Minireview

Inter-proteomic posttranslational modifications of the SARS-CoV-2 and the host proteins – A new frontier

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Impact statement

The pandemic of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has affected >69 million individuals worldwide among whom >15 million in the USA as of 10 December 2020, and has put major strains on health care resources all over the world. Nearly 1.6 million deaths have been reported in the world, including ~290 thousand in the USA. Currently, only physical barrier measures are used to prevent the spread of COVID-19, albeit with limited success. As concurrent sprints to produce vaccines and drugs are under way with mixed success, this review attempts to summarize known molecular mechanisms employed by SARS-CoV-2 to infect cells, leading to the COVID-19 morbidity and mortality. The mechanisms reviewed in this paper deal with interactions between viral and human proteins elicited in inter-proteomic studies, and may be useful for anti- SARS-CoV-2 therapies, which, if successful, would have undeniable impact in the fight against SARS-CoV-2.

Abstract

Posttranslational modification of proteins, which include both the enzymatic alterations of protein side chains and main-chain peptide bond connectivity, is a fundamental regulatory process that is crucial for almost every aspects of cell biology, including the virus-host cell interaction and the SARS-CoV-2 infection. The posttranslational modification of proteins has primarily been studied in cells and tissues in an intra-proteomic context (where both substrates and enzymes are part of the same species). However, the inter-proteomic post-translational modifications of most of the SARS-CoV-2 proteins by the host enzymes and *vice versa* are largely unexplored in virus pathogenesis and in the host immune response. It is now known that the structural spike (S) protein of the SARS-CoV-2 undergoes proteolytic priming by the host serine proteases for entry into the host cells, and N- and O-glycosylation by the host cell enzymes during virion packaging, which enable the virus to spread. New evidence suggests that both SARS-CoV-2 and the host proteins undergo inter-proteomic posttranslational modifications, which play roles in virus pathogenesis and infection-induced immune response by hijacking the host cell signaling. The purpose of this minireview is to bring attention of the scientific community to recent cutting-edge discoveries in this understudied area. It is likely that a better insight into the molecular mechanisms involved may open new research directions, and thereby contribute to novel therapeutic modality development against the SARS-CoV-2. Here we briefly discuss the rationale and touch upon some unanswered questions in this context, especially those that require attention from the scientific community.

Keywords: Covid-19, proteome, protein modifications, host reaction

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Introduction

Posttranslational modification (PTM) of proteins is a fundamental regulatory process in biology, which creates enormous diversity from a limited number of encoded proteins.[1] For example, 15 modifiable amino acids (out of 20-proteinogenic amino acids) are known to undergo over 100 different types of PTMs.[1] As, at any given time point, only one PTM can occupy a modifiable site, these multiple modifications at a common site likely occur in a mutually exclusive manner.[2] Moreover, a large number of proteins are subjected to multiple PTMs at different sites in a protein molecule, which often occur in clusters and appear to be interrelated in diverse ways.[2–5] Thus, the scope and complexity of PTMs is enormous, with current limited understanding.

In the past, studies of PTMs were limited due to lack of appropriate tools and techniques.[6–8] Technological advances in mass spectrometry over the past two decades have created opportunities to study PTM of proteins at a larger scale in cells and tissues.[9–11] Consequently, there has been a

growing interest in capturing different context-dependent types of PTMs (e.g. phosphorylation, acetylation, *N*- and *O*-glycosylation, etc.) to get new insights into biological and pathological processes,[12–15] and to identify potential therapeutic targets.[16] In the context of viral infection, such proteomic studies have primarily focused on identifying the host proteins that are substantially altered in response to the infection, or to map the virus-host protein interactome.[17–19] Some of the identified host proteins are essential for the viruses in infecting the host cells and in replicating within these cells, which may provide a basis for the development of vaccines and anti-viral therapeutics.

The unprecedented global impact of the SARS-CoV-2 pandemic has created an urgent need for innovative ideas beyond the classical route of anti-viral therapeutic and vaccine development, and to deploy a more diversified approach for a global pandemic of this magnitude.

