HHS Public Access

Author manuscript

Trends Microbiol. Author manuscript; available in PMC 2019 June 01.

Published in final edited form as:

Trends Microbiol. 2019 June ; 27(6): 477–479. doi:10.1016/j.tim.2019.03.001.

Influenza Neuraminidase: Underrated Role in Receptor Binding

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Summary:

Influenza virus neuraminidase cleaves sialic acid groups from cell glycoproteins, enabling release of the virus from host cells. Neuraminidase also contributes to virus binding to the sialic acid groups of cell glycoproteins, which could complement the receptor-binding function of hemagglutinin, enhancing enzymatic activities of neuraminidase, and facilitate virus infection.

Keywords

influenza A virus; influenza virus; neuraminidase; sialic acids; receptor; receptor binding

Influenza A Tropism Through Binding Affinity

Influenza A viruses (IAVs) infect humans and a wide range of animal species, including birds, pigs, horses, dogs, and marine mammals (e.g., seals and whales). The initial step in viral infection is the binding to sialic acid receptors, typically avian-like receptors (sialic acid- α2,3-galactose linkage, SA2,3Gal) and human-like receptors (sialic acid-α2,6-galactose linkage, SA2,6Gal) on the host cell surface glycoproteins, and the last step in viral infection is the cleavage of sialic acid receptors from glycoproteins and glycolipids by neuraminidase (NA) and release of virus particles after budding from the host cell plasma membrane. Gaining binding affinity to human-like SA2,6Gal receptors is considered a key prerequisite for avian IAVs that cause pandemics or epidemics among humans. The IAV glycoprotein hemagglutinin (HA) attaches to host receptors during virus entry, and the receptor-binding specificity of HA is well documented to affect IAV host tropism. We review recent studies demonstrating that NA, the other IAV glycoprotein, contributes to virus binding to the sialic acid receptors at host cell glycoproteins and that such receptor-binding contributions can complement those of HA, enhance NA enzymatic activities, and facilitate virus infection and host adaptation.

Receptor Binding of IAV NA

The NA of IAVs belongs to the exo-sialidase family, which cleaves the α-ketosidic linkage between synthetic substrates and the terminal sialic acid residues in oligosaccharides, glycoproteins, glycolipids, or colominic acids. It is generally accepted that the functions of HA and NA are, to some extent, contrary: HA binds to sialic acid receptors on host cells and

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mediates virus attachment and entry, whereas NA cleaves the α-ketosidic linkage between the terminal sialic acid residues and the remaining substructures. However, it has been reported that, in addition to their enzymatic functions, NA proteins, such as N1, N2, and N9, also have sialic acid receptor–binding functions. For example, subtype N9 from A/tern/Australia/G70C/75(H11N9) alone hemagglutinates animal erythrocytes by using a second receptor binding site (SRBS), which is also referred as hemadsorption site, that is distinct from the NA catalytic site [1].

Structural analyses suggested the SRBS of NA consist of three loops (367–372, 400–403, and 432) and interact with Neu5Ac moiety in chair conformation, which was similar to that by the RBS of HA [2]. In contrast, the NA catalytic site interacts with sialic acid receptor in a twisted boat conformation [2]. Sequence analyses showed that these residues in the SRBS of NA are mostly conserved among avian IAVs but not human and swine IAVs [2]. Of interest, the sialic acid binding function of the SRBS of NA has been so far identified primarily in avian IAVs [3]. On the other hand, sequence diversity in the SRBS across different NA subtype or even within the same NA subtype is well documented to affect the receptor binding ability of NA. For example, exchange of amino acids in the loops at positions 368–370 and positions 399–403 of N9 into those corresponding positions in the N2 by site-directed mutagenesis made N2 acquire the hemagglutination activity as shown in N9 [4]. The pandemic viruses A/Singapore/1/57(H2N2) and A/Brevig Mission/1/18 (H1N1) differed from their avian counterpart precursors with abolished or reduced hemadsorption activity due to variations in the SRBS of NA [3].

