PATHOGENS AND GLOBAL HEALTH
2021, VOL. 115, NO. 5, 292–299
https://doi.org/10.1080/20477724.2021.1906562

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SUMO: a novel target for anti-coronavirus therapy

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ABSTRACT

Over the past 20 years, humankind has encountered three severe coronavirus outbreaks. Currently ongoing, COVID-19 (coronavirus disease 2019) was declared a pandemic due to its massive impact on global health and the economy. Numerous scientists are working to identify efficacious therapeutic agents for COVID-19, although treatment ability has yet to be demonstrated. The SUMO (small ubiquitin-like modifier) system has diverse roles in viral manipulation, but the function of SUMO in coronaviruses is still unknown. The objective of this review article is to present recently published data suggesting contributions of the host SUMO system to coronavirus infection. These findings underscore the potential of SUMO as a novel target for anti-coronavirus therapy, and the need for a deeper understanding of coronavirus pathology to prepare and prevail against the current and emerging coronavirus outbreaks.

KEYWORDS

SUMO/coronavirus/COVID-19/Therapy

Introduction

Coronavirus disease 2019 (COVID-19) is a newly emerging human infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. COVID-19 has globally spread, with initial cases detected in Wuhan, China and major outbreaks in the United States, Brazil, Russia, India, and Europe [2]. As of 12 March 2021, the number of COVID-19 patients reached 117,799,584 with 2,615,018 deaths across over 250 countries and territories (WHO COVID-19 Dashboard, https://who.sprinklr.com/). About 80% of COVID-19 patients recovered without special treatment, whereas the remaining 14% and 6% of patients have severe disease and critical illness, respectively [3]. In particular, people who are elderly or have chronic medical conditions, such as lung or cardiovascular disease, cancer, diabetes, or immune disorders, are more likely to develop severe pneumonia with acute respiratory distress syndrome [4]. Currently, there is no well-established therapy for COVID-19, and we still face the challenge of developing safe and effective vaccines and antiviral therapeutics.

In the past two decades, three coronaviruses causing severe illness in humans, SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 have led sequentially to enormous public health and economic crises [1]. These coronaviruses belong to the *Betacoronavirus* genus in the *Coronaviridae* family [5]. They are pleomorphic RNA viruses consisting of a positive-sense, single-stranded RNA genome 27 to 32 kb in length (Figure 1). Two-thirds of the viral RNA genome is translated into two large polyproteins, which are further cleaved into 16 nonstructural proteins involved in transcription and replication, while the remaining viral genome encodes the structural proteins, such as spike (S), envelope (E), membrane (M), and nucleocapsid (N), and several accessory proteins [6,7]. RNA viruses generally have higher evolutionary rates than any other viruses due to their high polymerase error rate and shuffling by recombination and reassortment [8]. The SARS-CoV-2 genome has a sequence homology of 77.5% with SARS-CoV and 50% with MERS-CoV [9]. While SARS and MERS affected 8,422 people with a 11% morality rate and 2,494 people with a 34% morality rate, respectively, COVID-19 has been spreading more efficiently with a lower mortality rate of 6% [10].

While it is unclear when the COVID-19 pandemic will end, it is noteworthy to mention that the 1918 flu pandemic lasted from January 1918 to December 1920 [11]. Kassa and colleagues' mathematical model estimated the possibility of circulating COVID-19 outbreaks via resurgence in infection [12]. Furthermore, because various coronaviruses persist in wildlife reservoirs, it is almost impossible to completely prevent future outbreaks of new coronaviruses [7]. Therefore, preparing for future coronavirus epidemics and pandemics is essential. The systemic changes in prevention, diagnosis, and treatment are necessary for mounting a more efficient and effective response to