The PTM of proteins has primarily been studied in an intra-proteomic context where protein substrates and enzymes are part of the same species. The framework of PTMs in an inter-proteomic context, such as SARS-CoV-2-host cell interactions, or other similar pathogen-host interactions, is not well explored. SARS-CoV-2 carries a 30 kb genome with a 5′ and a 3′ untranslated regions, which contain open reading frames (ORFs) that encode 4 structural proteins, 15–16 non-structural proteins (Nsp 1–15 or 16), and at least 6 accessory proteins.[20–22] Nsp3 and Nsp5 derive from a polyprotein encoded by ORF1a and ORF1b at the 5′ region, and form the replicase/transcriptase complex.[20] Nsp3 and Nsp5 are also known as papain-like protease (PLpro) and main protease, respectively. There are, in addition, a dozen ORFs at the 3′ end of the SARS-CoV-2 genome, which encode structural proteins and accessory proteins.[20–22] The structural proteins are spike (S), envelop (E), membrane (M), and nucleocapsid (N) proteins. Five accessory proteins labeled ORF3a, 6, 7a, 7 b, and 8 are considered to play a role in viral pathogenicity, while the existence of accessory ORF proteins 3 b, 9, and 10 is still controversial. Interestingly, antibodies were detected against ORF3b protein, along with anti-ORF8 antibodies, starting early during the SARS-CoV-2 infection,[23] suggesting that ORF3b is indeed expressed in patients with COVID-19. Another study found that SARS-CoV-2 ORF3b is a potent interferon antagonist whose activity is further increased by a naturally occurring elongation variant,[24] supporting its role in the suppression of innate immunity by the virus.

It is conceivable that the SARS-CoV-2 enzymes (e.g. Nsp3 and Nsp5) may utilize the host proteins as substrates to manipulate the host cell's signaling system for their own benefit. The diversity created by inter-proteomic PTMs of the virus and the host proteins is expected to empower the SARS-CoV-2 to infect the host cells, evade the host immune defense, replicate within host cells, and eventually spread from one host to another. Recent cutting-edge discoveries of inter-proteomic PTMs of the SARS-CoV-2 and the host proteins, and their crucial roles in virus infection and pathogenesis[25–27] suggest that this is indeed the case. These ground-breaking discoveries may be seen as the product of the unprecedented need for innovative ideas and multi-pronged approach to fight SARS-CoV-2. Indeed, new evidence related to inter-proteomic PTMs have created exciting new research directions and opportunities to look beyond the traditional path of antiviral therapeutics and vaccine development. It is conceivable that inter-proteomic PTMs of the SARS-CoV-2 proteins by the host enzymes would substantially increase diversity and functions of viral proteins. Here we will briefly discuss recent exciting discoveries in this understudied area and touch upon some emerging and unanswered questions, which require attention from the scientific community.

SARS-CoV-2 infection-induced changes in phosphoproteome

Protein phosphorylation, which is dynamically regulated by diverse kinases and phosphatases, is the most common PTM and a key regulatory process that is crucial for almost every aspect of cell biology.[1] Consequently, dysregulated protein phosphorylation is often found in pathobiology and protein kinases are considered ideal drug targets, with some kinases having been successfully targeted for therapeutic purposes.[16] Thus, the study of protein phosphorylation has been a promising area in biomedical research.

