JBC EDITORS' PICK HIGHLIGHT

Finding proteases that make cells go viral

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The activation of influenza virus hemagglutinin (HA) glycoprotein via cleavage by host cell proteases is essential for viral infectivity, and understanding the mechanisms for HA protein cleavage and how they may differ depending on the biological context is important for the development of flu treatments. However, the HA proteases involved in the activation of many viral strains remain unidentified. In this issue, Harbig *et al.* identify a repertoire of proteases that cleave HA and determine the proteases' functionality against specific HA glycoproteins.

Influenza A and B viruses (IAV/IBV) annually account for 3–5 million severe illnesses and result in 350,000–650,000 deaths worldwide (World Health Organization, Influenza (Seasonal) Fact Sheet; https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal); accessed July 10, 2020). Entry of influenza virus into host cells is initiated through HA binding to cell-surface sialic acid. During infection, HA is transcribed as a fusion-incompetent precursor protein, which must be cleaved by host cell proteases into linked subunits to yield an active, pH-sensitive glycoprotein. Infected cells that lack the proper cellular proteases produce noninfectious viral particles (1). HA cleavage by proteases differs depending on viral strain, host species and type, and area of localized infection within the host cell. Despite intensive HA research over the last 90 years, cellular attributes that lead to the production of functional HA are still being uncovered. In their recent work, Harbig *et al.* (2) make a significant step forward in identifying how type and location of known and recently identified proteases result in functional HA in IAV and IBV.

A common feature among most influenza HAs is a monobasic cleavage site activated by trypsin-like proteases in the airways of mammals and in the respiratory and intestinal tissue of avian hosts, respectively. In 2006, Böttcher *et al.* (3) found that the transmembrane serine protease 2 (TMPRSS2) activates human IAV HA via cleavage of this monobasic site. More recently this protease has been shown as important for SARS-CoV-2 infection (4). It was later discovered that TMPRSS2-deficient mice do not develop pathogenic influenza infection to H1, H7, or H10 serotypes, indicating that TMPRSS2 is necessary for pathogenesis of multiple HA serotypes in mice (5, 6). Intriguingly, IBV strains and certain H3N2 IAV strains were pathogenic in these knockout mice, indicating the presence of other proteases that could yield proteolytic cleavage and activation of HA.

To identify candidate proteases responsible for activation of H3N2 and IBV strains, the authors determined the repertoire of viruses in the mouse lower airway by RNA sequencing of the trachea, bronchi, and lungs. This transcriptome analysis profiling detected dozens of candidate proteases with trypsin-like activity, a hallmark for potential activity for cleaving HA. To narrow the list of proteases, the authors infected murine lung cell lines, in the presence and absence of trypsin, to identify which proteases did not yield cleavage of HA using plaque assay analyses. They identified two cell lines: MLE-15, which did not yield successful production of IAV or IBV virions in the absence of trypsin but could be rescued with trypsin, and AECII, which could fully result in active infection and replication of IAV and IBV strains. Proteases present in AECII cells, but not in MLE-15 cells, were potential candidates, and proteases present in MLE-15 cells but not in AECII cells could be discounted. From this analysis, four highly likely candidate proteases were selected: hepsin, prostasin, TMPRSS4, and TMPRSS13.

Previous work using a double-knockout mouse line for the proteases TMPRSS2 and TMPRSS4 demonstrated a role for TMPRSS4 in HA cleavage (7). However, despite some deficiencies, H3 and IBV were still activated to an extent, indicating that at least one other protease is involved. To determine the extent of involvement of their candidate proteases, Harbig *et al.* used the protease inhibitors aprotinin, BAPA, MI-432, and α_1-antitrypsin in conjunction with transfection of TMPRSS13, hepsin, and prostasin in double-knockout *Tmprss2*$^{-/-}$*Tmprss4*$^{-/-}$ cells. Together, this highlighted differences in HA reliance on protease cleavage. Using a quantitative proteolytic cleavage fluorescence-based assay, the authors determined the range and intensity of specific protease inhibition, as highlighted in Fig. 1. From this work, they determined which proteases were impacted by which protease inhibitor and to what degree HA cleavage was impacted. Interestingly, when multicycle replication of IAV serotype H3 and IBV was measured following inhibitor treatment, IAV H3 and IBV HA responded with similar inhibition trends but with different intensities to protease inhibitor treatments. This indicates that IAV and IBV HA proteases overlap but facilitate HA cleavage differently. Finally, the authors tested how these results from the lower airways of mice compared with that of humans. It was found that human orthologs of hepsin and prostasin yielded significant cleavage of IBV HA but not of IAV H3 HA, which contrasts with the activation of both when using murine orthologs. This suggests differences between mice and humans in HA proteolytic specificity, which likely account for variances in infectivity of viral strains and host species.

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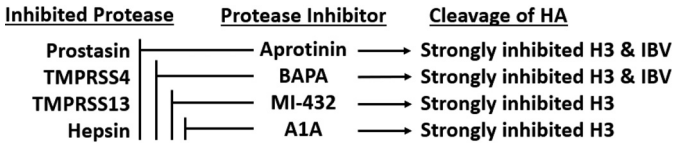

Figure 1. Serine protease inhibitors that suppress cleavage of HA. Inhibitors were selected to determine the specificity of host cell proteases on different influenza strains, uncovering various levels of overlap in strain specificities.

Harbig *et al.* showed resourcefulness and progressed the field. Overall, it was found that there are different yet overlapping sets of proteases that facilitate the cleavage of HA in IAV H3 and IBV influenza virus strains. H3 HA likely has a narrower range of proteases that work efficiently compared with the IBV strains, which showed a broader range in effectiveness of proteolytic cleavage and activation. Influenza viruses continue to cause widespread morbidity and mortality worldwide, despite increasing access to vaccines and antivirals. These vaccines and treatments need to be constantly renewed due to the high rate of viral changes from year to year. Determining specific host factors in the production of infectious viral particles is not only an essential aspect in unraveling the infection process but is also highly significant to the development of antiviral drugs by targeting host-derived machinery. For example, it has been observed that short-term respiratory treatment with the protease inhibitor aprotinin has reduced the time of influenza virus infection in humans (8), similarly to that observed with common neuraminidase inhibitors. Many medically significant viruses require proteolytic activation of the fusion protein to result in functional infectious particles, making the identification of proteases in this study highly relevant to both basic science and potential therapeutic applications.

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Abbreviations—The abbreviations used are: IAV, influenza A; IBV, influenza B; HA, hemagglutinin.

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