Pharmacological Research 159 (2020) 105038

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Review

Ephrin-Eph signaling usage by a variety of viruses

Esther C.W. de Boer[a], Janine M. van Gils[b,*], Marit J. van Gils[a,*]

[a] Department of Medical Microbiology, Amsterdam UMC, Amsterdam Infection & Immunity Institute, University of Amsterdam, Meibergdreef 9, Amsterdam, the Netherlands

[b] Einthoven Laboratory for Vascular and Regenerative Medicine, Department of Internal Medicine, Leiden University Medical Center, Leiden, the Netherlands

ARTICLE INFO

Chemical compounds studied in this article:
Dasatinib (Pubchem CID: 3062316)
Chloroquine (Pubchem CID: 2719)
Nicotine (Pubchem CID: 89594)

Keywords:
Ephrin-Eph signaling
Virus infection
Viral entry
Lymphocyte migration
Tyrosine kinase
Henipavirus

ABSTRACT

Ephrin-Eph signaling is a receptor tyrosine kinase signaling pathway involved in a variety of cellular mechanisms, of which many are related to the adhesion or migration of cells. Both the Eph receptor and ephrin ligand are abundantly present on a wide variety of cell types, and strongly evolutionary conserved. This review provides an overview of how 18 genetically diverse viruses utilize the Eph receptor (Eph), ephrin ligand (ephrin) or ephrin-Eph signaling to their advantage in their viral life cycle. Both Ephs and ephrins have been shown to serve as entry receptors for a variety of viruses, *via* both membrane fusion and endocytosis. Ephs and ephrins are also involved in viral transmission by vectors, associated with viral replication or persistence and lastly to neurological damage caused by viral infection. Although therapeutic opportunities targeting Ephs or ephrins do not seem feasible yet, the current research does propose two models for the viral usage of ephrin-Eph signaling. Firstly, the viral entry model, in which membrane molecules are used for viral entry, leading to cells being used for replication or as a transporter. Secondly, the advantageous expression ephrin-Eph signaling model, where viruses adapt the expression of Ephs or ephrins to change cell-cell interaction to their advantage. These models can guide future research questions on the usage of Ephs or ephrins by viruses and therapeutic opportunities.

1. Introduction

Viruses are highly variable, metabolically inert microbes, that parasitize on their host for metabolism and reproduction. They can infect every form of life and cause disease in a variety of organisms. With millions of viral infections in humans alone happening each year, they pose a serious threat to human, animal and plant health [1]. Viruses are dependent on their hosts for their replication and spread, by using host factors and mechanisms to aid the viral life cycle. One of the important questions around viral infections is which host factors are involved, as these are essential for the viral life cycle [2]. Due to the large variety in different types of viruses and their quick mutation rate, it remains difficult to treat and control viral disease. Some antiviral agents have been developed in the past years to treat viral infection, mainly suppressing further viral replication after infection. However, currently the main approach towards fighting viral disease is vaccination, to prevent infection taking place and minimize further spread of the virus. Deeper understanding of the molecular mechanisms behind different viral infections is necessary to aid in the development of future antiviral agents and vaccines [3].

An interesting group in this regard is the erythropoietin-producing hepatocellular (Eph) receptors and their ephrin ligands, as these are highly conserved and broadly expressed on different cells, by a wide range of organisms. The Eph receptor super family is the largest family of RTKs in humans. Receptor tyrosine kinases (RTK) are key regulators of a wide variety of cellular processes, from proliferation and differentiation to cell migration [4]. They can be found on the cell surface of all cells. The Eph receptors are activated *via* binding of their ligand; Eph family receptor interacting protein (ephrin). Ligands bind on the extracellular domain, whilst the intracellular domain contains the protein tyrosine kinase domain and regulatory regions. After activation by their corresponding ligands, the tyrosine residues in the receptors are phosphorylated and act as an assembly and activation site for intracellular signaling proteins [5]. Ephrin-Eph signaling has been linked in research

Abbreviations: CABYV, Cucurbit aphid borne yellows virus; CeV, Cedar virus; ECD, Extracellular domain; Eph, erythropoietin-producing human hepatocellular receptors; Ephrin, Eph family receptor interacting proteins; EBV, Epstein-Barr virus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HeV, Hendra virus; HIV, Human immunodeficiency virus; HNV, Henipavirus; IAPE, Intracisternal A-type Particles elements with an Envelope; IAV, Influenza A virus; IPNV, Infectious pancreatic necrosis virus; KSHV, Kaposi's sarcoma-assicated virus; MojV, Mòjiāng virus; NiV, Nipah virus; PEDV, Porcine epidemic diarrhoea virus; RRV, Rhesus monkey rhadinovirus; RSV, Respiratory syncytial virus; RTK, Receptor tyrosine kinase; SIV, Simian immunodeficiency virus; TuYV, Turnip yellow virus

* Corresponding authors.
E-mail addresses: j.m.van_gils@lumc.nl (J.M. van Gils), m.j.vangils@amsterdamumc.nl (M.J. van Gils).

https://doi.org/10.1016/j.phrs.2020.105038
Received 8 June 2020; Received in revised form 15 June 2020; Accepted 16 June 2020
Available online 18 June 2020

to a wide range of viruses, from highly pathogenic Hendra and Nipah viruses to plant-infecting turnip yellow virus.

This review will give an extensive overview of the utilization of ephrin-Eph signaling by different viruses in a variety of hosts and in human antiviral immunity. Viruses can utilize ephrin-Eph signaling for different purposes, although in most currently elucidated mechanisms ephrins or Eph receptors are used for viral entry. Both viral use of Ephs and ephrins for viral entry and for other purposes will be discussed, as well as possibilities in therapeutic targeting of Ephs and ephrins in viral disease. Altogether, this research leads to the proposal of two possible models explaining viral use of Ephs and ephrins, based on the current literature.

2. Ephrin ligands and receptors

RTKs are one of the biggest families of plasma membrane-associated proteins, playing an important role in cellular responses to signals from the cell exterior. Roles for RTKs can be found in a wide variety of cellular functions, from proliferation and differentiation to cell migration. Eph receptors are the largest family of RTKs and are highly conserved throughout evolution, from nematodes to vertebrates [5,6]. Their Ephrin ligands bind on the extracellular domain and can activate intracellular signalling proteins [5]. The most important downstream effects of ephrin-Eph signaling lie in the regulation of cytoskeletal dynamics and cell-cell adhesion, which are important in processes relying on cell motility and morphology, such as cell migration, neuronal pathfinding and tissue separation [7–10].

2.1. Eph receptor and ephrin structure

The members of the Eph receptor family are divided into two classes based on their binding preferences: EphA and EphB. EphA receptors bind preferentially to ephrin A ligands, whilst EphB receptors bind preferentially to ephrin B ligands [11,12]. The class of EphA receptors holds 10 members, named EphA1 - EphA10, the class of EphB receptors holds 6 members, defined as EphB1 - EphB6. Although the classification of the Eph receptors is based on their respective ligands, cross-interaction between the different classes has been demonstrated. EphA4 and EphB2 are able to bind ligands from the opposite class with high affinity [13].

Both classes of Eph receptors follow the same domain structure, consisting of 9 domains as described in Fig. 1 [12,14]. The tyrosine kinase domain is the active site of the receptor and contains most of the phosphorylation sites. Activation of the juxtamembrane region is an important autoregulator of Eph receptor activity, as the stabilization of the tyrosine kinase domain by the juxtamembrane region can block substrate binding and nucleotide access, but it also provides a binding site for SH2 domain-containing proteins so that the kinase can be stabilized in an active conformation upon autophosphorylation [7,15]. The SAM domain is also involved in regulation of the receptor, as it can bind SH2 domain-containing proteins too and holds some phosphorylation sites [15].

2.2. Ephrin-Eph signaling

What sets Ephrin-Eph receptor signaling apart from other RTK signaling pathways is that signaling can occur in three different directions: forward, reverse and bidirectional (Fig. 2) [16]. In forward signaling, ephrin ligands bind to an Eph receptor, the receptor autophosphorylates its intracellular tyrosine residues, inducing forward signaling. After activation adaptor proteins bind to the receptor to transmit signals further downstream into the cell. A wide range of adaptor proteins is known to be able to interact with the Eph receptor, such as Ras-GTPase-activating protein (RasGAP), phosphatidylinositol 3-kinase (PI3K) and Janus kinase 2 (Jak2) [7,16].

Reverse signaling has been shown for both ephrin-As and -Bs.

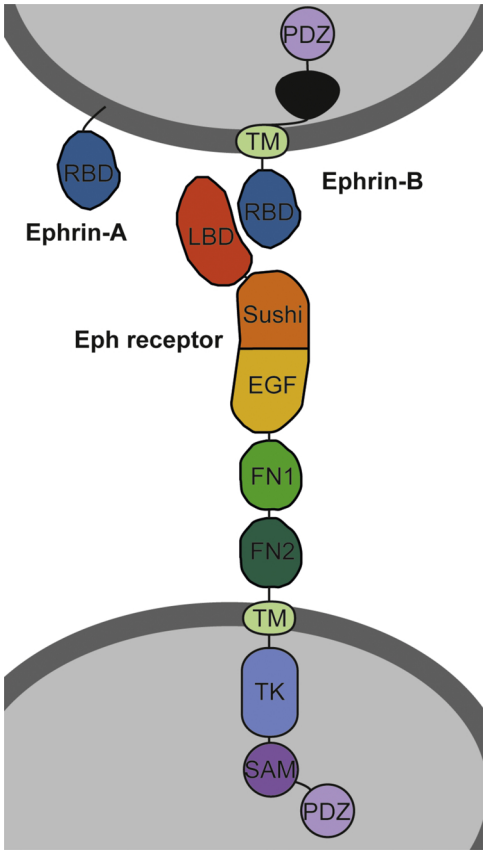

Fig. 1. Domain structure of the ephrin-A and ephrin-B ligand and the Eph receptor. The receptor contains 9 domains, from the extracellular side: the N-terminal ligand-binding domain (LBD), a cysteine-rich region comprised of a sushi-like and an EGF-like motif, and two fibronectin (FN) domains. After the transmembrane region (TM) and juxtamembrane region (JM), the tyrosine kinase domain (TK), sterile alpha motif (SAM) domain and PDZ domain follow on the intracellular side. Ephrin-A contains only of a receptor-binding domain and a GPI anchor, while ephrin-Bs are integral membrane proteins with a transmembrane region and a PDZ domain.