Recently, Bouhaddou *et al.*[25] reported a global phosphorylation landscape of SARS-CoV-2-infected cell lines, showing substantial changes in the phosphorylation of host proteins. They quantified 3036 human orthologous proteins and 4624 human orthologous phosphorylation sites. Notably, the majority of regulations linked to infection processes were found to be occurring at the level of phosphorylation and, in general, the viral proteins significantly increased, whereas the host proteins significantly decreased, in their relative abundance.[25] This coincided with upregulation of casein kinase II (CK2) and p38 MAP kinase, and downregulation of mitotic kinases, resulting in cell cycle arrest. Interestingly, the host cell's CK2 and the SARS-CoV-2's N protein co-localized at virus-induced filopodial protrusions, suggesting that N protein controls CK2 activity and disturbs cytoskeleton organization.[25] In addition, a marked regulation of phosphatidylinositol enzyme activities for PIK3CA, PLCB3, and PIKFYVE was noted, suggesting that an appropriate balance of phosphatidylinositol species plays a role in the infection process.[25] Thus, SARS-CoV-2 hijacks the host cell signaling system, alters host cell phosphoproteome, diverts host cell resources, and ultimately causes cell cycle arrest and cell death. The authors also addressed the therapeutic relevance of the identified signaling pathways by showing that inhibition of CK2, cyclin-dependent kinase (CDK), AXL and PIKFYVE kinases displayed antiviral efficacy in lung cell lines.[25] In aggregate, these findings provided insights into cell signaling pathways that may be required for diverting host cell resources to support rapid and uncontrolled replication of the virus. Collectively, these findings suggest an intimate and complex relationship between the viral and host cell proteomes (Figure 1).

Large changes in the host protein phosphorylation, as observed by Bouhaddou *et al.*,[25] highlight the degree to

