Transfer of therapeutic genes to patient cells has great potential to cure many types of diseases including monogenic disorders and cancer. Cell type-specific gene delivery, which restricts gene transfer to therapy-relevant cells, hence reducing unwanted side effects caused by the ectopic expression of transgenes by off-target cells, remains one of the biggest challenges to improve the safety and efficacy of gene therapy. Immune cells are a key target for genetic modification not only for gene therapy and immunotherapy, but also for functional genomics studies aimed at an improved understanding of the immune system. As the various cell types of the hematopoietic system can be readily distinguished by specific cell surface markers, restricting gene delivery at the step of cell attachment and entry is especially suited for immune system-related applications.

Among currently available gene delivery vehicles, lentiviral vectors (LVs) are unique in mediating long-term gene expression in resting as well as proliferating cells of the hematopoietic system. Moreover, genetic modifications introduced into stem cells will be transferred during differentiation to all their progeny. A flexible and specific cell targeting system for LVs is based on engineered measles virus (MV) glycoproteins, enabling LVs to use any surface molecule of choice for cell entry, and hence specifically transduce a cell type of interest. Cell type specificity is provided through a single-chain antibody (scFv) that recognizes a cell surface antigen selectively expressed on the cell type of interest fused to an MV hemagglutinin protein engineered to be deficient in natural receptor usage. Up to now, this approach has enabled the generation of a series of highly targeted LVs, including some with specificity for tumor antigens and for different types of hematopoietic cells. By displaying a CD20-specific scFv, we were able to develop LVs that are highly specific for human primary B lymphocytes, offering novel therapeutic options for B-cell based disorders or lymphomas. LVs targeted to MHC Class II molecules (MCH-II-LV) were very effective in delivering transgenes to dendritic cells (DCs) upon intravenous injection. DCs transduced by MHC-II-LV induced a robust immune response suggesting that this LV allows for the specific delivery of antigens to specific types of DCs in vivo. An example for a stem cell-targeting vector is represented by CD133-LV which delivers genes specifically to CD133+ hematopoietic stem cells (HSCs). CD133 defines HSCs with excellent long-term engraftment capabilities, which in the future may even be manipulated in vivo using CD133-LV. Below, we discuss our recent work on T lymphocyte-specific gene transfer, pointing to the potential usefulness of targeted LVs for cellular immunotherapy.

In an effort to improve the safety and efficacy of T cell receptor (TCR)-based gene therapy, we generated a CD8-specific gene delivery lentiviral vector, termed CD8-LV, to deliver genes exclusively and specifically to CD8+ T cells, one of the most important type of immune cell owing to its ability to directly recognize and kill tumor cells. The selectivity of CD8-LV for CD8+ cells relies on a scFv antibody fragment that was derived from the monoclonal antibody OKT8 and displayed on an engineered H protein. CD8-LV was highly efficient in transducing primary CD8+ cells ex vivo as well as in vivo, in humanized NOD-SCID-IL2Rγ− mice, while CD8− cells were protected from gene delivery. Moreover, in delivering more complex genes such as those coding for αβ TCRs, CD8-LV was more efficient than conventional non-targeted LVs (i.e., VSV-G-LV). When using CD8-LV for the transfer of a tyrosinase-specific TCR directed against melanoma, CD8-LV-transduced peripheral blood mononuclear cells (PBMCs) were substantially more efficient in killing melanoma tumor cells than PBMCs transduced by
Moreover, the unique property of OKT8 of inducing effector functions of CD8+ cells suggests that the multiple OKT8-derived scFv CD8 contacts formed during the entry of CD8-LV particles into CD8+ effector cells likewise trigger the activation of effector functions and enhance the killing activity of CD8-LV-transduced cells (Fig. 1). While further experimental data are required to validate this working hypothesis, our findings clearly demonstrate that the delivery of TCR-coding genes to CD8+ T cells as mediated by CD8-LV significantly enhances tumor cell killing and that this vector may therefore become a promising tool for immunotherapy.

Figure 1. Proposed mechanisms of enhanced tumor cell killing by CD8-LV-transduced T cells. For transduction with a tumor-specific TCR-coding gene, CD8-LV binds to CD8 via CD8-specific OKT8-derived fragments (blue triangle) displayed on the surface of the vector particle. CD8-LV-transduced cells express higher levels of CD8 than cells transduced by the conventional vector VSV-G-LV, which enters cells independently of CD8 (lower panel). The enhanced CD8 level (reflected by the number of CD8 molecules) and activation of T-cell effector functions promoted by the OKT8-derived fragment (depicted with a color change from blue to pink), enhances the sensitivity of the TCR expressed on CD8+ effector cells for peptide MHC (p-MHC) complex recognition and reduces the cell activation threshold. Consequently, upon tumor cell restimulation, CD8-LV-transduced cells become more activated (red color) than VSV-G-LV-transduced cells (orange color), producing higher levels of granzyme B (GrB) and perforin, and hence being more efficient at tumor cell lysis.

VSV-G-LV. This activity was accompanied by increased perforin and granzyme B levels. We altered the experimental set-up by normalizing for the number of effector cells and/or the number of non-effector cells in the transduced cell mixture, or using a pure culture of CD8+ T cells to exclude any effect mediated by CD4+ T cells. Remarkably, the strong antitumor response mediated upon CD8-LV transduction was uninfluenced.

These interesting and surprising findings prompted us to investigate the mechanisms responsible for the increased tumor cell killing by CD8-LV-modified T cells. Straightforward explanations including (1) a difference in the cell surface expression levels of transduced TCRs, (2) a difference in the release of cytokines such as interferon γ (IFNγ) and tumor necrosis factor α (TNFα), or (3) a different sensitivity of modified T cells for activation-induced cell death upon tumor antigen restimulation, were all excluded as causative. Instead, we identified a small population of CD8-LV-transduced T cells to be more activated and to express slightly but significantly higher levels of CD8 than their VSV-G-LV-transduced counterparts. This could be due to a preference of CD8-LV to transduce CD8high subpopulations and/or to the upregulation of CD8 expression upon CD8-LV binding. Since it is well established that the interaction between CD8 and MHC Class I molecules is critical for T-cell activation and hence for the lysis of target cells, we propose that higher levels of CD8, as observed in a small population of CD8-LV-transduced T cells but not among their VSV-G-LV-transduced cells, reduced the threshold for activation and increased lytic efficacy (Fig. 1). Moreover, the unique property of OKT8 of inducing effector functions of CD8+ cells suggests that the multiple OKT8-derived scFv/CD8 contacts formed during the entry of CD8-LV particles into CD8+ effector cells likewise trigger the activation of effector functions and enhance the killing activity of CD8-LV-transduced cells (Fig. 1). While further experimental data are required to validate this working hypothesis, our findings clearly demonstrate that the delivery of TCR-coding genes to CD8+ T cells as mediated by CD8-LV significantly enhances tumor cell killing and that this vector may therefore become a promising tool for immunotherapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
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