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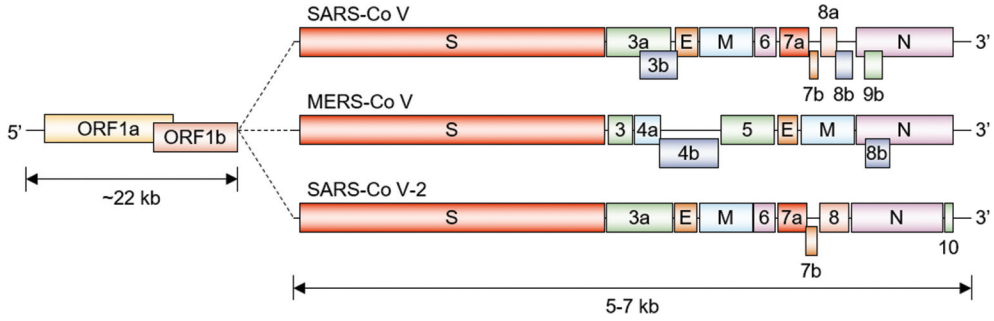

Figure 1. Schematic representation of the genome architecture of the three coronaviruses. SARS-CoV, MERS-CoV, and SARS-CoV-2 have a single-stranded RNA genome, which comprises ~22 kb of two large genes, ORF1a and ORF1b, and 5–7 kb of structural genes encoding structural proteins, spike (S), envelope (E), membrane (M), nucleocapsid (N), and accessory proteins. Different coronaviruses contain unique characteristics in terms of the number, genomic organization, and sequence of accessory genes.

the next epidemic or pandemic [13]. Thus, studying the diverse mechanisms governing coronavirus biology is essential to establish effective antiviral therapeutic strategies.

Viruses have gradually evolved and developed many strategies to exploit host cellular mechanisms to counteract antiviral responses and maximize survival and reproduction [14]. Among such cellular pathways, post-translational modification (PTM) by small ubiquitin-related modifier (SUMO) is an obvious control strategy that contributes to replication and propagation of a wide range of DNA and RNA viruses [15]. Numerous viral proteins are not only substrates of this reversible PTM but also modulators of the host SUMO pathway. It is clear from numerous studies that the SUMO machinery is important for both adaptation of virus to host cells and resistance to virus infection; however, only few reports have presented the correlation between SUMO and coronaviruses [16]. The human E2 SUMO-conjugating enzyme Ubc9 directly binds and links covalently SUMO to SARS-CoV nucleocapsid protein that is involved in viral RNP assembly and replication [17,18]. Recent proteomics analyses of SARS-CoV-2-infected cells have also revealed a significant decrease in the expression of E3 SUMO ligase RanBP2, which acts as a regulator of the retrovirus restriction factor TRIM5α, implying that TRIM5α-mediated antiviral activity may be regulated by SUMO upon SARS-CoV-2 infection [19,20].

This review presents an overview of the diverse roles of the SUMO system in viral infections, especially that of coronavirus, from the perspective of the host cell and the virus. In addition, SUMO is proposed as a novel target for anti-coronavirus therapy.

The SUMO system

The SUMO protein post-translationally modifies a diverse array of substrates that play important roles in various cellular processes, including transcription, DNA replication, cell cycle progression, nucleocytoplasmic transport, apoptosis, genome integrity, and stability maintenance, and cellular stress responses [21]. Humans have four genes that encode SUMO proteins, SUMO-1, –2, –3, and –4. SUMO-2 and SUMO-3 are almost identical and are hereafter referred to as SUMO-2/3; SUMO-2/3 shares about 50% similarity with SUMO-1 [22].

Their paralogs are first translated as precursors with a C-terminal extension. The precursors then undergo specific proteolytic cleavage to yield mature proteins with a pair of Gly residues. However, it is unclear whether SUMO-4's product can be covalently coupled to other proteins, because its precursor is not likely to be processed *in vivo* [23]. The mature form of SUMO is conjugated to lysine (K) side chains of substrate proteins via an enzyme cascade similar to that used for ubiquitin-protein conjugation [24,25] (Figure 2). A heterodimeric E1 SUMO-activating enzyme (SAE1/SAE2) first forms a thioester linkage through its active-site cysteine to the carboxyl terminus of SUMO, and then the SUMO moiety is transferred to the active-site cysteine of a E2 SUMO-conjugating enzyme (Ubc9). The SUMO is finally conjugated to the lysine side chain-(s) of a target protein with the aid of one of several E3 SUMO ligases. Site-specific proteases can disassemble SUMO chains on substrates [26].