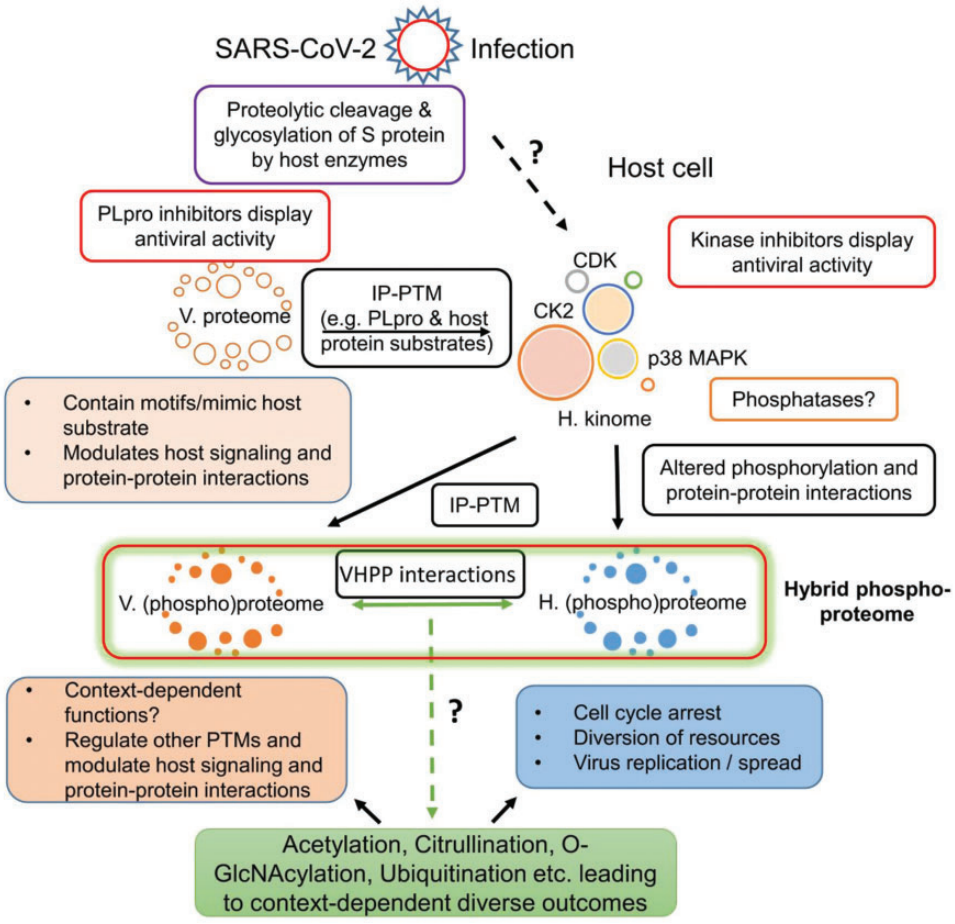

Figure 1. Inter-proteomic PTMs of the virus and host proteins, and hybrid phosphoproteome resulting from SARS-CoV-2 infection. A schematic showing SARS-CoV-2 infection in the host cell modulates the host cell kinome and leads to the generation of a hybrid phosphoproteome comprised of altered phosphoproteome of the host cells and newly phosphorylated virus proteins, which is critical for virus infection, replication and spread via filopodia. Targeting kinases identified by Bouhaddou *et al.*[25] and PLpro shown by Shin *et al.*[27] affect SARS-CoV-2 pathogenesis (red rectangle). As phosphorylation is dynamically regulated by both kinases and phosphatases, it is likely that phosphatases are also involved (golden rectangle). Infection-related changes in cell signaling and viral proteins both contribute to the modulation of phosphorylation regulating host enzymes. It is possible that virus and host phosphoprotein-protein interactions play roles in diverse outcomes because the composition of the hybrid phosphoproteomes is expected to vary in different cell types and in different states. It is likely that this framework could also apply to other PTMs that are dynamically regulated similar to phosphorylation, such as acetylation, deimination/citrullination, *O*-GlcNAcylation, and ubiquitination (green rectangle). H: human; V: virus; VHPP: virus and host protein–protein; IP-PTM: inter-proteomic posttranslational modification; PLpro: papain-like protease.

which the virus makes use of host enzymes to promote rapid changes in the host cell-signaling. However, the mechanisms involved remain unclear. It is likely that different mechanisms contribute to temporal changes in the host phosphoproteomes and its associated consequences. For example, the host proteins that directly interact with viral proteins may play a role in early events post infection, whereas an increase in the relative abundance of viral proteins with increased viral load over time may play a role in later events. Moreover, it is likely that the host's systemic environment in general and the cell-specific environment within the host in particular may vary substantially with age, sex, and preexisting conditions such as diabetes, obesity, chronic inflammation, immune deficiency, and autoimmune diseases, where distinct phosphoproteomes may be generated and contribute to diverse outcomes, as observed in epidemiological studies.[28–30] For instance, a poorly controlled glycemic state in patients with obesity and/or diabetes may provide a fertile ground for virus replication and infection-related cytokine storm, because of increased glucose availability to fuel altered cellular metabolism in inflammatory immune cells, which has emerged as a major regulator of different immune cell types.[31,32] Thus, this new knowledge about dramatic rewiring in the phosphorylation of host proteins has opened new research directions to explore therapeutic avenues against virus replication, its spread within the host, and infection-related diverse outcomes.

SARS-CoV-2 infection-induced PTMs other than phosphorylation

As cell signaling proteins are often regulated by multiple types of commonly occurring PTMs, which often influence and regulate each other, it is likely that the SARS-CoV-2 infection affecting host proteins will cause substantial changes in PTMs other than phosphorylation, e.g. deamination, O-GlcNAcylation, acetylation, ubiquitin modification, etc.

