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Review

Surface-Engineered Viral Vectors for Selective and Cell Type-Specific Gene Delivery

Christian J. Buchholz,[1,2,*] Thorsten Friedel,[1] and Hildegard Büning[3,4,5]

Recent progress in gene transfer technology enables the delivery of genes precisely to the application-relevant cell type *ex vivo* on cultivated primary cells or *in vivo* on local or systemic administration. Gene vectors based on lentiviruses or adeno-associated viruses can be engineered such that they use a cell surface marker of choice for cell entry instead of their natural receptors. Binding to the surface marker is mediated by a targeting ligand displayed on the vector particle surface, which can be a peptide, single-chain antibody, or designed ankyrin repeat protein. Examples include vectors that deliver genes to specialized endothelial cells or lymphocytes, tumor cells, or particular cells of the nervous system with potential applications in gene function studies and molecular medicine.

Challenges in Gene Delivery

Gene delivery has become one of the most important technologies in basic life sciences and modern medicine including gene therapy and regenerative medicine. Recent progress resulted in benefit not only for patients suffering from inherited monogenetic diseases but also for cancer patients. Exciting examples include complete remission in 90% of patients with relapsed or refractory acute B cell leukemia by chimeric antigen receptor (CAR) T cell therapy [1], cure of various forms of inherited severe combined immunodeficiency syndromes following retransplantation of functionally corrected autologous hematopoietic stem cells [2,3], a substantial reduction in the need of prophylactic factor IX concentrate injections for hemophilia B patients following liver-directed application of adeno-associated viral (AAV) vectors, or the first marketing authorization of a gene therapy medicinal product in Europe [2].

In recent years, in particular two delivery systems, lentiviral (LV) vectors and AAV vectors are in the spotlight. LVs belong to the family of retroviral vectors, which stably integrate their genetic information into the genome of the transduced cell. They are therefore preferred when stem cells or dividing cells are to be genetically modified. AAV vectors remain episomally, and this is why they are best suited for terminally differentiated cells or if short-term gene expression in dividing cells is required. LV vectors can package larger genomes (up to 10 kb) than AAV vectors (4.5–5 kb), while the latter can be generated at higher titers, which together with their smaller size (AAV, 25 nm; LV, 125 nm in diameter), usually results in a more extensive penetration through tissue. They are therefore preferred for ***in vivo* gene transfer** (see Glossary).

For both medical and research purposes, the decisive goal for gene transfer is to deliver genetic information with high efficiency and safety exclusively to the cell type of interest not only in a cell culture dish but also *in vivo* after local or systemic administration. Then, loss of vector particles to

Trends

Numerous receptor-targeted viral gene vectors have been described during the past years using distinct cell surface proteins for cell entry that are selectively expressed on defined cell types instead of their natural broadly expressed receptors.

Receptor-targeting strategies based on directed evolution or rational engineering have been established. The latter is equally applicable to non-enveloped and enveloped vectors involving the destruction of natural receptor usage followed by the addition of a high-affinity ligand mediating attachment to the desired surface protein.

Receptor-targeted vector particles can be as selective for their targeted cell type as antibodies for their antigen when applied systemically or locally in preclinical studies.

Receptor targeting opens up novel concepts in gene therapy and the cell type-specific delivery of genetic material in life sciences.

[1]Paul-Ehrlich-Institut, 63225 Langen, Germany
[2]German Cancer Consortium, 69120 Heidelberg, Germany
[3]Institute of Experimental Hematology, Hannover Medical School, 30625 Hannover, Germany
[4]Center for Molecular Medicine Cologne (CMMC), University of Cologne, 50931 Cologne, Germany
[5]German Center for Infection Research (DZIF), partner sites Bonn-Cologne and Hannover-Braunschweig, Germany

*Correspondence: christian.buchholz@pei.de (C.J. Buchholz).