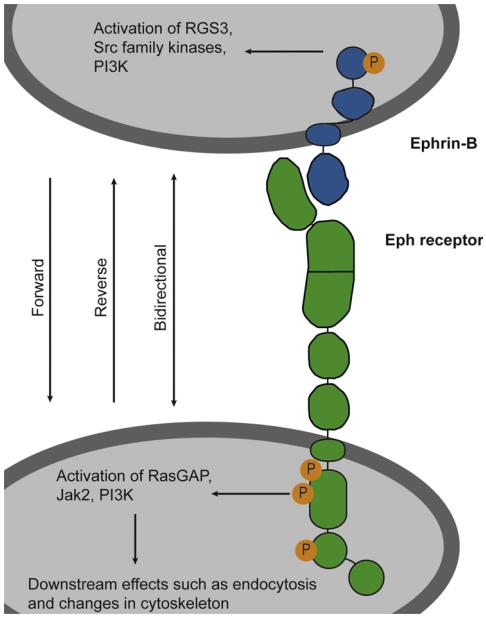

Fig. 2. Ephrin-Eph receptor signaling pathways. Main phosphorylation sites are marked with a P on the Eph and ephrin domains. Major downstream pathways are also listed.

Ephrin-A reverse signaling is mediated *via* co-receptors as it has no intrinsic intracellular domain. The role of co-receptor has been suggested for two neurotrophin receptors, tropomyosin receptor kinase B (TrkB) and p75 neurotrophin receptor (p75), and the Ret receptor tyrosine kinase [17]. Reverse signaling in ephrin-Bs takes place *via* the phosphorylation of tyrosine residues in the linker connecting the transmembrane domain to the PDZ domain. As the Eph receptor also contains a PDZ domain, it is not surprising that similar downstream pathways have been shown to be induced by reverse signaling as by forward signaling, such as PI3K, regulator of G-protein signaling 3 (RGS3) and Src family kinases [16].

Two Eph receptors do not seem to engage in the canonical forward signaling pathways that the other Eph receptors use. EphA10 and EphB6 are inactive kinases, denominated pseudo-kinases, as they miss the amino acids that catalyze the phosphoryl transfer from ATP in conventional protein kinases. The presence of these two catalytically inactive Eph receptors suggests that they play a regulatory role in relation to the other Eph receptors. Although the protein structures of EphA10 and EphB6 have not been elucidated yet, it is known that they show a protein sequence similarity of around 50 % to their closes homologues and have the same domain organization [15,16].

In some cases, the ephrin-Eph interaction leads to endocytosis of the receptor-ligand complex. During this process, termed trans-endocytosis, the full receptor-ligand complex is internalized into one of the two cells, of which the direction is determined by cytoplasmic determinants. The exact mechanisms behind ephrin-Eph mediated endocytosis have not been fully understood, but a link between ephrin-Eph signaling and both clathrin-mediated endocytosis and caveolin-mediated endocytosis has been established, although other endocytic pathways could also be at play. The destination of the bud off vesicles can be the cytosol, lysosome or Golgi apparatus, dependent on further signaling proteins [2,18,19]. The endocytosed product can generate intracellular ephrin or Eph fragments that have specific downstream signaling properties. It has been suggested that trans-endocytosis of the receptor-ligand complex enables the diverse effects of ephrin-Eph signaling as well as the ability to terminate and convert signals in a controlled way and fashion [18].

2.3. Eph receptors and ephrins are expressed in and function throughout many different cell types

The Eph receptors and ephrins are expressed in many different organisms and cell types. Most cell types that express Eph receptors and ephrin ligands do not just express the receptor or the ligand, but usually express both. With regards to ephrin and Eph receptor expression in context of viral infection, the epithelium, endothelium and immune cells are of specific interest. Expression for these tissues is described in Table 1 [11,20,21].

The epithelium is relevant as it usually is the first barrier to infection. The endothelium plays an important role in the guidance of immune cells. Co-localization studies have suggested that Eph receptors regulate the permeability of endothelial and epithelial as it has several mechanisms to regulate cell-cell adhesion *via* gap junctions, cell adherence and tight junctions. In an inflammatory setting, it has been proposed that the quick upregulation of Eph receptors and ephrins contributes to the disruption of vascular (endothelial) end epithelial barriers [11,22]. A well-described example is the role of ephrin-Eph signaling in barrier dysfunction in chronic obstructive pulmonary disease (COPD). Smoking contributes to COPD, of which the pathogenesis has been associated with disruption of the actin cytoskeleton in the respiratory epithelium (specifically: in the bronchial airway epithelial cell monolayer), resulting in a loss of epithelial barrier function. This epithelial barrier dysfunction was linked to increased EphA2 signaling, which downregulated E-cadherin, an adherence-inducing junction protein [23].

Ephrin-Eph signaling also effects the immune system in several ways, mainly *via* immune cell activation and immune cell trafficking. B and T lymphocyte as well as dendritic cell activation has been shown to be regulated *via* ephrin-Eph signaling [16,22]. The trafficking of immune cells is affected by ephrin-Eph signaling *via* its effects on barrier permeability, but also directly by providing guidance cues for lymphocytes in the vascular endothelium [10,11,22,24–26]. The expression of Ephs and ephrins can also be regulated by certain inflammatory cytokines and pathogen-associated molecular patterns [11,27,28]. Taken together, ephrin-Eph signaling has been shown to play a role in the attraction, activation and adhesion of immune cells, as well as the vascular endothelial transmigration, which is also linked to the permeability of the endothelial barrier.

3. Usage of ephrins and Eph receptors for viral entry

Usage of ephrins and Eph receptors as entry receptors for viral entry has been described for multiple viruses from different viral families and using different types of entry mechanisms. Viral entry can mechanistically take place *via* different pathways. The pathways are all mediated by viral receptors, which are molecules on the target cell surface to which the virus binds. The viruses found to this day to use Ephs or ephrins for viral entry, are enveloped viruses. For these viruses, the viral proteins binding to the viral receptor on the target cell are normally glycoproteins expressed on the viral envelope. A virus often has multiple viral receptors that determine which tissues and cells it can successfully infect. Binding to these viral receptors can induce viral entry in two different ways related to Ephs and ephrins: by inducing conformational changes of the viral glycoproteins that lead to membrane fusion or by transmitting signals that lead to endocytosis of the viral particle [2]. During membrane fusion, conformational changes of the viral glycoproteins induce the viral envelope and the viral membrane to fuse, leading to the release of the viral genome into the host cell. Often, a part of the fusion protein on the viral surface is inserted into the target cell membrane to obtain a starting point for fusion [2]. For endocytosis, viruses can use both clathrin-mediated endocytosis or clathrin-independent mechanisms, such as macropinocytosis. Entry *via* endocytosis is often followed by membrane fusion, when the content of the virion is released by fusion of the endocytic vesicle membrane and the viral membrane [2].

3.1. Ephrin usage by henipaviruses for viral entry is well-established

Henipaviruses are a newly discovered genus in the Paramyxoviridae family. The genus is composed of the Hendra (HeV) virus and Nipah (NiV) virus, which give the genus its name, and the more recently discovered Mòjiāng virus (MojV), Cedar virus (CeV) and African

Table 1
Expression of ephrins and Eph receptors in human tissues relevant for viral infection, because of their functions in the immune system.

Tissue	Expressed ephrins	Expressed Eph receptors	Refs
Intestinal epithelium	All ephrin-As, ephrinB1-B3	EphA1-A3, A5-A8, B1-B4, -B6	[11]
Endothelial cells	EprhinA1, EphrinB1, -B2	EphA2, B1-B4	[20,21]
Lymphocytes	EphA1, -A3, -A4, -A7, -B1, -B2.	EphB1-B4, -B6	[11]
Dendritic cells	EphA2, -A4, -A7	EphB1, -B3	[11]
Monocytes	EphA4, EphB1-B3, -B6	EphrinB1-B3	[11]

Table 2
Overview of the viruses that use Ephs and ephrins for viral entry.

