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Retargeting of herpes simplex virus (HSV) vectors

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Gene therapy applications depend on vector delivery and gene expression in the appropriate target cell. Vector infection relies on the distribution of natural virus receptors that may either not be present on the desired target cell or distributed in a manner to give off-target gene expression. Some viruses display a very limited host range, while others, including herpes simplex virus (HSV), can infect almost every cell within the human body. It is often an advantage to retarget virus infectivity to achieve selective target cell infection. Retargeting can be achieved by (i) the inclusion of glycoproteins from other viruses that have a different host-range, (ii) modification of existing viral glycoproteins or coat proteins to incorporate peptide ligands or single-chain antibodies (scFvs) that bind to the desired receptor, or (iii) employing soluble adapters that recognize both the virus and a specific receptor on the target cell. This review summarizes efforts to target HSV using these three strategies.

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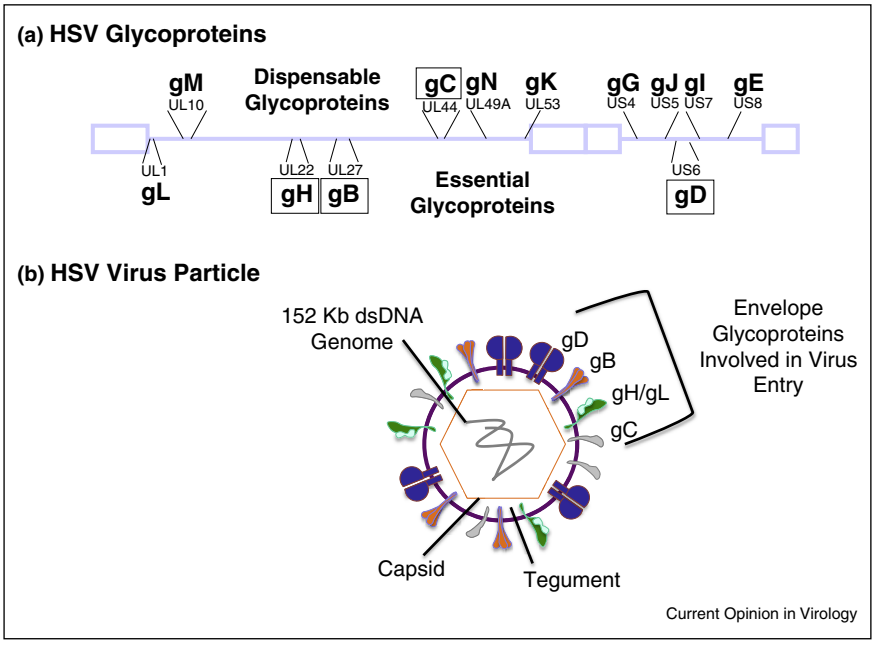
Introduction

With the approval of Glybera (alipogene tiparvovec) in Europe [1,2] for the treatment of lipoprotein lipase deficiency and IMLYGIC (T-VEC, talimogene laherparepvec) [3,4] in the US [5] for the treatment of malignant melanoma, gene therapy is beginning to show promise as an approved alternative to drug and radiation therapies in the treatment of human disease. However, further progress will require the refinement of gene therapy approaches before it becomes a true everyday therapeutic. Current gene therapy applications, such as Glybera, often rely on tissue-specific promoters to limit therapeutic gene expression to specific cells. Although this strategy helps minimize off-target effects, it does not ensure delivery of the viral vector to the intended cell or tissue. One strategy being employed to refine gene therapy is the use of transductional targeting to limit viral vector infection to only the desired target cell, a strategy called 'retargeting'.

Limiting vector transduction to specific cell types can benefit many gene therapy applications, including those for chronic pain and cancer. For example, in pain gene therapy, most standard approaches fail to limit infection to specific peripheral nerve fiber subtypes. Ideally, one only wants to silence the activity of C-fiber neurons that are the nociceptors involved in pain sensation, while avoiding nerve fibers that regulate proprioception, pressure or itch so that they will function normally. Cancer gene therapy approaches have recently drawn interest due to the success of T-VEC. However, even that oncolytic herpesvirus (oHSV) is not restricted to which type of cells it can transduce, but is designed to selectively replicate in rapidly dividing tumor cells based on the absence of the viral *ICP34.5* gene. As an alternative approach to limiting oHSV infection and lysis to tumor cells, our group and the groups of Campadelli-Fiume and Roizman have designed retargeted oHSV that preferentially infect breast or brain tumors. In this strategy, viral envelope glycoproteins that bind to widespread cellular receptors for HSV, are modified to ablate natural receptor binding and incorporate ligands or single-chain antibodies (scFv) that recognize the human epidermal growth factor (EGF) receptor 2 (HER2) [6••,7••], the interleukin-13 receptor IL-13Rα2 [8•], or the EGF receptor (EGFR) [9••,10••] that are often over-expressed in these and other tumors.