irrelevant or harmful cells (such as antigen-presenting cells) can be avoided and even the delivery of potentially problematic genes (toxic proteins, transcription factors) is envisaged. Since none of the naturally available viruses fulfills these requirements when converted into a viral vector, vector engineering is being performed. Here, two strategies can be distinguished, those modifying vector particle cell entry by vector surface engineering and those modulating post-entry steps by addressing regulatory sequences present on the **vector genome**. Post-entry strategies comprise the use of promotor sequences that are only active in the relevant cell type, while miRNA target sequences prevent gene expression in off-target cell types [4,5]. While such strategies can be effective, for example, in avoiding the induction of an immune response by preventing gene expression in antigen-presenting cells, they do not prevent loss of particles to irrelevant cells, which impacts on the efficiency of gene delivery, the immunogenicity of vector particles and the toxicity of certain gene products. Moreover, cell type-specific promoters are often large and therefore not compatible in particular with the restricted packaging capacity of AAV vectors. Engineering vector particles at their surface, by contrast, does not reduce the packaging capacity. By focusing on the first step in gene transfer, namely binding of the vector particle to its cell surface receptor, which mediates particle uptake, loss of vector particles to off-target tissue/cell types can also be diminished.

Concepts for Engineering Receptor Choice

The expression profile of membrane proteins that serve as viral receptors is an important determinant of vector **tropism** (others, such as virus restriction factors, also exist). Thus, cells deficient in receptor expression cannot be transduced. Notably, receptor expression may not only differ among different cell types but also depend on the activation status of the cell. Prominent examples are resting lymphocytes, which in contrast to activated lymphocytes, cannot be transduced with conventional vesicular stomatitis virus (VSV)–LV vectors pseudotyped with the glycoprotein G of the VSV. Switching the envelope glycoproteins can overcome this barrier (Box 1). Similarly, human hematopoietic stem cells have to be expanded prior to successful gene transfer by AAV vectors [6].

For LV vectors a large variety of envelope glycoproteins derived from different viruses can be used to modulate the tropism [7]. Likewise, the capsids of different AAV serotypes can be exploited to alter the receptor usage of AAV vectors [8]. For this purpose, AAV2-based vector genomes are packaged into the serotype capsid, which best suits the particular application (**pseudopackaging**), such as preferential **transduction** of liver (AAV2/8) or muscle (AAV2/1) after systemic or local injection. Although these AAV vectors exhibit a certain degree of preference for distinct organs, they are not selective for a defined cell type. Thus for both **pseudotyping** of LVs or pseudopackaging of AAV vectors the flexibility in receptor choice is restricted to the parental virus receptors that are expressed on many different cell types but do not define a distinct cell type.

Selective gene delivery exclusively into the cell type relevant for the desired application requires **receptor-targeted (RT) viral vectors**, which can be generated by engineering of the AAV capsid proteins or the glycoproteins incorporated into LV particles [9]. Rational-based and directed evolution-based engineering strategies have been established for both vector types. They differ fundamentally in the knowledge required about the targeted receptor present on the target cell. While rational engineering starts out from identifying a cell surface receptor that defines the relevant cell type, directed evolution relies on offering a large variety of viral particle variants from which those particles that deliver genes into the relevant cell type are selected [10] (Box 2). The cell surface receptor used for entry by the 'winner' of the selection can be identified thereafter, if required [11]. Moreover, selectivity of the resulting vector particles for its target cell type is mainly determined by the affinity and specificity of the targeting ligand and – in directed evolution – also by the conditions of the selection process.

Glossary

***ex vivo* gene transfer:** genetic modification of explanted cells in a cell culture dish before they will be retransplanted.
***in vivo* gene transfer:** gene transfer directly into an animal or a human.
Pseudopackaging: packaging the vector genome derived from one AAV serotype (often AAV2) into the capsid of another serotype.
Pseudotyping: incorporating envelope glycoproteins from one virus species into the envelope membrane of another virus species (often HIV-1 for LVs).
Receptor-targeted (RT) viral vector: viral vector that has been engineered to use a cell surface receptor different from its natural receptor for cell entry.
Transduction: introduction of genetic material into a cell by a viral vector.
Tropism: entirety of the cell types and tissues that are transduced or infected and in which transgene expression is accomplished.
Vector genome: encodes for the transgene expression cassette and contains a viral signal sequence (packaging signal) that marks this nucleic acid for packaging into viral capsids.