Virus	Viral characteristics	Eph or ephrin	Interaction	Entry pathway	Refs
Nipah virus	Enveloped -ssRNA *Paramyxoviridae*	Ephrin-B2,-B3	Entry receptor (or binding only)	Fusion and macropinocytosis	[33,36,38]
Hendra virus	Enveloped -ssRNA *Paramyxoviridae*	Ephrin-B2, -B3	Entry receptor	Membrane fusion	[33,36]
Cedar virus	Enveloped -ssRNA *Paramyxoviridae*	Eprhin-A2, -A5, -B1, -B2	Entry receptor	Membrane fusion	[32,37]
African henipavirus	Enveloped -ssRNA *Paramyxoviridae*	Ephrin-B2	Entry receptor	Membrane fusion	[34]
Mòjiāng virus	Enveloped -ssRNA *Paramyxoviridae*	No use of ephrins	–	–	[31]
Hepatitis C virus	Enveloped + ssRNA *Flaviviridae*	EphA2	Co-factor for entry	Clathrin-mediated endocytosis, followed by fusion	[44]
Rhesus monkey rhadinovirus	Enveloped dsDNA *Herpesviridae*	EphB2- & -B3	Entry receptor for B cells and endothelial cells	Receptor-mediated endocytosis, followed by fusion	[45]
Kaposi's sarcoma-associated herpesvirus	Enveloped dsDNA *Herpesviridae*	Eph-A2 &-A4	Entry receptor	Receptor-mediated endocytosis, followed by fusion	[46,47,48]
Epstein-Barr virus	Enveloped dsDNA *Herpesviridae*	EphA2	Entry receptor for endothelial cells	Membrane fusion	[49,50]
Mouse IAPE endogenous retrovirus	Non-enveloped ssRNA *Retroviridae*	All ephrin-As, preferentially ephrin-A4	Entry receptor	Reinfection	[51]

henipavirus (African HNV). HeV and NiV cause respiratory disease and sometimes neuronal disease in humans and other animals and can be lethal. The henipaviruses are zoonotic, with their main reservoir in bats, and have a broad host range. Viral infection starts with infection of the respiratory epithelial cells, then spreading to the endothelium, specifically to microvascular endothelial cells in the lungs. HeV and NiV can then also infect neurons and spread towards the central nervous system, a major factor in the high pathogenicity of the viruses. *Paramyxoviridae* conventionally attach to host cells *via* sialic acids, CD150 or nectin-4 cell surface receptors and enter *via* membrane fusion. Henipaviruses, however, do not bind to any of these molecules, meaning that another entry mechanism must be in place [29–35].

Henipaviruses mainly use two transmembrane glycoproteins for entry *via* fusion: G protein, for attachment, and F protein, for fusion [36]. Generally speaking, the G proteins interact with ephrins, although which ephrin is used differs per virus (see Table 2). HeV and NiV have both been shown to use ephrin-B2 and ephrin-B3 as entry receptors. The interaction with ephrin-B2 even has one of the highest affinities for viral envelope-receptor interaction currently known [33]. On the other hand, CeV and African HNV, do use ephrin-B2 but not ephrin-B3 for viral entry [34]. To add, CeV can use ephrin-A2, -A5 and -B1, which could be attributed to structural differences in the receptor-binding pocket of the G protein of CeV compared to HeV and NiV [32,37]. The usage of these strongly conserved receptors possibly explains the species tropism of henipaviruses, which is much broader than that of most other *Paramyxoviridae* [38]. Negrete et al. [38] hypothesize that the ability to bind to ephrin-B3 plays a role in the encephalopathic capacity and thus in the fatality of the virus, seeing as ephrin-B3 is expressed in the central nervous system. As NiV binds ephrin-B3 more efficiently than HeV, this would also explain NiVs higher encephalitic capacity [39].

Although the majority of NiV entry events occur *via* fusion, there is some evidence for endocytosis as an entrance mechanism *via* ephrin-B2 in CHO-K1 and VeroE6 cells, mimicking the EphB4/ephrin-B2 interaction. Like EphB4, NiV binds ephrin-B2 in the G-H loop, inducing endocytosis that leads to internalization of the full receptor and vial particle. More specifically, NiV seems to partake in macropinocytosis [40]. As to why entry *via* macropinocytosis is sometimes preferred over entry *via* fusion, Pernet and colleagues [40] hypothesize that endocytosis possibly occurs more rapidly, or that NiV-G might require a different confirmation for endocytosis than for fusion, determining which path is followed. This information indicates the high versatility of viral usage of Ephs and ephrins, even for a single virus.

Although successful infection in leukocytes has only been shown for dendritic cells, with low level viral replication, NiV does bind to ephrin-B2 and -B3 on all leukocytes. Interestingly, it has been suggested that NiV binding to leukocytes is used to disseminate throughout the body *via* the lymphatic and blood vessels [41]. Mathieu et al. do show that NiV-bound leukocytes in hamsters can establish transinfection, by transmitting the virus to cells that are susceptible to NiV infection, such as endothelial cells. This could also be the way in which NiV passes the blood-brain barrier. Transendothelial migration of immature dendritic cells has been shown to increase upon NiV infection, although no effect could be observed for other monocytic cell lines [42]. Potentially, this mechanism could play a role in the transmission of henipaviruses from the respiratory tract to the central nervous system, which causes the fatal encephalitis, as leukocytes can pass the blood-brain barrier. However, transmission of henipaviruses from leukocytes to neural cells has not been investigated yet.

Lastly, the Mòjiāng virus, which is a recently discovered and rare virus, does not seem to use ephrins as entry receptors at all. The virus is classified as a henipavirus based on gene structure and protein alignment, although nucleotide alignment with other henipaviruses is low. The attachment protein G of MojV only has a nucleotide sequence similarity of around 40 % with other henipaviruses, whilst this is 50–60 % for other viral proteins [43]. The MojV G protein has a similar

structure as NiV G protein, but the beta-propeller domain has a different arrangement, which renders binding to ephrin-B2 or -B3 impossible. The virus also does not bind to sialic acid or CD150, as conventional for *Paramyxoviridae*. This suggests either that novel entry route for henipaviruses is at play here and that the broad species tropism is not a characteristic of a specific viral entry receptor alone [31], or that the classification of MojV as a henipavirus is not justified.

3.2. Mouse IAPE endogenous retrovirus uses ephrin-A4 as a viral entry receptor

The only other virus identified to this date to use an ephrin as viral entry receptor is the Intracisternal A-type Particles elements with an Envelope (IAPE). These are murine endogenous retroelements of which many copies can be found in the mouse genome. It is the progenitor of intracellular IAP elements, posing the most successful and most active family of retrotransposons in the mouse, but also remaining as infectious elements. This means the virus can still generate fully functional particles that are then able to infect new cells and species. The virus uses its envelope protein IAPE Env for reinfection. From screening of a lentiviral library, IAPE Env has been shown to be able to interact with all ephrin-As. The most efficient receptor for entry is ephrin-A4, as solely interacting with ephrin-A4 allowed IAPE Env to establish viral entry. The authors also suggest that the fact that IAPE family uses ephrins as entry receptors, might be a reason why they are so successful and still remain present in mouse genomes. The broad functions of ephrins and their high expressions early in development make it impossible to completely downregulate ephrins and thus difficult to escape the IAPE family. This also allows the retroviral elements to insert early on in the germline, strongly contributing to its survival [51].

3.3. Viruses that use Eph receptors for viral entry via endocytosis and fusion

Hepatitis C virus (HCV) is a liver infecting virus that is transmitted through liver endothelial cells, but productive infection is targeted at hepatocytes and has also been shown to infect dendritic cells and B lymphocytes [52]. Viral entry for HCV takes place *via* clathrin-mediated endocytosis with attachment taking place *via* binding of glycoproteins on the virion surface to glycosaminoglycans on the host cell. The viral and endosomal membrane then fuse, releasing the nucleocapsid into the cell [53]. The entry of HCV has been shown to depend on the formation of a co-receptor complex on the target cell. Interaction between the CD81-claudin-1 coreceptor complex on the hepatocytic membrane with the virus is essential for and has been suggested to initiate viral entry *via* membrane fusion [54]. An siRNA screen identified EphA2 as one of the cofactors for HCV viral entry. EphA2 has been suggested to regulate the formation of the CD81-claudin-1 complex, thereby regulating viral entry of HCV. Inhibiting EphA2 using a kinase inhibitor, prevented viral entry, which seems to suggest that EphA2 is an essential cofactor for HCV entry [44].

Two different rhadinoviruses, a genus of herpesviruses, have been shown to depend on Eph receptors for viral entry: Kaposi's sarcoma-associated herpesvirus (KSHV) and rhesus monkey rhadinovirus (RRV), which both cause different cancerous malignancies. KSHV infects a range of cell types, from endothelial and epithelial cells to different immune cells, with B cells being their main site of persistence. Like other herpesviruses, it is an enveloped virus with a double-stranded DNA genome [47,48]. RRV has been shown to have a very similar infection pattern and pathogenesis, although it has not been linked to the formation of solid tumors [45].

Hahn and Desrosiers [45] tested Eph receptor binding and Eph-mediated entry for KSHV and RRV for all 14 Eph receptors. Ten different Eph receptors were shown to have an interaction with RRV, whilst KSHV only interacts with EphA2, -A4 and -B1. However, viral entry for RRV mainly seems EphB2 and EphB3 dependent. RRV also seems able to use an Eph-independent pathway to productively infect fibroblasts and epithelial cells, which was shown by adding soluble EphA2 receptor decoys or ephrins. Whilst this did reduce viral entry of KSHV, RRV viral entry into the mentioned cell types seemed unaffected [45]. Unlike entry for the henipaviruses mentioned above, KHSV entry can take place *via* both fusion and macropinocytosis, involving three types of glycoproteins. In its fusion process, the KSHV first attaches to heparan sulphate proteoglycans on the cell surface, after which it binds specifically to cell surface molecules on host cells, mainly integrins and Eph receptors, with the gHgL glycoproteins interacting with Eph receptors exclusively. This interaction leads to endocytosis of the virion, and then to fusion of the virion with the endocytic membrane, releasing the virions into the cell [48]. On endothelial and epithelial cells, gHgL binds EphA2, with EphA4-binding on epithelial cells as well [47,48]. The interaction with EphA2 is the strongest of these interactions and has been shown to be similar to the interaction of EphA2 with its natural ligand ephrin-A5 and has also been indicated in clathrin-mediated macropinocytosis. These processes are dependent on cofactors, such as the androgen receptor [46,55,56].

