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Inhibiting the Fusion of Enveloped Viruses

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Mini Review

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Introduction

Enveloped viral entry is a process that concludes with the delivery of genomic material into the host cell by the fusion of the lipid bilayers of the virion and the host cell, making membrane fusion a critical step of the enveloped viral life cycle. Spontaneous protein-free membrane fusion does not normally occur in nature due to the repulsive hydrophobic and electrostatic forces, and high elastic force to prevent membrane deformation. Thus, to overcome this barrier, entry is facilitated by fusion proteins, which require "priming" to allow fusion to occur. Depending on the mechanism by which priming occurs, they belong to three types: I) priming by cleaving a single trimeric precursor (*e.g.*, HIV) II) priming by cleavage of a heterodimeric chaperone protein that protects the fusion peptide (*e.g.*, Rift Valley fever virus) III) Priming in the absence of cleavage (*e.g.*, Epstein-Barr virus). Irrespective of the mechanism of fusion or type of fusion protein, targeting this step in the viral life cycle can inhibit infection. In this review, recent advancements in inhibition of viral entry by targeting membrane fusion are discussed in the context of the viruses with Class I membrane fusion proteins: HIV, Ebola virus and Zika virus which has a Type II membrane fusion protein.

Human Immunodeficiency Virus (HIV)

The fusion protein of HIV, Env, is a polypeptide precursor, gp160, which is cleaved by host furin-like protease into two components: gp120, the receptor binding fragment, and gp41, the fusion fragment [1-3]. The precursor polypeptide, gp160, is arranged such that the C-terminal region of gp41 (C-terminal heptad repeats - CHR) is inserted into the viral membrane. Cleavage of gp160 and binding of gp120 to its receptor (CD4) and coreceptors (CXCR4/CCR5) causes insertion of the N-terminal region of gp41 (N-terminal heptad repeats – NHR) into the target cell membrane (fusion intermediate). Three CHRs wrap around three NHRs to form a six helical bundle (6HB), and this brings the membranes close enough to allow for pore formation. This 6HB formation is integral to the fusion process, which is why it has been extensively studied as a target for inhibiting viral entry.

Peptides which resemble the CHR prevent the formation of this 6HB by competing with the natural CHR, targeting the exposed face of the trimer N-helix, thereby preventing fusion. Several peptides have been designed as drugs, but the only FDA approved fusion inhibitor is T20, a 36-mer HIV-1 LAI CHR-derived peptide, which targets the FP and NHR to prevent 6HB formation. The low drug ability of peptide inhibitors is owed to their low half-life and solubility in blood, and their requirement to be frequently injected. Additionally, HIV1 strains resistant to T20 have also been recorded [4,5]. A second-generation peptide inhibitor, FB006, approved by the Chinese Food and Drug Administration, overcomes the problem of low solubility and short half-life by modifying the 13th amino acid into 3-maleimidopropionic acid so that it can bind to serum albumin [6]. Using the T20 peptide as a template multiple third generation lipopeptide inhibitors have been designed to have better pharmacokinetics and less side effects, but are yet to be cleared in clinical trials [6,7]. Peptides derived from the NHR are not as extensively studied due to their aggregation in solution; however, some peptides which are mutated to not interact with the CHRs while still forming the NHR helix have been developed [8].

Peptides are poor drug candidates because of their low oral adsorption and membrane impermeability leading to low bioavailability, high immunogenicity, and in-*vivo* instability. This makes small molecule inhibitors an attractive alternative. A hydrophobic pocket in the central coiled coil formed by the three NHR has been shown to be an effective target to

inhibit the binding of the CHR and thereby, formation of the 6HB [9]. Computational screening approaches, using the DOCK program, have sped up the process by which millions of compounds can be screened for the ability to dock into this hydrophobic pocket with favorable energetics [10]. Multiple compounds that interact with the NHR and prevent either the formation of the NHR trimer [11,12] or its interaction with CHR [13] have been identified. However, their function is observed in the micromolar range, thereby making them poor drug choices that warrant further development.

Ebola Virus (EBV)

Fusion and entry of EBV is mediated by the envelope glycoprotein (GP, which is made of GP1 and GP2. Following endosomal internalization by micropinocytosis, cellular proteases cleave the GP1 domain to allowing binding to the major receptor, the Neimann-Pick Complex Me (NPC1). This causes conformational changes in GP2, which inserts its fusion loop into the endosomal membrane, and forms a 6HB (three CHRs wrapped around a core formed by three NHRs) like that formed by HIV [14]. Thus, this formation of the 6HB has been studied as a potential drug target extensively.

Short peptides derived from the GP2 region have shown the ability to competitively inhibit fusion in pseudoviruses; however, this effect was observed only at very high concentrations (2mg/mL) [15]. Peptides are poor drug candidates against Ebola as the peptide must be delivered into endosomes much deeper in the pathway, where entry and fusion occur for them to be able to exert any inhibitory effect. Although attempts have been made to enhance the activity and delivery using cholesterol [16], or by conjugation to the HIV-Tat sequence [17], their efficacy and safety in-*vivo* and in clinical trials is yet to be determined. Small molecule inhibitors, such as E64D, which act as protease inhibitors to prevent cleavage of GP1, and thereby inhibit fusion have been reported [18], but they remain to be tested in-vitro and they are expected to have off-target effects as this is a host cell target. Using computational modeling and the structural similarity of the EBOV 6HB to the HIV 6HB, small molecules which exert their effect at the micromolar range and prevent the formation of the EBOV 6HB and thereby prevent fusion and entry have been discovered [19]. The efficacy *in vivo* of these molecules remains to be determined.

Zika Virus (ZIKV)

Fusion of ZIKV is mediated by the interaction of its Type II membrane fusion E protein which fuses with the endosomal membrane. In the pre-fusion state, E-protein exists in a dimeric form, and it becomes trimeric during the fusion-state, which is triggered by the low pH of the endosome [20]. E protein is composed of four domains: the stem-transmembrane domain, domain II (DII) consisting of the fusion loop (FL) that gets inserted into the host cell membrane, and domain I (DI) linking DII and DIII. N-glycans conjugated to the surface of E-protein allow for the entry and uptake of the virus by clathrin-mediated endocytosis. Once it encounters the acidic pH of the late endosomes, the E-protein shifts conformationally from homodimer to homotrimer, which allow the FL to be exposed, and as it gets inserted into the target membrane, three homotrimers associate to form a trimeric structure, and this allows for pore formation and release of genomic material into the cytosol [21].

Existing FDA-approved drugs, such as atovaquone [22], chloroquine and other quinolones have shown high potency against ZIKV [23]. However, quinolones are not safe to be administered to pregnant women due to dangers to the fetus, which makes alternative drug discovery important. Computational modeling has shown that the ligand binding pocket of co-crystallized detergent β-octyl glucoside (BOG) molecule in the interface of DI and DII can be the targets of small molecule inhibitors, which led to the identification of multiple compounds that inhibited infection in the micromolar range [25]. Using Dengue virus as a model, it has been hypothesized that peptides derived from the stem region of E-protein can competitively inhibit fusion. This led to the development of Z2, which was effective both in-*vitro* and in-*vivo*, and could also cross the placental barrier to prevent infection of the fetus [25].

Conclusion

The discovery and efficacy of T20 in clinical settings sparked extensive research into mechanisms of viral fusion and targeting fusion to inhibit infection. The availability of modern technologies, such as computational modeling, has also allowed an exponential increase in the ability to screen for potential drug candidates. Combined, this has generated a wealth of information that can be used to develop novel inhibitors of viral fusion and infection. These candidates now need to progress to optimization and pre-clinical testing.

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