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Receptors and tropisms of envelope viruses

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Abstract

Envelope virus replication begins with receptor binding, followed by fusion of the viral envelope with the cell membrane. The binding and fusion steps are usually mediated by envelope proteins. The ability of envelope proteins of a particular virus to bind and fuse with target cells defines the host range of the virus, known as “viral tropism”. The mechanism(s) of fusion by the viral envelope is largely categorized as either pH-dependent or –independent. By redirecting the binding specificities of envelope proteins to desired target molecules while maintaining fusion activity, it is possible to redirect the tropisms of virus and viral vectors, enabling specific killing and/or transduction of desired cells *in vivo*. Recently, a lipid, phosphatidylserine, was also shown to mediate binding of virus, which affects the tropisms of viruses and viral vectors.

Introduction

Tropism of a virus pertains to the types of cells, tissues, and animal and plant species in which it can replicate [1,2]. Because various replication steps of viruses require host proteins, the expression levels of such host proteins in a cell affect tropism. In addition, expression levels of host proteins that inhibit replication of a virus can affect its tropism. Although the steps required for replication of viruses differ between species, replication of all animal viruses begins with attachment of the virus to host cells. Therefore, expression levels of the target receptor for a given virus largely determine the tropisms of viruses and viral vectors.

After attachment, viruses deliver their genomes into the cytoplasm of target cells. In the case of envelope viruses, fusion between the viral envelope and the cell membrane enables delivery of the genome. Binding and fusion of envelope viruses are usually mediated by viral envelope proteins. Although attachment of the virus is a typical step for replication of both envelope and non-envelope viruses, the fusion step is unique to envelope virus replication. The fusion mechanisms of most envelope viruses are categorized as either pH-

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dependent or -independent [3] (a few viruses, including ASLV [4] and HSV-1 [5], use more complicated fusion mechanisms, which will not be discussed in this review).

In the case of pH-dependent fusion, exposure of envelope proteins to the low pH of the endosome elicits fusion activity. Regardless of the types of receptors bound, fusion activity is elicited as long as the virus is endocytosed and exposed to low pH environment. In the case of pH-independent fusion, envelope proteins need to bind specific receptors to elicit fusion activity. Certain receptors can bind envelope proteins but cannot elicit fusion activity. Tropism of the envelope virus is affected by the efficiency of envelope proteins in mediating both binding and fusion.

Viral vectors are used to deliver diagnostic and therapeutic transgenes [6,7] and several types of replication-competent viruses [8] are used to kill cancer cells in clinical settings. However, if such viral vectors also deliver the transgene(s) to the cells and tissues where the action of transgenes should not take place, and if virus kills non-cancerous cells more efficiently than cancer cells, their adverse effects would exceed their therapeutic effects. The ability to control the tropisms of virus and viral vectors by making them specifically bind and fuse to desired cell and tissue types would make it possible to use viruses as tools to deliver transgenes specifically to intended sites of action for diagnostics and treatment and of diseases such as cancers.

This review describes the mechanisms of pH-dependent and -independent entry, using influenza virus and HIV as examples, respectively. The description of each entry mechanism will be followed by a strategy of modification of envelope proteins to redirect the tropisms of the viruses and viral vectors using that form of entry. In addition, we discuss a novel and newly described mechanism of virus binding, whereby envelope lipids can mediate binding of virus.

1A) pH-dependent entry

Influenza virus contains an envelope protein, hemagglutinin (HA), which mediates binding and fusion. HA consists of two subunits, HA1 and HA2 (Figure 1A) [9]. HA1 mediates virus binding and HA2 mediates fusion. The receptor for HA1 is terminal sialic acid (SA) of N- and O-linked glycans. Influenza virus binds to cells via an interaction between HA1 and sialic acid. The virus is subsequently endocytosed, and the low pH of the endosome elicits fusion activity of HA2 [10,11].

