Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Retroviral vector interactions with hematopoietic cells

Elizabeth M Everson[1] and Grant D Trobridge[1,2]

Hematopoietic stem cell (HSC) gene therapy using retroviral vectors is a powerful and promising approach to permanently correct many hematopoietic disorders. Increasing the transduction of quiescent HSCs and reducing genotoxicity are major challenges in the field. Retroviral vectors, including lentiviral and foamy vectors, have been extensively modified resulting in improved safety and efficacy. This review will focus on recent advances to improve vector entry, transduction efficiency, control of transgene expression and approaches to improve safety by modifying the retroviral integration profile.

Addresses
[1] Department of Pharmaceutical Sciences, Washington State University, Spokane, WA 99210, United States
[2] School of Molecular Biosciences, Washington State University, Pullman, WA 99164, United States

Corresponding author: Trobridge, Grant D (grant.trobridge@wsu.edu)

Current Opinion in Virology 2016, **21**:41–46

This review comes from a themed issue on **Viral gene therapy vector-host interaction**

Edited by **Roland Herzog** and **David Markusic**

http://dx.doi.org/10.1016/j.coviro.2016.07.010

Introduction

Hematopoietic stem cell (HSC) gene therapy using retroviral vectors has the potential to cure many diseases including primary immunodeficiencies, enzyme deficiencies and even acquired diseases like HIV. In this approach, gene modified HSCs self-renew and differentiate providing a life-long supply of hematopoietic cells with a therapeutic transgene. Although HSC gene therapy is promising, the appearance of serious adverse events in early HSC gene therapy clinical trials as a result of vector-mediated genotoxicity has limited its use clinically. It has also resulted in significant efforts to better understand the interactions of retroviral vectors with hematopoietic cells. This review will focus on recent advances made in improving vector entry, regulating expression of the therapeutic transgene, and reducing the genotoxicity of retroviral vectors for HSC gene therapy.

Vector entry

Retroviruses have an envelope protein on their outer surface that binds to host cell surface molecules and mediates viral entry. After binding, the retroviral and cellular membranes fuse, allowing the retroviral core to gain entry into the host cell cytoplasm. The retroviral envelope protein is a primary determinant of viral tropism. Envelope pseudotyping, replacing the native envelope protein with a heterologous envelope protein from a different enveloped virus, can expand, limit, or alter vector tropism. Pseudotyping can also increase the efficiency of gene transfer and the stability of vector particles. Various pseudotypes have been incorporated into retroviral vectors including vesicular stomatitis virus glycoprotein G (VSV-G), gibbon ape leukemia virus, feline endogenous RD114 virus and the cocal pseudotype [1,2,3,4,5••]. Of these, VSV-G is the most popular and well-studied due in part to its broad tropism. The VSV-G pseudotype mediates efficient gene transfer into large animal long term repopulating cells [6,7] and allows the vector to be efficiently concentrated *via* centrifugation [1]. However, VSV-G pseudotyped vectors are toxic, which has led to difficulty in developing stable vector-producing cell lines [8]. Also, VSV-G can be inactivated by human sera, limiting it's efficacy for *in vivo* delivery [9].

The cocal vesiculovirus envelope glycoprotein is an intriguing alternative to VSV-G. The cocal virus is a member of the genus *Vesiculovirus* and causes vesicular stomatitis primarily in cattle and horses [4]. The cocal virus glycoprotein is 71.5% identical at the nucleic acid level to VSV-G Indiana and is thought to use the same receptor as VSV-G for cell entry [4]. Similar to VSV-G, the cocal envelope has a broad tropism and mediates efficient gene transfer to human primary cells derived from blood, lung fibroblasts, retinal epithelia, bone epithelia, skin fibroblasts and stroma [4]. Cocal pseudotyped vector virions are more resistant to inactivation by human sera than VSV-G pseudotyped vector virions and may be more effective for gene delivery *in vivo*. Recently, a high titer 3rd generation self-inactivating lentiviral producer cell line was generated based on the cocal envelope [5••]. In a direct comparison, cocal pseudotyped lentiviral vectors were produced in packaging cells at higher titers than with the VSV-G pseudotype [5••]. Cocal pseudotyped lentiviral vectors also transduce human and non-human primate $CD34^+$ and $CD4^+$ cells more efficiently than VSV-G [4,5••].

Approaches to increase transduction efficiency

Cell cycle requirements play a key role in determining how effective the retroviral vector will be for use in HSC gene therapy where the target cell is quiescent. Gammaretroviral vectors can only transduce actively dividing target cells [10]. Lentiviral vectors are able to transduce

quiescent cells [11], however, they are much more efficient at transducing cells in the G_{1b} stage of the cell cycle [12]. Foamy viral vectors require mitosis for transduction, however, a major benefit of foamy viral vectors is that they can form a stable intermediate that can be maintained in quiescent cells [13]. Currently, cytokines are commonly used during *ex vivo* culture to stimulate cells to progress into the cell cycle. Commonly, fms-like tyrosine kinase 3, stem cell factor, granulocyte stimulating factor, thrombopoietin, interleukin-3 and interleukin-6 are used [14,15]. The use of CH-296 fibronectin, a recombinant fragment of human fibronectin, increases transduction and engraftment of gene-modified cells in patients by promoting co-localization of the retroviral vector with hematopoietic cells and also by maintaining HSC engraftment capacity [16,17]. An immobilized Notch ligand Delta-1 can also be used to accelerate hematopoietic engraftment in combination with cytokines [18].