Functions of the SUMO pathway in viral infection

In eukaryotes, SUMO is an essential regulator of homeostasis when cells respond to stresses, such as osmotic shock, hypoxia, heat, oxidative stress, nutrient deprivation, genotoxic stresses, or viral infection [27], and protein sumoylation levels by SUMO-1 and/or SUMO-2/3 are increased sharply in response to such stimuli [28]. There are two aspects of SUMO function during infection [16]. First, sumoylation of specific immune response factors mediates an antiviral effect, thus preventing viral load and contributing to viral elimination. Second, numerous viruses can exploit SUMO

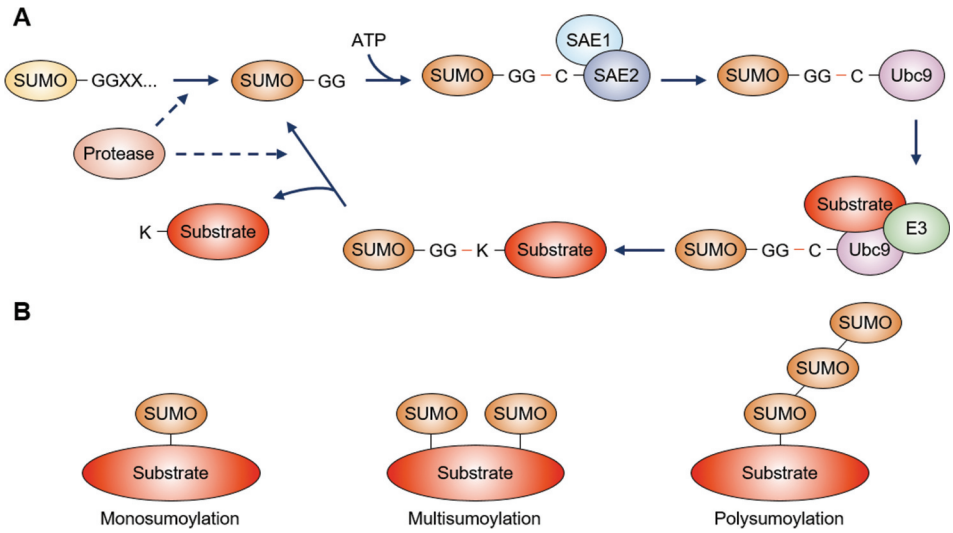

Figure 2. The SUMO pathway. (**A**) The precursor form of SUMO is processed by a SUMO-specific protease, creating a C-terminal Gly-Gly (GG) motif. In the presence of ATP, mature SUMO is activated by the heterodimeric E1 SUMO-activating enzymes, SAE1 and SAE2, through the cysteine (C) residue of SAE2. Subsequently, SUMO transferred to the cysteine residue of the E2 SUMO-conjugating enzyme (Ubc9) is finally conjugated to the lysine (K) residues of substrate proteins by aid of an E3 SUMO ligase. The SUMO protease deconjugates SUMO from proteins or edits SUMO chains, and then SUMO is recycled through the conjugation system.(**B**) SUMO is covalently linked to a single lysine residue of a protein (monosumoylation) or multiple lysine residues of a single protein (multisumoylation). Repeated sumoylation cycles build SUMO chain(s) attached on a substrate (polysumoylation).

modification to invade and replicate within host cells, resulting in several viral proteins and their targets becoming SUMO substrates.