The structures of terminal SA are known to be important for the tropisms of human and avian influenza viruses. Human influenza virus HA1 preferentially binds the SA of the α 2–6 linkage, while avian influenza virus HA1 prefers SA of the α 2–3 linkage. Human tracheal and bronchial epithelia predominantly express α 2–6 SA. α 2–3 SA is abundantly expressed at the sites of infection in birds, so it is likely that expression levels of α 2–6 SA and α 2–3 SA at the portal sites for virus entry contribute to the differing tropisms of human and avian influenza viruses [12].

Recently, DC-SIGN, a C-type lectin expressed on antigen-presenting cells, was shown to bind HA. DC-SIGN binds to N-glycans of HA, and can mediate entry and infection of SA-negative cells by influenza virus [13]. These results demonstrate that binding of HA to SA is not mandatory for entry of influenza virus. As such, entry via the pH-dependent mechanism is likely to be mediated by any receptor, providing the virus is endocytosed.

1B) Redirecting virus entering cells via the pH-dependent mechanism

Redirecting the tropisms of virus and viral vectors requires both elimination of the original receptor-binding regions of envelope proteins and conferring binding specificity by conjugating targeting ligands with viruses and viral vectors. Any receptor that mediates endocytosis of virus is sufficient for triggering pH-dependent fusion. Thus, artificial binding mediated by conjugated ligands can trigger fusion activity of viruses that enter cells via the pH-dependent mechanism. Ohno et al. redirected the tropism of Sindbis virus vectors [14]. Sindbis virus enters cells in the pH-dependent manner after binding to the heparin sulfate and laminin receptors [15–17]. Mutation of the original receptor-binding regions of the Sindbis virus envelope protein abolishes infectivity for its native host. Conjugation with targeting ligands enables infection of specific types of cells recognized by the targeting ligands (Figure 1B). Since the Sindbis virus envelope protein allows specific and efficient targeting, it was also utilized to redirect other types of virus and viral vectors. Bergman et al. redirected the tropism of vesicular stomatitis virus by pseudotyping it with modified Sindbis virus envelope proteins [18]. We also redirected γ -retroviral and lentiviral vectors by pseudotyping them with modified Sindbis virus envelope proteins [19]. Since fusion of the Sindbis virus envelope proteins does not require an interaction with its original receptors, we extensively mutated its original receptor-binding regions, which reduced the transduction of untargeted tissues, including the liver and spleen, and enabled targeted transduction of desired cells types *in vivo* [20]. We conjugated targeting ligands with various regions of the envelope proteins, and the tropism of the virus was redirected regardless of the sites of ligand conjugation [21,22]. The targeting ligands conjugated independently of the viral envelope protein using their own transmembrane domains, such as the transmembrane domain of membrane-immunoglobulin, could also redirect the vectors [23]. We used many different targeting ligands, including non-covalently conjugated antibodies and a soluble protein (transferrin), covalently conjugated integrin-targeting peptides, single-chain antibodies (ScFv), and high-mannose structure N-glycans that bind DC-SIGN. Magnets conjugated with the virus could also redirect the tropism of the virus when magnetic force was applied to mediate binding of virus and cells [24]. These results demonstrated that pH-dependent entry could be accomplished by binding with any receptor and by any means of binding.

2A) pH-independent entry

HIV is generally thought to enter cells in a pH-independent manner. The HIV envelope protein, gp160, consists of two subunits, gp120 and gp41. gp120 mediates binding, and gp41 mediates fusion. The fusion activity of gp41 is elicited by a signal from gp120, which is generated by the interaction of gp120 with its receptors. gp120 binds to CD4, which induces conformational changes of gp120 [25]. These conformational changes enable gp120 to bind to its co-receptor(s), CXCR4 and/or CCR5 (Figure 2A) [26,27]. The binding of gp120 to co-receptors elicits the signal that activates fusion activity of gp41. Selective killing of CD4-positive cells occurs during HIV infection, which can be explained in part by HIV tropism. The types of co-receptors used by HIV vary between strains. gp120 of X4-tropic strains can use CXCR4 as its co-receptor, but not CCR5, whereas gp120 of R5-tropic strains of HIV can use CCR5 but not CXCR4. The differences in co-receptor usage determine the tropisms of HIV strains.