Deimination/citrullination

Recent studies have proposed a role for posttranslational deimination by peptidylarginine deiminases (PADs) in SARS-CoV-2-infection.[33–35] Deimination converts arginine residue in a protein into citrulline, and allows the modified protein to carry out multifaceted functions, a phenomenon termed "protein moonlighting." There are five PADs in humans, with different tissue expressions and deimination activities, and they are involved in multiple diseases, including autoimmune diseases and viral infections. An *in silico* analysis of SARS-CoV-2-infected lung tissues and cell lines has identified that SARS-CoV-2-infection modulates the expression of PADs, predominantly PAD-2 and PAD-4, with link to inflammatory pathways.[33] Other studies have lent strong support to this notion by demonstrating that SARS-CoV-2 leads to activation of neutrophils and triggers the release of neutrophil extracellular traps (NETs) in the circulation[34,35] and organ tissues.[34] NETs are extracellular webs of DNA, proteins and histones, and their release was found to be dependent on ACE2, serine protease, virus replication, and PAD-4 activation.[34] NETs cause lung injury in COVID-19 patients[34] and propagate inflammation and microvascular thrombosis.[35] Apparently, histone-3 in NETs is deiminated/citrullinated in COVID-19 patients and correlates with platelet levels, whereas the DNA component correlates with acute-phase reactants, such as C-reactive protein, D-dimer and lactate dehydrogenase, and neutrophil count.[35] It is not known how SARS-CoV-2 activates PADs and triggers NETs release; however, protein citrullination appears to be a consequence of PAD activation in host cells by the infectious process. Intravascular NETs can drive thrombosis by activating both the contact and intrinsic pathways of coagulation, although coagulopathy in COVID-19 is generally considered to be multifactorial, including not only NETosis, but also vasculopathy and cytokine storm, which can lead to multi-organ failure. Of interest, SARS-CoV-2-induced release of NETs was abrogated by the PAD inhibitor Cl-amidine,[34] suggesting that blocking NETs and PAD could be useful in COVID-19 treatment.

Remarkably, NETosis is considered to trigger the formation of autoantibodies in rheumatoid arthritis, lupus, and vasculitis and aggravates these conditions, with the contribution of autoantigens hypercitrullination, which results in the formation of anti-citrullinated protein antibodies. These antibodies are used in the diagnosis of rheumatoid arthritis, but can also be found in other forms of arthritis, vasculitis, interstitial pneumonitis, and multiple sclerosis.[36] Patients with COVID-19 display an abnormal immune response with increased cytokine production and hyperactivation of immune cells, similar to patients with autoimmune diseases. This exaggerated immune response was thought to add to the already overactive immune response of patients with autoimmune diseases such as lupus, resulting in more symptomatic COVID-19 and aggravating the underlying autoimmune disease. This, however, did not materialize in practice when these patients continued taking their usual treatment.[37] Nevertheless, COVID-19 through above mechanisms and through molecular mimicry can lead to the formation of autoantibodies and the development of Guillain-Barré syndrome, autoimmune hemolytic anemia, immune thrombocytopenic purpura, and Kawasaki disease.[38] It is also of interest that SARS-CoV-2 acting through PAD-4 can result in post-Covid-19 rheumatoid arthritis with strong anti-citrullinated peptide antibody titer, as demonstrated in a recent case report.[39]

O-GlcNAcylation

Epidemiological studies have indicated that hyperglycemia is an aggravating factor in the prognosis of COVID-19[29,30] and this may be mechanistically similar to what occurs in influenza infection. It has been reported that patients infected with influenza A virus have higher blood glucose levels and *O*-GlcNAcylation of interferon regulatory factor (IRF)-5 than healthy controls.[31] Glucose influx through the hexosamine biosynthetic pathway increases *O*-GlcNAcylation of IRF5, which is critical for pro-inflammatory cytokine production. Since SARS-CoV-2, like influenza, can cause cytokine storm, it is possible that the degree of alteration of glucose metabolism contributes to the variety of COVID-19 outcomes, including injury to internal organs and coagulopathy. Moreover, altered *O*-GlcNAcylation of proteins has been implicated in mitochondrial dysregulation,[40] as well as in cardiac, neurological, and other diseases.[40–42] Because of the role of cell metabolism in immune cell functions, it is likely that the impact of altered glucose metabolism on heightened induction of inflammatory cytokines is not limited to influenza and coronaviruses, but is also involved to various extents in other viral infections.