It has even been suggested that an interplay between EphA2 and KSHV aids in Kaposi's sarcoma pathogenesis. Kaposi's sarcomas have some histological characteristics that are linked to atypical angiogenesis, such as dilated abnormal vessels with thinned endothelium, and extravasation of erythrocytes. EphA2 is a known inducer of angiogenesis. The cancerous angiogenesis in Kaposi's sarcoma has also been linked to ephrin-B2, which was upregulated indirectly by KSHV [48,57,58].

Epstein-Barr virus (EBV), another *Herpesviridae* family member, mainly infects human B lymphocytes and epithelial cells. The primary infection with the virus is linked to infectious mononucleosis, but in the long run the virus is also oncogenic. The virus has a different entry mechanism for epithelial cells and B lymphocytes, mediated by surface glycoproteins. EphA2 has been shown to act as both an entry and a fusion receptor for EBV in epithelial cells. In epithelial cells, fusion at the plasma membrane is the entry pathway, whilst for B cells endocytosis occurs before fusion. The intracellular domain of EphA2 was found not to be essential for viral entry, which it is for KSHV [49,50].

4. Utilizations of Eph receptors and ephrins by viruses aside from vial entry

Apart from the use of Eph receptors and ephrins for viral entry, some other utilizations of ephrin and Eph receptors by a variety of viruses have been identified. There are direct indications for the functionality from their involvement, for others only correlations have been found. This section will give an overview of the associations of Ephs and ephrins with viral functions other than entry (for full overview, see Table 3).

4.1. Eph receptors play a role in aphid transmission

Plant viruses are usually transmitted between hosts *via* vectors and express proteins on their surface that allow for the interaction between the virus and the vector. The family of *Luteoviridae* are viruses transmitted by aphid (*Myzus persicae)* vectors that infect plants of the cabbage family (*Cruciferae*), but do not infect the cells of the vector [59,60]. The luteoviruses express two capsid proteins: major coat protein (CP) and minor capsid protein (RT*) that play a role in aphid transmission. For turnip yellow virus (TuYV) and cucurbit aphid borne yellows virus (CABYV),the aphid EphB receptor type 1 has been shown to be a binding partner for RT*. Silencing this EphB receptor using RNA interference also gave a reduction in transmission of TuYV, which could also be demonstrated for a few other poleroviruses for which the direct interaction of RT* with EphB type 1 has not been shown, suggesting a broader role for EphB in polerovirus transmission [60,61].

Table 3
Overview of viral utilisations of Ephs and ephrins other than viral entry, including the organism in which it has been shown.

Virus	Viral characteristics	Eph or ephrin	Found link	Refs
Turnip yellows virus	Non-enveloped + ssRNA virus in plants of the *Cruciferae* family; *Luteoviridae*	EphB (aphid vector)	The aphid EphB showed to play a role in vector-mediated transmission of the virus.	[59,60]
Cucurbit aphid borne yellows virus	Non-enveloped + ssRNA virus in plants of the *Cruciferae* family; *Luteoviridae*	EphB (aphid vector)	The aphid EphB showed to play a role in vector-mediated transmission of the virus.	[59,60]
Human immunodeficiency virus	Enveloped + ssRNA virus in humans, *Retroviridae*	EphB1	A decrease in EphB1 expression is associated with (reversible) damage to neurons in HIV dementia.	[62]
Simian immunodeficiency virus	Enveloped + ssRNA virus in apes, *Retroviridae*	Ephrin-B3	Ephrin-B3 was increased in different type of cells in the white matter of the frontal cortex in SIV brain infection.	[63]
Influenza A virus	Enveloped -ssRNA virus in humans, *Orthomyxoviridae*	EphB6	EphB6 was found as a host factor for viral replication in a genome-wide *in vitro* RNAi screen.	[64,65]
Respiratory syncytial virus	Enveloped -ssRNA virus in humans, *Paramyxoviridae*	Ephrin-B2	Ephrin-B2 expression was associated with viral replication.	[66,67]
Infectious pancreatic necrosis virus	Non-enveloped dsRNA virus in salmonids, *Birnaviridae*	Ephrin-B1	Upregulation of ephrin-B1 takes place in embryonic salmon cells persistently infected. Upregulation could take place because ephrin-B1 is a possible entry receptor, or linked to its involvement in T cell or gap junction regulation.	[68,69]
Porcine epidemic diarrhoea virus	Enveloped + ssRNA virus in pigs, *Coronaviridae*	Ephrin-B2	An epigenome and transcriptome analysis of the jejunum of infected piglets showed increased histone methylation and expression of *EFNB2* compared to healthy piglets.	[70,71]
Hepatitis B virus	Enveloped dsdDNA virus infecting humans, *Hepadnaviridae*	EphA3	The rs9310117 *EFNA3* polymorphism, an intron mutation, was associated with susceptibility to chronic severe hepatitis.	[72,73]

4.2. Ephrin-Eph signaling seems to be involved in SIV and HIV dementia

Human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) are two closely related lentiviruses respectively infecting humans and apes. Both viruses mainly affect the immune system, but can also cause damage to the central nervous system by infecting macrophages and microglia, which can lead to HIV dementia. HIV and SIV infection both lead to a similar encephalitis, damaging the central nervous system by the release of neurotoxic agents by infected macrophages and microglia. Although damage to the central nervous system in adults is generally often difficult to reverse, improvement of the functional impairment of the neurons has been shown in HIV dementia. Two different studies have described involvement for ephrin-Eph signaling in the neuronal involvement of SIV and HIV [62,63,74].

Westmoreland et al. [63] investigate the involvement of GAP-43 and ephrin-B3 in the brains of rhesus macaques infected with SIV. GAP-43 induction has been linked to ephrin-B3/B3 pathway activation in injured neurons. GAP-43 and ephrin-B3 are increased in certain brain regions of SIV-infected macaques, but differ per brain region. Ephrin-B3 increase was mainly shown for the white matter in the frontal cortex and the increased ephrin-B3 in these regions came from macrophages, microglia and multinucleated giant cells. The model they propose behind this hypothesis is that the microglia and macrophages infected with SIV express ephrin-B3 to stimulate astrocytes to produce nerve growth factor, which then induces the expression of GAP-43 in neurons. The interaction between ephrin-B3 on microglia and macrophages, and the astrocytes is suggested to be mediated through EphA4 or EphB3 on the astrocytes [63].

A study by Cao et al. [62] on infection of the central nervous system by HIV focused on the use of nicotine to reverse the changes in gene expression in HIV-1 infected rat brains. One of the changes in gene expression in the brain that they found was a decrease in EphB1 expression upon HIV infection in the prefrontal cortex and dorsal striatum. This change in expression could be reversed using nicotine, indicating that this is a (majorly) reversible change in both the prefrontal cortex and dorsal striatum [62].

4.3. Ephrin-Eph signaling plays a role in influenza A and respiratory syncytial virus replication

Influenza A virus (IAV) is a subtype of influenza, causing mainly respiratory disease and the cause of all known pandemics of highly pathogenic influenza to this date [64]. A genome-wide RNAi screen after infection in HEK293 T cells, revealed Eph receptor B6 as a host factor contributing to influenza A replication for two different IAV strains. No mechanisms or purposes were described for EphB6 in IAV replication [65]. It is likely that the link between EphB6 expression and IAV replication is an indirect one, as the replication of IAV takes place in the nucleus of the cell, while EphB6 is membrane bound. A possible explanation for this upregulation of EphB6 could be that this receptor is used in activation of the cell or to establish the antiviral state [18,64,65].

Respiratory syncytial virus (RSV) is one of the main causes of respiratory infections in children and mainly infects lung epithelial cells *via* fusion. The fusion mechanism is similar to that of henipaviruses, which come from the same order, using CX3CR1 as main entry receptor [66,75]. Shirato et al. [67] investigated viral replication of RSV *in vitro* using a cDNA library, and showed ephrin-B2 expression associated with increased viral replication. They do not propose any mechanistic links between RSV infection and ephrin-B2 replication. Unlike IAV replication, the replication complex of RSV forms on the intracellular side of the cell membrane [75]. It is therefore a possibility that there is a direct interaction between ephrin-B2, which has an intracellular domain, and the viral replication process. Other *Paramyxoviridae,* as described in this review, with similar entry mechanisms as RSV, use ephrin-B2 as an

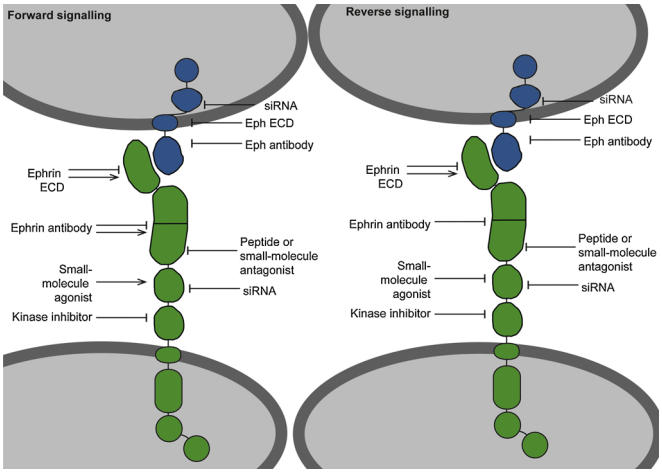

Fig. 3. Different classes of agents targeting ephrin-Eph signaling and their effects on forward (left) and reverse (right) signaling. Recombinant ephrin extracellular domains (ECDs) can have both an inhibiting and activating effect on forward signaling. For forward signaling, multimeric ECDs are usually activating, while monomeric ECDs are mainly inhibiting. Monomeric Eph ECDs always have an inhibitory effect on reverse signaling, where multimeric ECDs always have an activating effect. siRNA: small-interfering RNA.

entry receptor, which would be an interesting hypothesis to investigate for RSV too.

