Inhibiting SARS-CoV-2 papain-like protease may stop viral spread

By Samantha Black, PhD, The Science Advisory Board staff writer

July 30, 2020 -- Another nonstructural protein, papain-like protease, has been identified as a SARS-CoV-2 therapeutic target with the potential to block viral replication, according to an article published in *Nature* on July 29.

During infection viruses must overcome host immune defenses, including innate immune responses where cytokines can modulate other parts of the immune system and upregulate antigen presentation. These innate immune systems include type I interferons (IFNs) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways.

Coronaviruses can suppress innate immune responses and promote their own replication. SARS-CoV-2 relies on the activity of viral proteases -- specifically the main protease and papain-like protease -- to generate a functional replicase complex and enable viral spread.

SARS-CoV-2 papain-like protease preferentially reduces Isgylation of substrates -- the protein modification process by which a cytokine and intracellular ubiquitin modifier, interferon-stimulated gene 15 (ISG15), forms covalent conjugates with cellular proteins. By preferentially cleaving ISG15, SARS-CoV-2 blocks the production of host IFNs.

Papain-like proteases have been studied in SARS-CoV, and now a group of German and Dutch researchers has been able to explore the effect of SARS-CoV-2 papain-like protease on immune responses in cell culture experiments.

Understanding how papain-like protease blocks innate immune responses

First, the researchers determined the crystal structure of SARS-CoV-2 papain-like protease in complex with ISG15. In comparing a remote catalytic S2 site with papain-like proteases of SARS-CoV and other human coronaviruses, the team found that the site is poorly conserved and varies in hydrophobicity, which could influence substrate specificity among different coronaviruses. For example, while SARS-CoV papain-like protease preferentially binds to ubiquitinated substrates, SAS-CoV-2 papain-like protease more strongly binds to ISG15.

Next, the team analyzed the cellular interactome of SARS-CoV-2 papain-like protease. The enzyme decreases Isgylation of cellular proteins leading to decreases in phosphorylation of critical components of the IFN pathways including interferon regulatory factor 3 (IRF3) and Tank-binding kinase 1 (TBK1; activates NF-kB pathways for upregulation of inflammatory signaling). This finding is in agreement with clinical data that suggests that COVID-19 is associated with lower interferon responses than other diseases such as severe acute respiratory syndrome (SARS) or influenza.

Rescuing innate immune responses with a papain-like protease inhibitor

Lastly, to support efforts to identify potential therapeutic strategies against COVID-19, the research team tested the effect of a drug that was previously developed for SARS-CoV for potency against SARS-CoV-2.

"We used the compound GRL-0617, a noncovalent inhibitor of papain-like protease, and examined its mode of action very closely in terms of biochemistry, structure, and function," said senior author Ivan Dikic, PhD, director of the Institute of Biochemistry at University Hospital Frankfurt. "We concluded that inhibiting papain-like protease is a very promising double-hit therapeutic strategy against COVID-19. The further development of papain-like protease -inhibiting substance classes for use in clinical trials is now a key challenge for this therapeutic approach."

GRL-0617 is a naphthalene-based inhibitor that does not inhibit other host proteases. The researchers hypothesized that the drug would bind to conserved residue, Tyr268, of SARS-CoV-2 papain-like protease to block the entry of ISG15 to the catalytic site. They found that GRL-0617 effectively blocked SARS-CoV-2 papain-like protease, which led to increased levels of Isgylated proteins, including IRF3, TBK1, and p65 (a marker of NF-kB pathway activation).

The team infected Caco-2 cells with SARS-CoV-2 and treated them with GRL-0617 to see if the drug could block viral replication as measured by cytopathogenic effect. They found a gradual dose-dependent inhibition of SARS-CoV-2. The treatment reduced active viral replication and consequently decreased the release of viral particles from infected cells into the supernatant.

The authors noted that while GRL-0617 appears to be a valuable therapeutic target of SARS-CoV-2 papain-like proteases, additional work is needed to develop more potent and selective inhibitors. They also suggested that combining drugs targeting both the SARS-CoV-2 main protease and the papain-like protease may be a successful therapeutic option in the future.

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