Ubiquitin modification

SARS-CoV-2, like other coronaviruses, uses modulation of ubiquitin and ubiquitin-like molecules to alter IRF3 and NF-κB functions and escape the host cell immune response.[27,43,44] Through this mechanism, ubiquitin or the ubiquitin-like interferon-stimulated gene 15 protein (ISG15) is removed from the target proteins by Nsp3/papain-like protease, which is part of the replicase/transcriptase system, as mentioned above. This is one of the mechanisms used by SARS-CoV-2 and other viruses to reduce the host cell innate immune response.[27,43,44] Recently, it was reported that ORF10 protein of SARS-CoV-2 interacts with the ubiquitin ligase complex, suggesting that ORF10 may hijack the ligase for ubiquitination and degradation of restriction factors to facilitate infection.[20]

Acetylation

It has been recently reported that TGF-beta-induced protein can be acetylated during COVID-19 and that the acetylated product level correlates with the severity of the disease and could be used as diagnostic tool.[45] This protein can upregulate and activate signaling of NF-κB, and its acetylation is reportedly followed by its secretion into the circulation, with possible enhancement of inflammation. Whether other proteins are acetylated during COVID-19 infection is unknown.

Inter-proteomic PTMs of the SARS-CoV-2 proteins

The best-known example of inter-proteomic PTM of the SARS-CoV-2 and other coronavirus proteins is the proteolytic priming of the S protein by the host's serine proteases ACE2 and TMPRSS2, which is critical for virus entry into the host cells.[46] ACE2 is expressed in several host cells such as pneumocytes, epithelial cells and endothelial cells, which is one of the factors explaining the widespread infection of SARS-CoV-2 to many organs. In addition, N- and O-glycosylations of the S protein by the host cell enzymes during virion packaging have been identified, which play a role in the virus life cycle.[47,48] Among other SARS-CoV-2 proteins, the nucleocapsid (N) protein, which is an important structural protein responsible for viral replication and genome packaging, is posttranslationally phosphorylated in the host cell by several kinases, among which cyclin-dependent kinase, C-TAK1, glycogen synthase kinase-3, MAP kinase, and CK2. These kinases target specific serine residues and it appears that N protein phosphorylation by C-TAK1 on serine-218 facilitates its sequestration by protein 14–3-3 and allows its localization in the cytoplasm. Thus, N protein phosphorylation is thought to permit its shuttling between cellular compartments and has been proposed as a host cell response to control the virus.[49] In fact, mutations in the phosphorylation sites of N protein have been reported in SARS-CoV-2 isolated from various populations and geographic locations,[49] and this diversity caused by mutations might allow the virus to evade host defense mechanisms. Thus, there is an enormous interest in developing therapeutics that target the N protein and its phosphorylation. Whether other proteins of the SARS-CoV-2 and other coronaviruses also undergo different types of PTMs by host enzymes remains largely unexplored. It is possible that some SARS-CoV-2 proteins are acetylated, similar to MERS-CoV pp1ab protein.[50] More notably, during phosphoproteomic analysis of the host cell proteins, Bouhaddou *et al.*[25] also identified 25 phosphorylation sites in SARS-CoV-2 viral proteins; five of them were detected in a cluster within a short C-terminal region of M protein. This finding, in combination with another recent report,[51] has now created a catalog of 49 phosphorylation sites across seven viral proteins, including structural and non-structural proteins.

As different kinases often phosphorylate protein substrates at specific consensus motifs, the identified phosphorylation sites in SARS-CoV-2 proteins led to prediction of potential host kinases for these proteins. The top kinase families predicted by phospho motifs analyses of protein sequence to regulate these sites included CK2, CDK, and protein kinase C[25]. Of note, serine-186 in the N protein of the SARS-CoV-2 is a CK1 phosphorylation site and serine-197 is a phosphorylation site for Aurora A and B kinases.[49] As these kinases are known to have roles in cell cycle regulation, it is possible that N protein may alter the host cell cycle to favor SARS-CoV-2 replication. However, the functional consequences of the phosphorylation of viral proteins by the host kinases remain unexplored.