5. Targeting ephrin-Eph signalling using therapeutics

Previous sections have described the broad prevalence and functions of ephrin-Eph signaling throughout the body, and how these are relevant in viral infections. This section will investigate the potential to target ephrin-Eph signaling in fighting viral infection.

5.1. Different types of agents available to target ephrin-Eph signaling

Six different types of agents are available that target the receptor or the ligand and use forward or reverse signaling, or both, to manipulate the function of the receptor and/or ligand. Most of these inhibitors or activators have been used quite extensively in *in vitro* research, but have not been tested much in *in vivo* or in clinical settings. They can be divided into six different classes: extracellular domains, antibodies, peptides, small-molecules, kinase inhibitors and siRNAs, as summarized in Barquilla and Pascale [76] and in Fig. 3. However, as Eph and ephrins are expressed on many different cells and involved in a variety of essential cellular processes, targeting them could result in undesirable side effects.

Recombinant extracellular domains (ECDs) are very efficient in targeting ephrin-Eph interactions in a way that closely resembles the receptor-ligand interaction. They have been used in several studies to test how ephrin-Eph signaling affects viral infections. The usage of an EphA2 ECD could inhibit KSHV entry *in vitro*, which is normally mediated by EphA2. This inhibition probably occurred *via* competition between soluble and membrane bound EphA2 [57]. ECDs could potentially also be used to inhibit viral entry in other viruses that use similar entrance mechanisms as KSHV. To this end however, it should be ascertained that the ECD will only affect target cells, to avoid side effects coming from the broad expression of Ephs and ephrins.

The potential for ephrin or Eph targeting antibodies for anti-viral therapy has yet to be determined, as up to now only one monoclonal antibody is widely used clinically to treat a viral disease, namely palivizumab in RSV [77]. Antibodies against Ephs and ephrins have been used extensively in research, but have not yet been tested as antiviral therapeutic *in vivo* or clinically. Examples of successful use of antibodies in preventing or reducing viral infection *in vitro* can be found for HCV, EBV and KSHV. Treatments with an antibody targeting the HCV glycoprotein, or EphA2 and ephrin-A1 antibodies in EBV and KSHV infection, showed inhibition of infection *in vitro* [44,47,49].

Dasatinib is a kinase inhibitor and small molecule antagonist able to inhibit EphA2 signaling. It has been demonstrated effective as an HCV entry inhibitor *in vitro*, but not *in vivo*. EphA2 is a regulator for the formation of certain co-receptor complexes that are essential for HCV entry. Using dasatinib the co-receptor complex formation could be inhibited, thus preventing viral entry [44]. This functionality of dasatinib could also be related to the inhibition of Abl, another RTK associated with HCV entry, which would mean that the effect of dasatinib is twofold and not just dependent on Eph inhibition [78]. Dasatinib is currently used as a therapeutic in humans for different types of leukaemia and has an acceptable side effect profile for these diseases [79], although it should be noted that in oncological cases the bar for acceptable side effects often lies higher than in infectious disease. Dasatinib has also been studied *in vivo* for some viral infections, which have other RTKs as host factors. Dasatinib was tested for treatment of vaccinia, variola and monkeypox virus and HIV infection in mice, but showed no protection against these viruses. Although these viruses use RTKs other than Ephs as a host factor, on which dasatinib works, their ineffectiveness was suggested to be linked to its immunosuppressive activity. This would explain why there were promising results for this inhibitor *in vitro*, but why it's effects did not show beneficial effects for viral infections *in vivo* [80]. A positive effect of dasatinib in mice was seen for the prevention for acute HIV-1 infection, if given preventively, allowing for the establishment of viral control in an early stage of infection. This was possibly mediated by decreased activation of $CD4^+$ T cells by RTK inhibition, meaning that there is less proliferation of infected T cells, which decreases reservoir formation [81].

Lastly, antisense oligonucleotides and siRNAs can bind highly selectively to specific Ephs or ephrins and efficiently block the activity of their targets, and many studies escribed in these review use siRNAs for research purposes (for example in [23,45,67]). Their high selectivity would make them attractive agents to use as therapeutics, keeping possible side targets to a minimum, but their delivery *in vivo* is highly inefficient [76].

5.2. Possible future therapeutic targets with regards to ephrin-Eph signaling

Although chloroquine is not identified as an agent affecting ephrin-Eph signaling, it has been tested as a possible inhibitor of an Eph-induced endocytosis pathway. Chloroquine is a small molecule normally used to treat malaria infections and is amongst others thought to disrupt membrane function [82]. Preliminary *in vitro* tests showed that chloroquine inhibited viral entry *via* endocytosis. They therefore suggest that using chloroquine or amiloride, which are widely utilised medicines against malaria and hypertension, could be a low-cost antiviral therapy against NiV, a highly pathogenic virus for which currently no treatments are available. Chloroquine also came up in a henipavirus multicycle replication assay as possible therapeutic to treat henipavirus infection [82]. However, two *in vivo* animal studies could not show an improvement using chloroquine with regards to viral replication and pathology [83,84].

Another potential for targeting ephrin-Eph signaling, as described by Barquilla and Pasquale [76] lies within the nervous system. Ephrin-Eph signaling plays an important role in the developing nervous system and has been linked to several neuropathologies. However, many parts of the signaling pathway have also been shown to be upregulated after nervous system injury, to induce repair processes. Signaling between ephrin-B3 and EphA4 and EphB3 in brain cells damaged by SIV infection can reverse neuronal impairment that comes with SIV dementia [63]. Potentially, targeting of ephrin-Eph signaling could be utilized to optimize recovery after SIV or HIV dementia, a form of neural damage which is known to be (partially) reversible with a role for ephrin-Eph signaling. The desired effect of the therapeutics in this case would be to

increase signaling from ephrin-B3 to EphB3 and EphA4 in astrocytes. According to the overview provided by Barquilla and Pasquale [76], an increase in Eph forward signaling can be achieved using a multimeric ephrin ECD, an Eph activating antibody or an Eph small-molecule agonist. As the targeted cells in this case would lie behind the blood-brain barrier, the delivery of the agents can be challenging. Smaller molecules could potentially be delivered intranasally or transfer the blood-brain barrier *via* transmembrane diffusion if they are lipid-soluble [85].

6. Conclusions and future directions

The aim of this review was to outline the current knowledge on interactions of viruses with Eph receptors or ephrins. Up to now, 18 different viruses have been described to have a relation with Eph receptors or ephrins relevant for their viral life cycle, summarized in Table 2. Based on the overview of viral interactions with Ephs and ephrins given in this literature review, we propose two different models for the utilization of Ephs and ephrins by viruses: the viral entry model and the advantageous expression of Ephs/ephrins model. These models could be used by viruses both individually and combined.

6.1. Viral entry model

The viral entry model is based on the usage of Ephs or ephrins as entry receptors for viruses, as described in Section 3. Important to note here is that the usage of Ephs or ephrins as entry receptors could contribute to their differential expression as well. This seems plausible especially for endocytosis-mediated entry, where both the receptor and the ligand are internalized and thus no longer expressed on the cell membrane. Future research should point out whether this indeed contributes to a significant change in expression of Ephs and ephrins on cells. Another possibility is that the Ephs or ephrin receptors are not the only or main entry receptors into the cell for a virus, but could just be an adhesion mechanism. A weak binding to a receptor on the cell membrane would slow a virus down on the cell surface, allowing for interactions with the actual entry receptors to establish. This could also explain why some viruses with different entry mechanisms seem to have (weak) interactions with Ephs or ephrins, or show a correlation for Ephs/ephrins with infection rates.

That both Eph receptors and ephrins can be used as entry receptors is not unexpected, as the ephrin-Eph signaling pathways are known to be bidirectional, and mechanisms like trans-endocytosis can also occur in both the receptor- and the ligand-expressing cell. The use of ephrins or Ephs as entry receptors has multiple evolutionary benefits for different viruses, as they are expressed on many different cells, involved in a variety of essential cellular processes and viruses can often utilize multiple receptors for entry. It is unlikely that the host will adapt all these broadly important receptors at once. Indeed, a wide range of Eph receptors and ephrins have been shown to be associated with different viral functions, in a wide range of organisms. While some of these interactions are well-described, most still require further characterization, leaving many open questions and indicating the need for further research on this topic.