All viruses display a natural tropism for specific cell types, tissues and organs within the body. Tropism-determining factors include how the virus (i) encounters the host, (ii) attaches to host cell receptors that enable entry, (iii) establishes itself within the host, (iv) influences pathogenesis and disease, and (v) counteracts the host immune response. Retargeting can be performed to either expand the tropism of viruses that infect only a very limited number of cell types or, more commonly, to restrict the tropism of viruses that infect many cells of the host. Non-enveloped viruses employ one or more viral capsid proteins to interact with the host cell, while enveloped viruses use one or more glycoproteins to bind to and enter host cells. Although many enveloped viruses, such as measles, influenza or HIV, employ a single or two glycoproteins to achieve cell binding and entry, members

Figure 1



HSV glycoproteins. **(a)** The location of the HSV-1 encoded viral glycoproteins within the HSV-1 genome is depicted. Those listed below the viral genome are essential for virus replication, while those above it are non-essential and their deletion does not block virus replication in culture or *in vivo*. However, these dispensable glycoproteins can contribute to virus host range, pathogenesis and the host response to viral infection *in vivo*. Glycoprotein K (gK) is not found in the envelope of mature virus particles. Glycoproteins in boxes have been altered in efforts to retarget HSV. **(b)** The HSV particle is composed of an icosahedral-shaped capsid containing a 152-kb double-stranded linear DNA genome. The viral nucleocapsid is surrounded by an amorphous tegument layer consisting of both viral and cellular proteins. The virus envelope contains 12 viral glycoproteins, those essential for entry (gD, gB, gH/gL) and modified for vector retargeting (gD, gB, gC, gH) are depicted.

of the herpesvirus family rely on an array of glycoproteins to enter cells and spread from infected to uninfected cells. Herpes simplex virus (HSV) encodes 12 different glycoproteins (Figure 1a) and uses glycoproteins B, C, D, E, H, I, K and L for entry and cell-to-cell spread within the host (reviewed in [11–13]). Most of these glycoproteins contribute to viral tropism, making retargeting of HSV a distinct challenge. HSV retargeting is a worthwhile pursuit, however, given the attractive features HSV offers as a gene therapy platform, including a capacity to accommodate very large or multiple transgenes and infection without integration into host chromosomes.

Years ago we performed some of the first retargeting studies with HSV by genetic fusion of full-length erythropoetin (EPO) to HSV glycoprotein C (gC) that had been N-terminally truncated to eliminate attachment of the virus to cellular heparan sulfate proteoglycans (HS or HSPG) in the background of a virus lacking the HS-binding region of gB [14]. Although this resulted in entry of the gC-EPO virus into EPO receptor-bearing cells, the virus was still able to enter cells that possess the natural receptors for HSV entry. Thus, we had expanded HSV's tropism rather than restricting it to EPO receptor-bearing cells. In order to achieve full retargeting of HSV, virus interaction with the canonical HSV entry receptors must be blocked or eliminated and functionally replaced with alternate ligand–receptor interactions. The choice of alternate receptors is limited to candidates that are markers for the target cell and are recognized by peptide ligands or single-chain antibodies (scFvs) that preferentially do not activate the normal physiological function of the receptor. As summarized below, studies by a number of laboratories over the past 20 years have greatly enhanced out understanding of HSV entry, ultimately allowing the development of a first generation of fully retargeted HSV vectors.

HSV attachment and entry

The HSV particle (Figure 1b) is composed of an icosahedral-shaped nucleocapsid containing the 152-kb double-stranded DNA genome (Figure 1a), an amorphous tegument layer consisting of viral and cellular proteins, and a lipid envelope acquired from the host cell that contains the 12 viral glycoproteins. The glycoproteins are grouped according to whether they are essential or non-essential for HSV entry [15–18]. Another group of important players in the HSV entry process are the cellular receptors that HSV normally engages to achieve attachment and entry. Initially, HSV gB and gC bind via arginine-rich and lysine-rich regions to negatively-charged HSPG present on