Inter-proteomic PTMs of the host proteins

The discovery of inter-proteomic PTMs of the SARS-CoV-2 proteins raises an obvious question—whether the host proteins are also subjected to inter-proteomic PTMs, such as proteolytic cleavage by the virus proteins, as coronavirus genome is known to produce the papain-like (Nsp3) and other proteases (Nsp5)[27,.52,53] The papain-like protease is an essential coronavirus enzyme required for processing viral polyproteins to generate functional replicase complex,[20,27] which has been shown to act on the host's proteins.[27,52,53] Recently, the biochemical structure and functional analysis of papain-like protease revealed that it plays a role in controlling host interferon and NF-κB pathways, e.g. via deubiquitination/deISGylation.[27,43,44] Interestingly, despite having a high degree of sequence homology, papain-like proteases from SARS-CoV and SARS-CoV-2 were found to exhibit different host substrate preference.[27] In this context, it is important to note that the Nsp3 encoding sequence is highly variable in different coronaviruses and has been proposed to possess other functions, such as regulating ADP-ribosylation of STAT1.[54] Thus, a better understanding of the inter-proteomic PTMs of the host proteins has the potential to shed new light on differences in the pathogenicity and the host responses to different coronaviruses and likely other viruses. Importantly, targeting papain-like protease of the SARS-CoV-2 with small molecule inhibitor was found to impair the virus-induced cytopathogenic effect and to foster the anti-viral interferon response pathway, reducing viral replication in infected cells.[27] Taken together, these promising findings on targeting the virus proteases[27] and the host kinases[25] have created new opportunities and provided a rationale for simultaneous targeting of inter-proteomic PTMs of the SARS-CoV-2 and the host proteins in our fight against SARS-CoV-2.

Major questions raised by the discovery of SARS-CoV-2-induced "hybrid phosphoproteome" (and potentially other PTMomes) are how the virus leads to substantial changes in the host phosphoproteome, what are the roles of SARS-CoV-2 phosphoproteome, and what are the relative pathological consequences of these PTMomes. Theoretically, there are many potential implications of the phosphorylation and other commonly occurring PTMs of virus proteins by the host enzymes, ranging from competing with the host substrates and altering their functions to sterically blocking enzyme (e.g. kinases and phosphatases) access, which may be utilized by the virus to prioritize the needs of the virus over the host. Moreover, an increase in relative abundance of viral proteins may shift different PTM regulating enzyme activities away from their typical substrates and may change the dynamics of protein–protein interactions. Moving this forward will take significant amount of work to delineate its pathological consequences. Importantly, as protein phosphorylation and its associated functions are dynamically regulated by protein kinases and phosphatases, it would be interesting to know whether SARS-CoV-2 proteins that undergo phosphorylation by the host kinases can also be dephosphorylated by manipulating the host's protein phosphatases and potentially reverse the

pathological consequences of the phosphorylated virus proteins. This framework may also apply to other PTMs that are dynamically regulated similar to phosphorylation, such as acetylation, ubiquitination, *O*-GlcNAcylation, etc.

SARS-CoV-2 infection in cells was also found to shutdown mitotic kinases resulting in cell cycle arrest.[25] This finding along with other findings related to N protein phosphorylation and their relationship with cell cycle regulatory proteins makes sense because such changes are expected to allow diversion of the host cell's resources to support the replication and growth of the virus within the host. In aggregate, these findings on proteolytic priming of the S protein by the host serine proteases during infection, its N- and O-glycosylation within host cells, and the phosphorylation of different SARS-CoV-2 proteins suggest that the virus proteins contain motifs that mimic the host substrates and are recognized by the host enzymes—an evolutionary strategy called "molecular mimicry," which is adopted by viruses to exploit the host's cellular machinery.[26] However, how viruses evolve this strategy remains a mystery.