6.2. Advantageous expression of Ephs/ephrins model

The model of advantageous expression of Ephs and ephrins is more complex and based on a combination of current knowledge about viruses and the role of ephrin-Eph signaling in epithelial and endothelial barrier function, cell migration, immune activation and guidance (Fig. 4). In this model, viruses manipulate the expression or signaling of Ephs or ephrins on cells they interact with, which can have broad downstream effects. That ephrin-Eph signaling can be altered by extracellular factors other than their ligands, has already been

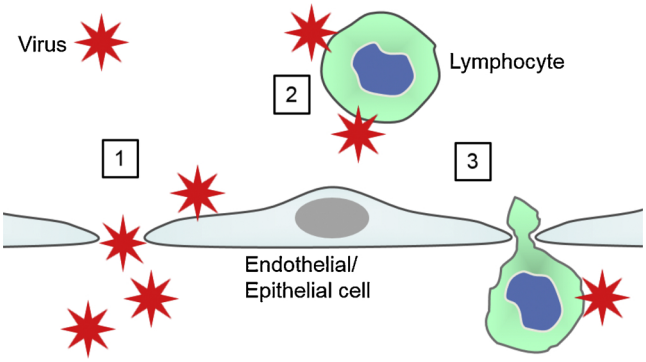

Fig. 4. Advantageous expression of Ephs and ephrins model. Virus binding to or inducing Eph/Ephrin signaling to help the virus to further advance the infection by 1) reduce endothelial or epithelial barrier function increasing viral spread to underlying tissues, 2) inhibit lymphocyte migration resulting in less recruitment to sites of infection, 3) hitch hike along to spread to other tissues and cells.

demonstrated, for example by the effects of tobacco smoke on COPD [23]. Many viruses discussed in this review have a correlation between increased expression for certain Ephs or ephrins, and some viral functionality. However, it is often unclear whether cells with increased expression are more likely to be infected, or whether the increased expression is caused by the virus itself. This can have several potentially beneficial effects for the virus. Firstly, a loss of barrier integrity for both the epithelium and endothelium could increase viral entry into a tissue. Ephrin-Eph signaling have been indicated as a regulator for barrier integrity, *via* cell-cell adhesion and tight junction formation. A worthy line of investigation would be whether it is necessary for viruses to infect a cell to have an effect on the barrier, or whether just binding to Ephs or ephrins on the cell membrane would be sufficient to induce changes in signaling that lead to a reduced barrier integrity by widening tight junctions. Secondly, changes in ephrin-Eph interactions can affect cellular migration. This could be beneficial for the virus in two ways. These changes could repel immune cells, thus avoiding detection by the immune system. Further research on Eph and ephrin expression on specific lymphocyte subtypes is needed to hypothesize further on this possibility. Another possibility is that viruses use ephrins or Ephs to “hitch a ride” to different sides of the body, using the receptor-expressing cell as a transporter, as has been shown for NiV and leukocytes [41,42].

Leukocyte infection could encourage the spread of the virus to more tissues and organs, as they travel the blood and lymphatic vessels extensively, often interacting with the endothelium by rolling. This might initiate infection of the endothelium in multiple sites, or have an effect on the integrity of the endothelium *via* ephrin-Eph signaling, making infection of underlying organs and tissues easier. It would be very interesting to investigate whether this is also the mechanism ensuring infection of the CNS. Tiong et al. [42] show *in vitro* that for immature dendritic cells infected with NiV transendothelial migration is increased compared to non-infected cells. The authors do not directly suggest a mechanism that mediates the increased permeability of this *in vitro* blood-brain barrier, but do report an increased permeability upon TNF-α treatment. TNF-α has been shown to increase ephrin-A1, ephrin-B1 and ephrin-B2 on endothelial cells, of which the last two have been shown to increase the permeability of the endothelial barrier [26,86]. They found that increased permeability upon TNF-α treatment could thus also be ephrin-Eph mediated. An interesting hypothesis for future research, would be whether NiV binds to ephrins on lymphocytes and use them to disseminate throughout the body, as well to activate ephrin-Eph signaling that decreases integrity of the endothelial barrier. It would also be interesting to see whether this mechanism of using ephrins on leukocytes to hitch a ride through the body is also utilized by other viruses that use ephrins as an entry receptor.

6.3. Future directions

Together these different possibilities of viruses using Ephrin or Eph interactions in their advantage described in this review provide guidance in the formulation of future research questions to increase our understanding of the life cycles of a variety of viruses, as well as aid in the development of therapies. For therapeutic options, it seems that there are many variants of agents available to target Ephrin- or Eph-virus interaction and/or induced Ephrin-Eph signaling, distributed over six different classes. Although promising results for multiple agents and viruses were found *in vitro*, the current *in vivo* results seem disappointing. This is likely linked to the fact that Eph receptors and ephrins are widespread and regulate a variety of different mechanisms, making it difficult to control what will be targeted by therapeutics. Further characterization of the effects of ephrins and Ephs in antiviral immunity seems vital, as immunosuppressive side effects are detrimental in fighting viral infections.

Funding

No funding.

Declaration of Competing Interest

The authors declare no conflicts of interest.