Current studies suggested that, through its glycan binding, the SRBS of NA can increase the enzymatic activities on multivalent substrates but not monovalent substrates [3]. NA of a human H7N9 virus enhanced overall virus binding to SA2,6Gal but not to SA2,3Gal via the SRBS of NA within the context of virus particle [5]. Interestingly, another study, when using recombinant NA proteins, showed that the NA of human H7N9 virus, with a mutation A401T in the SRBS of N9, increased virus binding and cleavage activities to SA2,3Gal, and none of these wild type or mutant NA recombinant proteins had significant binding affinities to SA2,6Gal [6]. Interestingly, the T401A substitution of N9 protein was acquired prior to the other two mutations G186V and Q226L in the RBS of H7 protein, which altered receptor binding properties of the novel H7N9 virus. Of note, the binding specificity was determined by biolayer interferometry in [5] whereas by glycan array in [6]. The conflicting results in these studies could be affected by experimental conditions (i.e. whole virus particle or recombinant proteins) and/or the glycan binding detection methods. Although the role of SRBS of NA in the interaction between influenza virus and the receptors of the host cells during virus infection remains largely unknown, these findings suggested mutations in the SRBS of NA could affect the HA-NA balance and facilitate the H7N9 avian IAVs to cross the host species barriers.

While the receptor binding via the SRBS of NA enhances the NA enzymatic activity, receptor binding via the catalytic site of NA displaying low enzymatic activity may complement HA receptor binding and serve as a receptor-binding protein during virus entry. In 2005, the N2 protein of human seasonal H3N2 virus gained a D151G substitution at the NA catalytic site, enabling the viruses to agglutinate erythrocytes in an oseltamivir-sensitive

manner, increasing binding affinities to 3′-sialyllactosamine and 6′-sialyllactosamine higher than the corresponding HA affinities to those sialosides [7]. The introduction of mutation D151G into N1 and N2 reduces NA enzymatic activity and enables preferential binding to SA2,3Gal [7, 8]. Of note, the entry of human H3N2 viruses with mutation D151G into Madin-Darby canine kidney cells was inhibited by oseltamivir, which can block the enzymatic site of NA [8]. Sequence comparison between clinical samples and virus isolates suggested that D151G was acquired during virus isolation and propagation for these recent human H3N2 viruses, which replicate poorly in MDCK cells, and the increased receptor binding due to D151G at the catalytic site of NA could help cell adaptation and facilitate viral infection [9]. Of note, the mutation D151G was not identified in the isolates recovering using MDCK-siat1 cells, which overexpresses human SA2,6Gal. On the other hand, H150R at the catalytic site of NA enabled hemagglutination ability for H3N2 viruses, and H150R was demonstrated as not a cell-adapted mutation (like D151G) but a natural mutation that emerged in 2005 and has become predominant in recent H3N2 viruses [11]. For human H1N1 viruses, G147R in the NA catalytic site of N1 which occurs at low frequencies in human pandemic and seasonal H1N1 viruses enables an HA binding–deficient mutant to use N1 as the receptor-binding protein [10]. Guo et al. [12] showed by using a biolayer interferometry assay, that a low activity NA can contribute to virion-receptor binding by its catalytic site in a NA inhibitor sensitive manner. Nevertheless, the function of these NA substitutions in the process of virus entry remains largely unknown.

Concluding Remarks

In summary, recent studies suggested that NA plays important roles in virus host adaptation and evolution by serving as a receptor-binding protein. Increasing evidence suggests that possibly all avian NAs have a SRBS distinct from but close to the catalytic site. However, the receptor-binding function of NA in IAV biology is underrated and needs further research. First, the roles of NA receptor binding in host/tissue/cell tropism is far from being understood; the relationship between receptor binding properties of NA and those of HA during virus entry is not clear. Prior studies suggested that the affinity to certain types of sialic acid receptors might be higher for NA protein than for HA protein, and further studies are needed to determine whether NA could affect the spectrum of receptors to which the viruses can bind. In addition, the role of the SRBS of NA in the interaction between influenza virus and its host receptors during virus infection is unclear, although the receptor binding activities through the SRBS of NA can enhance the enzymatic activities of NA over multivalent substrates. Finally, the effect(s) of the balance between HA and NA functional activities during virus evolution (e.g., compatibility between HA and NA during genetic reassortment) is still not fully understood.

Acknowledgement

This study was supported by the National Institutes of Health (grant number R01AI116744).

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