Many viruses benefit from protein sumoylation. Latent membrane protein 1 (LMP1), a viral oncoprotein, directly binds and modulates Ubc9 to increase SUMO conjugation in Epstein–Barr virus (EBV) infected cells [29,30]. LMP1-induced protein sumoylation regulates nuclear accumulation, stability, and function of interferon regulatory factor 7 (IRF7), a master regulator of the innate immune response, during latent infections [31]. Also, IRF family transcription factors undergo diverse post-translational modification leading to regulation of their properties. For example, whereas phosphorylation of IRF3 and IRF7 stimulates transcription of type I interferon (IFN) [32], their sumoylation negatively regulate IFN gene expression [33]. Infection of types A and B influenza viruses (IAV and IBV) triggers not only sumoylation of influenza viral proteins but also global reprogramming of host protein sumoylation [34,35]. To note, 76 SUMO1 substrates and 117 SUMO2 substrates demonstrated increased sumoylation states upon IAV infection, and IAV RNA polymerase activity in the nucleus contributed to remodeling of the host SUMO system [34]. Viral proteins are also substrates of sumoylation during viral infection. For example, Ubc9 binding and subsequent sumoylation of UL44, a human cytomegalovirus (HCMV) DNA polymerase subunit, results in its decreased localization in viral DNA replication compartments via altering intranuclear distribution and conversely causing a positive effect on viral replication and production [36]. Similarly, the hantavirus nucleocapsid protein interacts with Ubc9 during infection, and its sumoylation mediates assembly of structural proteins with the viral genome and localization at the perinuclear regions for viral replication [37,38]. Sumoylation of IAV nonstructural protein NS1 accelerates viral replication via enhancing NS1's stability [39]. In addition, human papillomavirus (HPV) E2 and EBV Rta proteins serve as targets of Ubc9-mediated sumoylation, which specifically stimulate the transcription of their target genes, completely contributing to viral replication [40,41]. Furthermore, several E3 ligases, PIAS1, PIASxβ, and RanBPM, also promote sumoylation of the Rta protein [42–44].

Conversely, some viruses benefit from impaired sumoylation. The CELO adenovirus Gam1 protein promotes degradation of Ubc9 or SAE1/SAE2 in a proteasome-dependent manner [45,46], which is crucial for viral replication and transcriptional regulation of specific genes via inactivating histone deacetylase 1 (HDAC1) [47,48]. HPV-16/18 E6 oncoprotein mediates proteasomal degradation of Ubc9, resulting in decreased global sumoylation levels and perhaps subsequent development of cervical cancer [49], and inhibits E3 SUMO ligase PIASy-induced senescence and sumoylation of its substrates [50]. In addition, in HeLa cells, coxsackievirus B5 protein alters Ubc9's cellular localization and eventually contributes to decreased sumoylation status of specific substrates [51]. Widespread reduction of cellular sumoylated protein levels, including antiviral proteins promyelocytic leukemia and Sp100, is induced by herpes simplex virus type 1 (HSV-1) ubiquitin ligase ICP0 in a proteasome-dependent way and is critical to suppressing intrinsic

immunity [52,53]. The EBV protein kinase BGLF4 functions to suppress global SUMO levels, and its SUMO binding capacity is required to induce the cellular DNA damage response and to facilitate lytic EBV replication [54].

Sumoylation of coronavirus proteins

Although protein sumoylation is a critical factor in virus-induced pathogenesis, the relationship between sumoylation and coronaviruses has yet to be established. There are, however, a limited number of reports suggesting a potential role of sumoylation in SARS-CoV infection. During the SARS infection, the nucleocapsid protein binds to the viral RNA genome to form the ribonucleoprotein core, which is able to interact with a number of host proteins [55,56]. Since the nucleocapsid is expressed abundantly in infected cells, it has often been investigated as a target of SARS rapid diagnosis kits [57,58]. The SARS-CoV nucleocapsid protein is 422 amino acids long, consisting of the N-terminal structural domain (NTD) and C-terminal structural domain (CTD) sandwiched between three intrinsically disordered regions (IDRs) [59,60]. The flexible linker region (LKR) between NTD and CTD has a Ser/Arg-rich region that contains a number of potential phosphorylation sites directly related to nucleocapsid oligomerization [61–65].