References

[1] L.W. Enquist, Virology in the 21st century, J. Virol. 83 (2009) 5296–5308, https://doi.org/10.1128/jvi.00151-09.
[2] L.W. Enquist, V.R. Racaniello, Virology: from contagium fluidum to virome, in: D.M. Knipe, P.M. Howley (Eds.), Fields Virol. 6th ed., Lippincott Williams & Wilkins, Philadelphia, PA, 2013, pp. 1–20.
[3] L.C.P. Vilas Boas, M.L. Campos, R.L.A. Berlanda, N. de Carvalho Neves, O.L. Franco, Antiviral peptides as promising therapeutic drugs, Cell. Mol. Life Sci. 76 (2019) 3525–3542, https://doi.org/10.1007/s00018-019-03138-w.
[4] G. Haqshenas, C. Doerig, Targeting of host cell receptor tyrosine kinases by intracellular pathogens, Sci. Signal. 12 (2019) 1–10.
[5] M.A. Lemmon, J. Schlessinger, Cell signaling by receptor tyrosine kinases, Cell 141 (2010) 1117–1134, https://doi.org/10.1016/j.cell.2010.06.011.
[6] B.T. Eaton, C.C. Broder, D. Middleton, L.F. Wang, Hendra and Nipah viruses: different and dangerous, Nat. Rev. Microbiol. 4 (2006) 23–35, https://doi.org/10.1038/nrmicro1323.
[7] K. Kullander, R. Klein, Mechanisms and functions of Eph and ephrin signalling, Nat. Rev. Mol. Cell Biol. 3 (2002) 475–486, https://doi.org/10.1038/nrm856.
[8] J. Huai, U. Drescher, An ephrin-A-dependent signaling pathway controls integrin function and is linked to the tyrosine phosphorylation of a 120-kDa protein, J. Biol. Chem. 276 (2001) 6689–6694, https://doi.org/10.1074/jbc.M008127200.
[9] S. Jellinghaus, D.M. Poitz, G. Ende, A. Augstein, S. Weinert, B. Stütz, et al., Ephrin-A1/EphA4-mediated adhesion of monocytes to endothelial cells, Biochim. Biophys. Acta - Mol. Cell Res. 1833 (2013) 2201–2211, https://doi.org/10.1016/j.bbamcr.2013.05.017.
[10] J. Braun, S.C. Hoffmann, A. Feldner, T. Ludwig, R. Henning, M. Hecker, et al., Endothelial cell EphrinB2-dependent activation of monocytes in arteriosclerosis, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 297–305, https://doi.org/10.1161/ATVBAHA.110.217646.
[11] A. Ivanov, A. Romanovsky, Putative dual role of ephrin-Eph receptor interactions in inflammation, Life 58 (2006) 389–394, https://doi.org/10.1080/15216540600756004.
[12] A. Kania, R. Klein, Mechanisms of ephrin-Eph signalling in development, physiology and disease, Nat. Rev. Mol. Cell Biol. 17 (2016) 240–256, https://doi.org/10.1038/nrm.2015.16.
[13] S.D. Funk, A.W. Orr, Ephs and ephrins resurface in inflammation, immunity, and atherosclerosis, Pharmacol. Res. 67 (2013) 42–52, https://doi.org/10.1016/j.phrs.2012.10.008.
[14] E. Seiradake, K. Harlos, G. Sutton, A.R. Aricescu, E.Y. Jones, An extracellular steric seeding mechanism for Eph-ephrin signaling platform assembly, Nat. Struct. Mol. Biol. 17 (2010) 398–402, https://doi.org/10.1038/nsmb.1782.
[15] L.-Y. Liang, O. Patel, P.W. Janes, J.M. Murphy, I.S. Lucet, Eph receptor signalling: from catalytic to non-catalytic functions, Oncogene 38 (2019) 6567–6584, https://doi.org/10.1038/s41388-019-0931-2.
[16] T.K. Darling, T.J. Lamb, Emerging roles for Eph receptors and Ephrin ligands in immunity, Front. Immunol. 10 (2019) 1–15, https://doi.org/10.3389/fimmu.2019.01473.
[17] E.M. Lisabeth, G. Falivelli, E.B. Pasquale, Eph receptor signaling and ephrins, Cold Spring Harb. Perspect. Biol. Med. 5 (2013) 1–20, https://doi.org/10.1101/cshperspect.a009159.
[18] M.E. Pitulescu, R.H. Adams, Eph/ephrin molecules - a hub for signaling and endocytosis, Genes Dev. 24 (2010) 2480–2492, https://doi.org/10.1101/gad.1973910.
[19] E.R. Andersson, The role of endocytosis in activating and regulating signal transduction, Cell. Mol. Life Sci. 69 (2012) 1755–1771, https://doi.org/10.1007/s00018-011-0877-1.
[20] H.U. Wang, Z.F. Chen, D.J. Anderson, Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4, Cell 93 (1998) 741–753, https://doi.org/10.1016/S0092-8674(00)81436-1.
[21] J. Torres-Vázquez, M. Kamei, B.M. Weinstein, Molecular distinction between arteries and veins, Cell Tissue Res. 314 (2003) 43–59, https://doi.org/10.1007/s00441-003-0771-8.
[22] M.G. Coulthard, M. Morgan, T.M. Woodruff, T.V. Arumugam, S.M. Taylor, T.C. Carpenter, et al., Eph/ephrin signaling in injury and inflammation, Am. J. Pathol. 181 (2012) 1493–1503, https://doi.org/10.1016/j.ajpath.2012.06.043.
[23] N. Nasreen, N. Khodayari, P.S. Sriram, J. Patel, K.A. Mohammed, Tobacco smoke induces epithelial barrier dysfunction via receptor EphA2 signaling, Am. J. Physiol. - Cell Physiol. 306 (2014) 1154–1166, https://doi.org/10.1152/ajpcell.00415.2012.
[24] J.M. Van Gils, B. Ramkhelawon, L. Fernandes, M.C. Stewart, L. Guo, T. Seibert, et al., Endothelial expression of guidance cues in vessel wall homeostasis dysregulation under proatherosclerotic conditions, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 911–919, https://doi.org/10.1161/ATVBAHA.112.301155.
[25] J.M. Van Gils, M.C. Derby, L.R. Fernandes, B. Ramkhelawon, T.D. Ray, K.J. Rayner, et al., The neuroimmune guidance cue netrin-1 promotes atherosclerosis by inhibiting the emigration of macrophages from plaques, Nat. Immunol. 13 (2012) 136–143, https://doi.org/10.1038/ni.2205.
[26] H. Liu, K. Devraj, K. Möller, S. Liebner, M. Hecker, T. Korff, EphrinB-mediated reverse signalling controls junctional integrity and pro-inflammatory differentiation of endothelial cells, Thromb. Haemost. 112 (2014) 151–163, https://doi.org/10.1160/TH13-12-1034.
[27] V.M. Dixit, S. Green, V. Sarma, L.B. Holzman, F.W. Wolf, K. O'Rourke, et al., Tumor necrosis factor-α induction of novel gene products in human endothelial cells including a macrophage-specific chemotaxin, J. Biol. Chem. 265 (1990) 2973–2978.
[28] J.G. Wohlfahrt, C. Karagiannidis, S. Kunzmann, M.M. Epstein, W. Kempf, K. Blaser, et al., Ephrin-A1 suppresses Th2 cell activation and provides a regulatory link to lung epithelial cells, J. Immunol. 172 (2004) 843–850, https://doi.org/10.4049/jimmunol.172.2.843.
[29] E. Villar, I.M. Barroso, Role of sialic acid-containing molecules in paramyxovirus entry into the host cell: a minireview, Glycoconj. J. 23 (2006) 5–17, https://doi.org/10.1007/s10719-006-5433-0.
[30] K. Xu, Y.P. Chan, K.R. Rajashankar, D. Khetawat, L. Yan, M.V. Kolev, et al., New insights into the hendra virus attachment and entry process from structures of the virus g glycoprotein and its complex with Ephrin-B2, PLoS One 7 (2012) 1–11, https://doi.org/10.1371/journal.pone.0048742.
[31] I. Rissanen, A.A. Ahmed, K. Azarm, S. Beaty, P. Hong, S. Nambulli, et al., Idiosyncratic Mòjiang virus attachment glycoprotein directs a host-cell entry pathway distinct from genetically related henipaviruses, Nat. Commun. 8 (2017) 1–11, https://doi.org/10.1038/ncomms16060.
[32] E.D. Laing, M. Amaya, C.K. Navaratnarajah, Y.R. Feng, R. Cattaneo, L.F. Wang, et al., Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species, Virol. J. 15 (2018) 1–12, https://doi.org/10.1186/s12985-018-0964-0.
[33] O. Pernet, B. Lee, Henipavirus receptor usage and tropism, Curr. Top. Microbiol. Immunol. 359 (2012) 59–78, https://doi.org/10.1007/82.
[34] B. Lee, O. Pernet, A.A. Ahmed, A. Zeltina, S.M. Beaty, T.A. Bowden, Molecular recognition of human ephrinB2 cell surface receptor by an emergent African henipavirus, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) E2156–E2165, https://doi.org/10.1073/pnas.1501690112.
[35] O. Escaffre, V. Borisevich, B. Rockx, Pathogenesis of Hendra and Nipah virus infection in humans, J. Infect. Dev. 7 (2013) 308–311, https://doi.org/10.3855/jidc.3648.
[36] K. Xu, C.C. Broder, D.B. Nikolov, Ephrin-B2 and ephrin-B3 as functional henipavirus receptors, Semin. Cell Dev. Biol. 23 (2012) 116–123, https://doi.org/10.1016/j.semcdb.2011.12.005.
[37] E.D. Laing, C.K. Navaratnarajah, S. Cheliout, D. Silva, S.R. Petzing, Y. Xu, et al., Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus, PNAS 116 (2019) 1–9, https://doi.org/10.1073/pnas.1911773116.
[38] O.A. Negrete, E.L. Levroney, H.C. Aguilar, A. Bertolotti-Ciarlet, R. Nazarian, S. Tajyar, et al., EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus, Nature 436 (2005) 401–405, https://doi.org/10.1038/nature03838.
[39] O.A. Negrete, D. Chu, H.C. Aguilar, B. Lee, Single amino acid changes in the Nipah and Hendra virus attachment glycoproteins distinguish EphrinB2 from EphrinB3 usage, J. Virol. 81 (2007) 10804–10814, https://doi.org/10.1128/jvi.00999-07.
[40] O. Pernet, C. Pohl, M. Ainouze, H. Kweder, R. Buckland, Nipah virus entry can occur by macropinocytosis, Virology 395 (2009) 298–311, https://doi.org/10.1016/j.virol.2009.09.016.
[41] C. Mathieu, C. Pohl, J. Szecsi, S. Trajkovic-Bodennec, S. Devergnas, H. Raoul, et al., Nipah virus uses leukocytes for efficient dissemination within a host, J. Virol. 85 (2011) 7863–7871, https://doi.org/10.1128/jvi.00549-11.
[42] V. Tiong, M.H. Shu, W.F. Wong, S. Abubakar, L.Y. Chang, Nipah virus infection of immature dendritic cells increases its transendothelial migration across human brain microvascular endothelial cells, Front. Microbiol. 9 (2018) 1–12, https://doi.

