into cancer cells by using EGF-SU-modified retroviral vectors whose infectivity can be activated by cell surface-associated host proteases. Indeed, the MMP-targeted vectors have been shown to display a strong selectivity for MMP-rich human tumour xenografts in vivo in mice [52].

A current limitation of the protease-activatable vectors is the engineering of an optimal linker peptide that serves as an efficient protease cleavage site on the vectors used to target genes into the tumour type of interest. Until now, substrate cleavage site motifs have been identified only for a few proteases present in or on tumour cells, such as the MT-MMP. MLV display libraries developed recently may resolve this problem. Based on the concept of protease activation a molecular evolution approach has been developed that allows the in vivo selection of linker peptides that are efficiently cleaved by a given target cell type [53]. Further improvement of this system may allow the generation of vector particles specific for individual tumour cell types.

Perspectives

Gene therapy commenced in 1990 with a clinical trial focusing on the treatment of Adenosine Deaminase Deficiency (ADA), thus aiming at the treatment of inherited diseases. Since then, the focus of gene therapy has been extended to cancer, cardio-vascular and infectious diseases. The number of clinical trials performed world wide has exceeded 500 involving more than 3000 patients (www.wiley.com/genmed). However, the cure of a patient enrolled in any of these trials has not been demonstrated until very recently when a successful gene therapy trial on another inherited immunodeficiency, the Severe Combined Immunodeficiency Disease (SCID), has been published [1]. SCID is caused by a defective gene encoding the γ-cytokine receptor subunit of interleukin receptors. This success is believed to be a major breakthrough in human gene therapy, encouraging increasing efforts in the development of gene therapy strategies and methods, including improvement of C-type-derived retroviral vectors.

Further gene transfer medicinal products can be expected. Currently, many gene therapy trials are focused on the treatment of cancer using suicide genes [54, 55] or tumour suppressor genes [56]. Other strategies comprise stimulation of anti-tumoural immune responses, by transferring tumour cells expressing cytokine genes [57, 58] or displaying tumour antigens in the context of co-stimulatory molecules [59]. Furthermore, by delivery of the multidrug-resistance-gene into hematopoietic cells enhanced doses of chemotherapy may be tolerated and may allow a more aggressive treatment of certain cancers [60].

Efficient gene delivery is believed to be crucial for the future success of gene therapy. Among others, the development of cell targeting vector particles and vectors allowing efficient gene delivery to quiescent cells are important problems to be solved. Lentiviral vectors may allow an efficient gene delivery into a variety of human cells and long-term transgene expression. Particularly, experimental progress has been made on gene delivery to the eye, the pancreatic islet cells and CD34+ hematopoietic stem cells. Some of the most promising work involves gene transfer to the central nervous system for the potential treatment of Parkinsons disease. Encouraging developments in lentiviral vector design such as the establishment of inducible HIV-1 packaging cells and self-inactivating lentiviral transfer vectors are continuing. Furthermore, progress has been made in the generation of retroviral cell targeting vectors. Indeed, a first clinical trial using a targeted retroviral vector that localises to the proteins of the extracellular matrix of tumour cells is currently being initiated [61]. Combining lentiviral vectors with cell targeting envelopes may result in further progress.

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Buchbesprechung

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Umweltgifte

Ecomed Verlagsgesellschaft, Landsberg, CD-ROM, ISBN 3-609-58780-6, DM 98,–

So sind eben die modernen Zeiten. Jetzt gibt es das Handbuch der Umweltgifte auch in einer modernen CD-ROM Edition. Bequem und modern, die Installation gelingt ganz ohne Schwierigkeiten und im Handumdrehen stehen uns alle Umweltgifte auch für eine elektronische Recherche zur Verfügung.

Aber es ist nicht das ganze, bisher publizierte, Loseblattwerk, so wie es der unvoreingenommene User annehmen könnte, sondern es sind nur ausgewählte Teile und Kapitel aus dem Handbuch für Umweltgifte wie z. B. die Umweltmedizin aus der Sicht der klinischen Toxikologie, Erkrankungen durch Umweltgifte, Diagnostik-Therapie-Prävention, Labor, Recht, Einzelstoffinformationen und natürlich ein umfangreiches Glossar. Nur dies steht dem Leser zur Verfügung. Es ist sozusagen eine „abgespeckte" Version des Handbuchs. Schade eigentlich, so kann dann der ausgesprochene Vorteil einer CD-ROM, nämlich die elektronische Recherche, nicht in einem ausreichenden Umfang zur Verfügung stehen!

So wie es bereits aus seinen Loseblattwerken bekannt ist, stellt sich auch die Umweltmedizin auf dieser CD-ROM in ihrer der eigenen Logik und Polemik nach dem klinischen Toxikologen Max Daunderer dar. Zu den gefährlichsten Umweltgiften zählt er die Gifte, die während des Nachtschlafs auf den Menschen einwirken: Folgerichtig residieren dann Zahngifte bei ihm an erster Stelle, der Tabakrauch aber erst an vierter.

Die CD gibt einen großen Überblick über viele Bereiche der Umweltmedizin, d.h. von Anforderungen an den leitenden Notarzt für den Umwelteinsatz bis über das „Multiple Chemikalienüberempfindlichkeits-Syndrom" (MCS) bis hin zu diffusen Giftquellen. Aber gerade bei den Angaben zu den Giftquellen fällt auf, dass Herr Daunderer wahrscheinlich über Jahre keine eigenen aktuellen Produkt- oder Noxenrecherchen mehr betrieben hat: Blei im Benzin spielt heutzutage keine Rolle mehr, Bakelit kommt so gut wie nicht mehr zur Anwendung, Asbest in Dachpappen, Fensterkitt, Fliesenkleber, Katzenstreu und in Elektrogeräten ist mittlerweile vollständig ersetzt. Terpentinöle in Fußbodenklebern hat es wahrscheinlich nur vor sehr langer Zeit gegeben, ebenso wie die halogenierten Kohlenwasserstoffe in Fußbodenreinigern. Penta-Chlor-Phenol (PCP) findet sich sicher nicht mehr im Holzleim, ebenso wie Polychlorierte Biphenyle (PCB) in Leuchtstoffröhren und in Staubsaugern. Ebenso ist Methanol in Nitroverdünnern schon lange „out". Die Liste dieser Ungereimtheiten ließe sich noch lange fortsetzen. Hier wäre wohl eine kompetente Überarbeitung des gesamten Loseblattwerkes angebracht, wobei auch unbedingt die Literatur durch einen Lektor kritisch betrachtet werden müsste.

Die Frage stellt sich nun, welche Funktion die vorliegende CD-ROM hat und für welches Publikum sie gedacht ist? Sie ist lediglich ein Torso und stellt ein unvollständiges Nachschlagewerk für umweltmedizinische Fakten dar, wobei aber die Inhalte unbedingt einer kritischen Wertung bedürfen. Deshalb sollte der Ecomed-Verlag den Autor in die Verantwortung nehmen. Wenn unter dem Stichwort Prophylaxe lediglich: „Alle Gifte meiden! Verursacher offen legen, Schadenersatz" vermerkt ist, spricht eine derartige Darstellung nicht für eine ausreichend kompetente und differenzierte Eignung als Fachbuchexperte für Umweltfragen.

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