DC-SIGN also binds to N-glycans of gp120, although it cannot induce conformational changes of gp120 [28]. DC-SIGN captures HIV on cells and increases the concentration of the virus on the cell surface, which facilitates gp120 interaction with CD4 and HIV co-receptors [29]. Therefore, DC-SIGN expression can enhance replication of the virus in cells expressing CD4 and co-receptors. However, if DC-SIGN-positive cells lack CD4 and/or co-

receptors for HIV, the virus can still bind to the cells, but cannot mediate fusion and infect the cells. While influenza viruses do not require SA for DC-SIGN-mediated infection, HIV requires its cognate receptors for entry, even though virus attachment is effectively mediated by other types of receptors.

2B) Redirecting virus entering cells via the pH-independent mechanism

Because the pH-independent entry mechanism requires a series of interconnected events, beginning with the envelope protein binding with specific receptors, it has been difficult to redirect the tropisms of viruses that use this entry pathway. Redirecting the tropism of this type of virus/viral vector was initially performed using γ -retroviruses [30]. Although specific binding of γ -retroviral vectors can be mediated by conjugation of targeting ligands, this artificial binding could not efficiently mediate the fusion step; thus, the redirected viruses were poorly infectious [31]. Successful redirection of pH-independent entry has been demonstrated with the measles virus. The measles virus envelope protein consists of H and F proteins, which mediate binding and fusion, respectively. The original receptors of measles virus are CD46 and CD150 [32] [33]. Binding of the H protein to these receptors elicits the signal that triggers the fusion activity of F proteins [34,35]. Nakamura et al. mutated the H protein to eliminate its interaction with CD46 and CD150, and conjugated it with ScFv at its C-terminus (Figure 2B) [36]. Binding of the conjugated ScFv with target antigens could elicit the signal triggering fusion activity of the F protein, thereby successfully redirecting the tropism of the virus. Redirected measles virus envelope proteins are also used for pseudotyping lentiviral vectors and modifying their tropisms [37]. These results indicate that measles virus fusion activity can be induced by artificial binding mediated by any targeting ligands. However, recent studies showed that: 1) targeting ligands inserted into certain regions of the H protein could not induce fusion despite mediating binding [35]; and 2) N-glycans of measles virus envelope proteins could bind DC-SIGN but that binding could not induce fusion [38]. Therefore, regulating the fusion step of retargeted measles virus envelope proteins still requires a particular means of binding, which has not yet been fully elucidated.

3) Virus binding mediated by envelope lipids

As described above, binding of viruses has been thought to be mediated by envelope proteins and/or its N-glycans. We recently found that one type of envelope lipid, phosphatidylserine (PtdSer), can also mediate attachment of virus [39]. PtdSer is one of the components of the cellular lipid bilayer. It usually resides in the inner leaflet of the cell membrane; however, it is exposed on dead cells, and detection of exposed PtdSer has been used for identifying apoptotic cells [40]. PtdSer is known to be exposed on the surface of several envelope viruses, including vaccinia virus [41], HIV [42], and Pichinde virus [43]. Exposed PtdSer on these viruses was shown to facilitate replication of these viruses, although the role of PtdSer in virus replication is unknown.