org/10.3389/fmicb.2018.02747.
[43] Z. Wu, L. Yang, F. Yang, X. Ren, J. Jiang, J. Dong, et al., Novel henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012, Emerg. Infect. Dis. 20 (2014) 1064–1066, https://doi.org/10.3201/eid2006.131022.
[44] J. Lupberger, M.B. Zeisel, F. Xiao, C. Thumann, I. Fofana, L. Zona, et al., EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy, Nat. Med. 17 (2011) 589–595, https://doi.org/10.1038/nm.2341.
[45] A.S. Hahn, R.C. Desrosiers, Rhesus monkey rhadinovirus uses Eph family receptors for entry into B cells and endothelial cells but not fibroblasts, PLoS Pathog. 9 (2013) 1–14, https://doi.org/10.1371/journal.ppat.1003360.
[46] A.S. Hahn, R.C. Desrosiers, Binding of the Kaposi's sarcoma-associated herpesvirus to the ephrin binding surface of the EphA2 receptor and its inhibition by a small molecule, J. Virol. 88 (2014) 8724–8734, https://doi.org/10.1128/jvi.01392-14.
[47] G. Rappocciolo, M. Jais, P.A. Piazza, D.C. DeLucia, F.J. Jenkins, R. Rinaldo, Human herpesvirus 8 infects and replicates in Langerhans cells and interstitial dermal dendritic cells and impairs their function, J. Virol. 91 (2017) 1–15.
[48] S.J. Dollery, Towards understanding KSHV fusion and entry, Viruses. 11 (2019) 1–17.
[49] J. Chen, K. Sathiyamoorthy, X. Zhang, S. Schaller, B.E. Perez White, T.S. Jardetzky, et al., Ephrin receptor A2 is a functional entry receptor for Epstein-Barr virus, Nat. Microbiol. 3 (2018) 172–180, https://doi.org/10.1038/s41564-017-0081-7.
[50] H. Zhang, Y. Li, H.B. Wang, A. Zhang, M.L. Chen, Z.X. Fang, et al., Ephrin receptor A2 is an epithelial cell receptor for Epstein-Barr virus entry, Nat. Microbiol. 3 (2018) 164–171, https://doi.org/10.1038/s41564-017-0080-8.
[51] M. Dewannieux, C. Vernochet, D. Ribet, B. Bartosch, F.L. Cosset, T. Heidmann, The mouse IAPE endogenous retrovirus can infect cells through any of the five GPI-Anchored EphrinA proteins, PLoS Pathog. 7 (2011) 1–11, https://doi.org/10.1371/journal.ppat.1002309.
[52] H. Barth, T.J. Liang, T.F. Baumert, Hepatitis C virus entry: molecular biology and clinical implications, Hepatology 44 (2006) 527–535, https://doi.org/10.1002/hep.21321.
[53] M.P. Manns, M. Buti, E. Gane, J.M. Pawlotsky, H. Razavi, N. Terrault, et al., Hepatitis C virus infection, Nat. Rev. Dis. Prim. 3 (2017) 1–19, https://doi.org/10.1038/nrdp.2017.6.
[54] H.J. Harris, C. Davis, J.G.L. Mullins, K. Hu, M. Goodall, M.J. Farquhar, et al., Claudin association with CD81 defines hepatitis C virus entry, J. Biol. Chem. 285 (2010) 21092–21102, https://doi.org/10.1074/jbc.M110.104836.
[55] X. Wang, Z. Zou, Z. Deng, D. Liang, X. Zhou, R. Sun, et al., Male hormones activate EphA2 to facilitate Kaposi's sarcoma-associated herpesvirus infection: implications for gender disparity in Kaposi's sarcoma, PLoS Pathog. 13 (2017) 1–23, https://doi.org/10.1371/journal.ppat.1006580.
[56] P. Dutta, M. Botlani, S. Varma, Water dynamics at protein-protein interfaces: molecular dynamics study of virus-host receptor complexes, J. Phys. Chem. B 118 (2014) 14795–14807, https://doi.org/10.1021/jp5089096.
[57] A.S. Hahn, J.K. Kaufmann, E. Wies, E. Naschberger, J. Panteleev-Ivlev, K. Schmidt, et al., The ephrin receptor tyrosine kinase A2 is a cellular receptor for Kaposi's sarcoma-associated herpesvirus, Nat. Med. 18 (2012) 961–966, https://doi.org/10.1038/nm.2805.
[58] G. Mariggiò, S. Koch, T.F. Schulz, Kaposi sarcoma herpesvirus pathogenesis, Philos. Trans. R. Soc. B Biol. Sci. 372 (2017) 1–20, https://doi.org/10.1098/rstb.2016.0275.
[59] A.E. Whitfield, B.W. Falk, D. Rotenberg, Insect vector-mediated transmission of plant viruses, Virology 479–480 (2015) 278–289, https://doi.org/10.1016/j.virol.2015.03.026.
[60] M. Mulot, B. Monsion, S. Boissinot, M. Rastegar, S. Meyer, N. Bochet, et al., Transmission of Turnip yellows virus by Myzus persicae is reduced by feeding aphids on double-stranded RNA targeting the ephrin receptor protein, Front. Microbiol. 9 (2018) 1–15, https://doi.org/10.3389/fmicb.2018.00457.
[61] M. Mulot, S. Boissinot, B. Monsion, M. Rastegar, G. Clavijo, D. Halter, et al., Comparative analysis of RNAi-based methods to down-regulate expression of two genes expressed at different levels in Myzus persicae, Viruses 8 (2016) 1–19, https://doi.org/10.3390/v8110316.
[62] J. Cao, S. Wang, J. Wang, W. Cui, T. Nesil, M. Vigorito, et al., RNA deep sequencing analysis reveals that nicotine restores impaired gene expression by viral proteins in the brains of HIV-1 transgenic rats, PLoS One 8 (2013) 1–10, https://doi.org/10.1371/journal.pone.0068517.
[63] S.V. Westmoreland, L. Annamalai, M.R. Lentz, E.M. Ratai, B. Assaf, K. Boisvert, et al., Growth-associated protein-43 and ephrin B3 induction in the brain of adult SIV-infected rhesus macaques, J. Neurovirol. 17 (2011) 455–468, https://doi.org/10.1007/s13365-011-0047-0.
[64] F. Krammer, G.J.D. Smith, R.A.M. Fouchier, M. Peiris, K. Kedzierska, P.C. Doherty, et al., Influenza, Nat. Rev. Dis. Prim. 4 (2018) 1–21, https://doi.org/10.1016/B978-0-323-44887-1.00027-4.
[65] A. Karlas, N. MacHuy, Y. Shin, K.P. Pleissner, A. Artarini, D. Heuer, et al., Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication, Nature 463 (2010) 818–822, https://doi.org/10.1038/nature08760.
[66] A.T. Borchers, C. Chang, M.E. Gershwin, L.J. Gershwin, Respiratory syncytial virus - a comprehensive review, Clin. Rev. Allergy Immunol. 45 (2013) 331–379, https://doi.org/10.1007/s12016-013-8368-9.
[67] K. Shirato, M. Ujike, M. Kawase, S. Matsuyama, Identification of CCL2, RARRES2 and EFNB2 as host cell factors that influence the multistep replication of respiratory syncytial virus, Virus Res. 210 (2015) 213–226, https://doi.org/10.1016/j.virusres.2015.08.006.
[68] I.S. Marjara, B.J. Thu, Ø. Evensen, Differentially expressed genes following persistent infection with infectious pancreatic necrosis virus in vitro and in vivo, Fish Shellfish Immunol. 28 (2010) 845–853, https://doi.org/10.1016/j.fsi.2010.02.001.
[69] M.K. Dahle, J.B. Jørgensen, Antiviral defense in salmonids – mission made possible? Fish Shellfish Immunol. 87 (2019) 421–437, https://doi.org/10.1016/j.fsi.2019.01.043.
[70] W. Li, F.J.M. van Kuppeveld, Q. He, P.J.M. Rottier, B.J. Bosch, Cellular entry of the porcine epidemic diarrhea virus, Virus Res. 226 (2016) 117–127, https://doi.org/10.1016/j.virusres.2016.05.031.
[71] H. Wang, L. Yang, H. Qu, H. Feng, S. Wu, W. Bao, Global mapping of H3K4 trimethylation (H3K4me3) and transcriptome analysis reveal genes involved in the response to epidemic diarrhea virus infections in pigs, Animals 9 (2019) 523, https://doi.org/10.3390/ani9080523.
[72] P. Karayiannis, Hepatitis B virus: virology, molecular biology, life cycle and intrahepatic spread, Hepatol. Int. 11 (2017) 500–508, https://doi.org/10.1007/s12072-017-9829-7.
[73] H. Yang, X.X. He, Q.L. Song, M. Chen, J. Li, M.Y. Wang, et al., Association of Ephrin receptor A3 gene polymorphism with susceptibility to chronic severe hepatitis B, Hepatol. Res. 42 (2012) 790–797, https://doi.org/10.1111/j.1872-034X.2012.00977.x.
[74] S.E. Beck, S.E. Queen, K.W. Witwer, K.A. Metcalf Pate, L.M. Mangus, L. Gama, et al., Paving the path to HIV neurotherapy: predicting SIV CNS disease, Eur. J. Pharmacol. 759 (2015) 303–312, https://doi.org/10.1016/j.ejphar.2015.03.018.
[75] C. Griffiths, S.J. Drews, D.J. Marchant, Respiratory syncytial virus: infection, detection, and new options for prevention and treatment, Clin. Microbiol. Rev. 30 (2017) 277–319, https://doi.org/10.1128/CMR.00010-16.
[76] A. Barquilla, E.B. Pasquale, Eph Receptors and Ephrins: Therapeutic Opportunities, Annu. Rev. Pharmacol. Toxicol. 55 (2015) 465–487, https://doi.org/10.1146/annurev-pharmtox-011112-140226.
[77] L.M. Walker, D.R. Burton, Passive immunotherapy of viral infections: "super-antibodies" enter the fray, Nat. Rev. Immunol. 18 (2018) 297–308, https://doi.org/10.1038/nri.2017.148.
[78] S. Min, Y.S. Lim, D. Shin, C. Park, J.B. Park, S. Kim, et al., Abl tyrosine kinase regulates hepatitis C virus entry, Front. Microbiol. 8 (2017) 1–12, https://doi.org/10.3389/fmicb.2017.01129.
[79] G.M. Keating, Dasatinib: a review in chronic myeloid leukaemia and Ph+ acute lymphoblastic leukaemia, Drugs 77 (2017) 85–96, https://doi.org/10.1007/s40265-016-0677-x.
[80] P.M. Reeves, S.K. Smith, V.A. Olson, S.H. Thorne, W. Bornmann, I.K. Damon, et al., Variola and monkeypox viruses utilize conserved mechanisms of virion motility and release that depend on abl and src family tyrosine kinases, J. Virol. 85 (2011) 21–31, https://doi.org/10.1128/jvi.01814-10.
[81] M. Salgado, J. Martinez-Picado, C. Gálvez, S. Rodríguez-Mora, B. Rivaya, V. Urrea, et al., Dasatinib protects humanized mice from acute HIV-1 infection, Biochem. Pharmacol. (2019) 113625, https://doi.org/10.1016/j.bcp.2019.113625.
[82] M. Porotto, G. Orefice, C.C. Yokoyama, B.A. Mungall, R. Realubit, M.L. Sganga, et al., Simulating henipavirus multicycle replication in a screening assay leads to identification of a promising candidate for therapy, J. Virol. 83 (2009) 5148–5155, https://doi.org/10.1128/jvi.00164-09.
[83] A.N. Freiberg, M.N. Worthy, B. Lee, M.R. Holbrook, Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection, J. Gen. Virol. 91 (2010) 765–772, https://doi.org/10.1099/vir.0.017269-0.
[84] J. Pallister, D. Middleton, G. Crameri, M. Yamada, R. Klein, T.J. Hancock, et al., Chloroquine administration does not prevent nipah virus infection and disease in ferrets, J. Virol. 83 (2009) 11979–11982, https://doi.org/10.1128/jvi.01847-09.
[85] W.A. Banks, Characteristics of compounds that cross the blood-brain barrier, BMC Neurol. 9 (2009) 5–9, https://doi.org/10.1186/1471-2377-9-S1-S3.
[86] G. Ende, D.M. Poitz, E. Wiedemann, A. Augstein, J. Friedrichs, S. Giebe, et al., TNF-α-mediated adhesion of monocytes to endothelial cells-The role of ephrinA1, J. Mol. Cell. Cardiol. 77 (2014) 125–135, https://doi.org/10.1016/j.yjmcc.2014.10.010.