Another approach to improving transduction is to overcome the restriction mechanisms that cells have evolved to inhibit viral infection. Inclusion of the drug rapamycin during *ex vivo* culture has shown promise in this regard. Rapamycin inhibits the mammalian target of rapamycin (mTOR) kinase and is commonly used as an immunosuppressant to prevent transplant rejection. The mTOR pathway regulates HSC quiescence. Rapamycin greatly increased lentiviral gene delivery without impairing engraftment of both primitive mouse HSCs and human primitive SCID-repopulating $CD34^+$ cells [19••]. Rapamycin appears to increase vector transduction by enhancing cytoplasmic entry events after vector binding [19••].

Obtaining appropriate transgene expression

For gene therapy to be successful, sufficient transgene expression must occur to correct a disease phenotype. However, a strong promoter can dysregulate nearby genes leading to oncogenesis. House-keeping promoters with less oncogenic potential, such as phosphoglycerate kinase 1 (PGK) and elongation factor-1α (EF1α), are being proposed for clinical trials. A systematic comparison of the most commonly used constitutive promoters demonstrated that EF1α promoters appear to be consistently strong in a variety of cell types [20]. A direct comparison in a competitive repopulation experiment in X-SCID canines showed that PGK was more effective than EF1α for immune system reconstitution [21]. X-SCID canines were simultaneously injected with equal titers of foamy viral vectors carrying a therapeutic gene driven by a PGK or EF1α promoter and a corresponding reporter gene. The PGK-driven vectors achieved lymphocyte marking of over 30% by 50 days post treatment and expanded to roughly 80% by 300 days post treatment. In comparison, EF1α resulted in approximately 10% lymphocyte marking at all time points [21]. These studies suggest that disease-specific testing *in vivo* may be necessary to identify an ideal promoter.

Several approaches are being developed to reduce the expression of the therapeutic transgene in non-target cells. While off-target expression does not appear to be deleterious for many diseases, some diseases require very specific transgene expression. For example, in the case of Wiskott-Aldrich syndrome, expression of the transgene is highly toxic in non-hematopoietic cells. In a recent Phase I/II clinical trial for Wiskott-Aldrich syndrome, a lentiviral vector driven by the Wiskott-Aldrich syndrome protein gene-specific promoter resulted in robust and stable multilineage engraftment of gene-corrected hematopoietic cells in three patients [22]. Transcriptional targeting using microRNAs (miRNAs) can also be used to reduce off-target transgene expression. The combination of similar miRNA target sequences for a specific cell type has been shown to cooperatively reduce gene expression by 100-fold in human U937 monocytes and primary dendritic cells [23,24]. For more details Gentner *et al.* provide an excellent review on the various applications of miRNA manipulation [25]. For HSC gene therapy, retroviral vectors can be modified to contain sequences in the transgene cassette that are complementary to miRNAs specifically found in off-target cell types. Thus, if the transgene is expressed in the wrong cell type or blood lineage, the specific miRNAs will bind and inactivate the mRNA transcript (Figure 1).

Several miRNA targets have been discovered that are lineage and/or differentiation-stage specific to reduce off-target expression for HSC gene therapy. miR-223 can de-target transgene expression from myeloid cells [24] whereas miR-150 can de-target transgene expression from mature T and B lymphocytes [26]. miR-144/451 is a positive regulator of late erythroid maturation and could have applications for hemoglobinopathies [26]. Examples of differentiation-stage specific miRNA targets include miR-181a, miR-155, miR-126 and miR-130a. miR-181a can be used to inhibit expression in immature T-cells while allowing for transgene expression in mature T-cells which may be beneficial in cancer immunotherapy [27]. miR-155 can be used to inhibit expression in immature dendritic cells while allowing for expression in mature dendritic cells [24], which might be helpful in correcting autoimmune diseases. miR-126 and miR-130a are both selectively expressed in immature HSCs but not prevalently expressed in differentiated hematopoietic cells. Thus, adding a target sequence for miR-126 into a retroviral vector can allow for transgene expression in differentiated hematopoietic cells while inhibiting expression in primitive HSCs. The addition of four tandem miR-126 target sequences strictly targeted expression of the therapeutic transgene to differentiated hematopoietic cells, sparing HSCs and progenitors completely [28].

There have been recent successes in the use of dual regulated retroviral vectors [29,30••] that employ a gene or tissue-specific promoter to target the correct tissue and miRNA targets to eliminate expression in off-target cells. A