We found that a serum protein, Gas6, enhances infection of replication-competent vaccinia virus and lentiviral vectors pseudotyped with various types of envelope proteins, including Sindbis virus, Ross River virus, and baculovirus envelope proteins. Gas6 was originally known to mediate phagocytosis and to remove dead cells by bridging exposed PtdSer on dead cells to Axl (one type of tyrosine kinase receptor) expressed on phagocytes (Figure 3) [44]. We found that the viral envelope of PtdSer also binds Gas6, and Gas6 mediates binding of virus to Axl on target cells. This means of entry, apoptotic mimicry, plays a dominant role when the binding of virus mediated by viral envelope proteins is weak; therefore, this entry mechanism can expand the host range (tropism) of the virus. Indeed, ectopic expression of Axl was shown to transform non-susceptible cells to be susceptible for

Ebola virus infection [45]. The concentrations of Gas6 and expression levels of Axl are reported to be up-regulated by blood vessel injury and inflammation. Therefore, this entry pathway may contribute to infection by viruses that efficiently use the sites of inflammation and/or injury as portal sites. We showed viral entry mediated by four types of viral envelope proteins is enhanced by this apoptotic mimicry pathway. All of the envelope proteins we tested used pH-dependent entry. Since PtdSer should be present on many types of envelope viruses, we should test more types of virus and viral envelope proteins using this entry pathway to fully understand the tropisms of the viruses.

As described in the sections 1B and 2B, the previous attempts to redirect envelope viral vectors were focused on both eliminating original receptor-binding regions of the viral protein and conjugating virus with proteins that specifically bind target cells and tissues. Since a lipid can mediate binding of virus, modification of envelope lipids should be considered when eliminating original tropism of viral vectors and/or redirecting viral tropism.

Perspective

We have described how the molecular mechanisms of attachment and fusion define the tropisms of viruses. Understanding the binding and fusion mechanisms of viruses has enabled development of virus and viral vectors that can transduce specific cell and tissue types *in vivo*. Because attachment and fusion are necessary for envelope viruses to replicate in target cells, blocking these steps by inhibitors can effectively block viral infection. For example, an anti-PtdSer antibody was shown to inhibit replication of Pichinde virus *in vivo* [43]. Studies of viral apoptotic mimicry mechanisms used by other viruses will broaden the application of anti-PtdSer antibodies to other viral diseases. Entry inhibitors have become well developed for therapy of HIV infection. HIV entry inhibitors are categorized into three types according to target steps of viral entry: 1) the interaction of gp120 and CD4; 2) the interaction of gp120 with co-receptors; and 3) the fusion activity of gp41 [46]. The therapeutic effects of these inhibitors were demonstrated in clinical settings, emphasizing how understanding viral entry mechanisms can contribute to new therapeutic approaches for virus infection. Identification of novel virus entry pathways commonly used by various types of viruses could suggest innovative antiviral approaches that can be explored as potentially effective treatments for various types of viral diseases [47] [48]. Further investigation of virus entry mechanisms will contribute to both the ability to treat viral infections and develop therapeutic applications that use viruses.

Highlights

- Receptors for envelope viruses mediate binding and fusion of virus.
- The fusion step is mediated in either a pH-dependent or –independent manner.
- Viral tropism can be determined by expression levels of viral receptors on target cells.
- The tropisms of viruses can be changed by modifying their receptor-binding specificity.
- The envelope lipid, phosphatidylserine, can mediate binding of virus.

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• of special interest

•• of outstanding interest

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Figure 1.

A) pH-dependent entry of influenza virus. The HA protein is shown as a monomer to simplify the entry mechanism; however, it forms trimers. B) pH-dependent entry of retargeted Sindbis virus. The heterodimer of E2 and E1 is shown as a monomer to simplify the entry mechanism; however, the heterodimer forms trimers.

Figure 2.

A) pH-independent entry of HIV. gp160 is shown as a monomer to simplify the entry mechanism; however, it forms trimers. B) pH-dependent entry of retargeted measles virus. H and F are shown as a monomer to simplify the entry mechanism; however, H forms tetramers and F forms trimers.

Figure 3.
Schematic representations of the mechanism by which hGas6 mediates phagocytosis of dead cells and the apoptotic mimicry pathway for virus entry.