It is known that protein phosphorylation also serves as a regulator for other PTMs and has diverse relationships with them. For example, phosphorylation has a relationship with acetylation, *O*-GlcNAcylation, and ubiquitination, and these modifications often occur in clusters.[2–4] Thus, the likelihood that SARS-CoV-2 proteins undergo other PTMs in host cells is very high. Certainly, N protein of SARS-CoV and 3b protein of SARS-CoV-2 have been shown to undergo acetylation, phosphorylation, sumoylation, and ADP-ribosylation,[55–57] and inhibition of several kinases was shown to impact the localization of N protein,[58] highlighting the need for inter-proteomic PTM of the virus proteins to carry out its function within the host cells. Consequently, there has been a growing interest in identifying and understanding the role of PTMs of SARS-CoV-2 proteins, including the S protein because of its potential importance in pharmacotherapy. For example, in a recent systematic *in silico* analysis, numerous PTMs have been predicted in different SARS-CoV-2 proteins.[59] In this context, it is also important to note that mutations have been identified at known/predicted PTM sites in S and N proteins of the SARS-CoV-2. Thus, future work is urgently needed to get insights into the diverse nature of inter-proteomic PTMs of the virus proteins.

As the major purpose of PTMs is to create diversity from a limited number of proteins, the finding that SARS-CoV-2 proteins serve as substrates for the host enzymes may create diverse functional consequences from the limited number of the viral proteins. Such diversity may include the modulation of cellular processes that are common between different cell types to support virus replication and spread within the host (Figure 1), whereas the modulation of cell type specific functions, such as immune cell functions, may allow the virus to escape the host's defense mechanisms and contribute to diverse infection-related outcomes in a context-dependent manner (e.g. age, sex and preexisting conditions), because of differences in the cellular milieu in different cell types and different pathophysiological states. Thus, inter-proteomic studies are incredibly important in light of these possibilities.

Recently, Gordon *et al.* [20] published a map of protein–protein interaction between SARS-CoV-2 and human proteins. This map was achieved by expressing recombinant virus proteins in HEK293 cells and comprised of 332 human proteins interacting with 27 viral proteins. Out of 332 human proteins identified, 40 were found significantly and differentially phosphorylated in Vero E6 cells upon SARS-CoV-2 infection.[25] Collectively, this would imply that the increased abundance of viral proteins plays a crucial role in the host cells, including the modulation of the host kinome. This makes sense in the light of increased severity with amplified virus load. As the permissiveness of different host cell types can vary substantially from the cell models used, it is likely that the hybrid phosphoproteome will also vary. Thus, caution is required due to limitations in the different cell models used (e.g. Vero E6, Caco-2, or A549-ACE2 cells). For example, it is very challenging to explain sex- and age-related diverse immune responses observed in epidemiological studies simply on the basis of recent findings from proteomic studies from cell lines. Future investigation is expected to clarify these limitations and answer any pertinent questions. Despite these limitations, new findings in this field have created many exciting opportunities and new research directions, including inter-proteomic phosphorylation and hybrid phosphoproteome. As protein phosphorylation is a key regulatory process in cell biology, the hijacking of the host kinome by the virus proteins may be the best method for a coordinated exploitation of the host cells to a multipronged benefit of the virus. This theme may also hold the key to the development of successful therapeutics.

Concluding remarks

As the SARS-CoV-2 pandemic moves from epidemiological and observational to mechanistic studies, the host proteome, particularly the host proteins that are targeted by the virus, are becoming central players. Moreover, the discoveries of inter-proteomic PTMs of the virus proteins by the host enzymes and *vice versa* have opened exciting opportunities for future research directions. However, as the permissiveness of different host cell types can vary substantially from the cell models used in various studies described here, it is likely that the virus-host protein interactome and hybrid PTMome will also vary substantially. Thus, caution is required in extrapolating findings in cell models to humans. Despite these limitations, the proteomic discoveries on SARS-CoV-2 infection to the host cells have created many promising opportunities and have opened new research directions, including inter-proteomic PTMs of the virus and the host proteins. As PTM of protein by phosphorylation is a key regulatory process in cell biology, hijacking the host kinome by the virus proteins makes sense for a coordinated exploitation of the host cells for a multipronged benefit for the virus. This theme may also hold the key for future studies and the development of successful therapeutics.

AUTHORS' CONTRIBUTIONS

SM and BLGN wrote the manuscript. GB did the literature search used for the review and designed the illustration.

DECLARATION OF CONFLICTING INTERESTS

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