In addition to phosphorylation, biochemical characterization revealed that the nucleocapsid has a motif for binding hUbc9 and is post-translationally modified by SUMO, in which the major site is the K62 within NTD and strongly promotes homo-oligomerization of the protein [17,18]. Moreover, this modification is required for normal subcellular localization of nucleocapsid and interference of host cell division [18]. Therefore, this lysine sumoylation of nucleocapsid may not be a result of the antiviral host defense system but instead is likely due to viral hijacking of the cellular SUMO system to enhance viral replication and pathogenesis [66]. Coronaviruses do not encode SUMO conjugation enzymes; however, evolution of coronaviruses may have conferred the means for these coronaviruses to hijack this modification in the host to promote survival. Although the sumoylation site of SARS-CoV -2's nucleoscapsid has yet to be determined, two distinct bioinformatics tools, SUMOplot and GPS-SUMO, predict the same SUMO conjugation site at the K124 as SARS-CoV along with two additional sites, at the K15 and K19 lysine positions. These sites need to be further explored and understood to elucidate the role of SUMO modification in SARS-CoV-2 infection.

Alterations of SUMO profile in coronavirus-infected cells

Similar to the changes in the SUMO proteome in response to various cellular stresses, there is a dramatic change in the SUMO proteome in virus-infected host cells [15]. However, to our knowledge, alteration in SUMO conjugation patterns has not been determined in cells infected with coronaviruses, even though a transcriptome analysis revealed that COVID19 patients exhibited changes in the expression of genes regulating ubiquitin or SUMO conjugation [67]. Instead of a SUMO-focused analysis, Bojkova and colleagues carried out proteomic studies to monitor the protein levels in human Caco-2 cells upon infection by SARS-CoV-2 [20]. They revealed the reestablishment of central pathways, including translation, splicing, carbon metabolism, and nucleic acid metabolism. Such research results provoked further study, and Ortea and Bock assessed host cell proteomic data upon SARS-CoV-2 infection [19]. This study additionally suggested a new network of proteins involved in the inflammatory response or mitotic chromosome segregation in the host proteome altered by SARS-CoV-2. Particularly, the nuclear pore complex protein RanBP2 (E3 SUMO protein ligase) is placed at the center of this identified network [19]. The RanBP2/SUMO-modified RanGAP1/Ubc9 complex directly interacts with TRIM5α, a cytoplasmic protein that recognizes specific retroviral capsids and prevents its invasion into the host cell cytoplasm [68], and regulates its antiretroviral activity and localization by sumoylation [69] (Figure 3). Moreover, some COVID-19 patients have mutations in *RANBP2* [70] and TRIM5 is positively correlated with the proliferation of natural killer cell response in COVID-19 [71]. Missense mutations in *RANBP2* were identified as a major cause of familial and recurrent acute necrotizing encephalopathy [72], suggesting that such mutations may also contribute to the acute necrotizing encephalopathy reported in some COVID-19 patients [73–75]. Since TRIM5α promotes the premature disassembly of HIV-1 capsid in the cytoplasm of the host cell and the replication and propagation of coronavirus are also mediated in the cytoplasm [68], the role of RanBP2-mediated TRIM5α sumoylation in coronavirus infection deserves further investigation.

The sumoylation of TRIM28 typically suppresses the transcription of endogenous retroviral (ERV) genes. IAV infection induces the loss of SUMO-modified TRIM28, a transcriptional repressor, thus promoting the expression of endogenous retroviral (ERV) RNAs that are sensed as non-self by host pattern recognition receptors (PRRs) [76,77] (Figure 4). Consequently, the derepression of

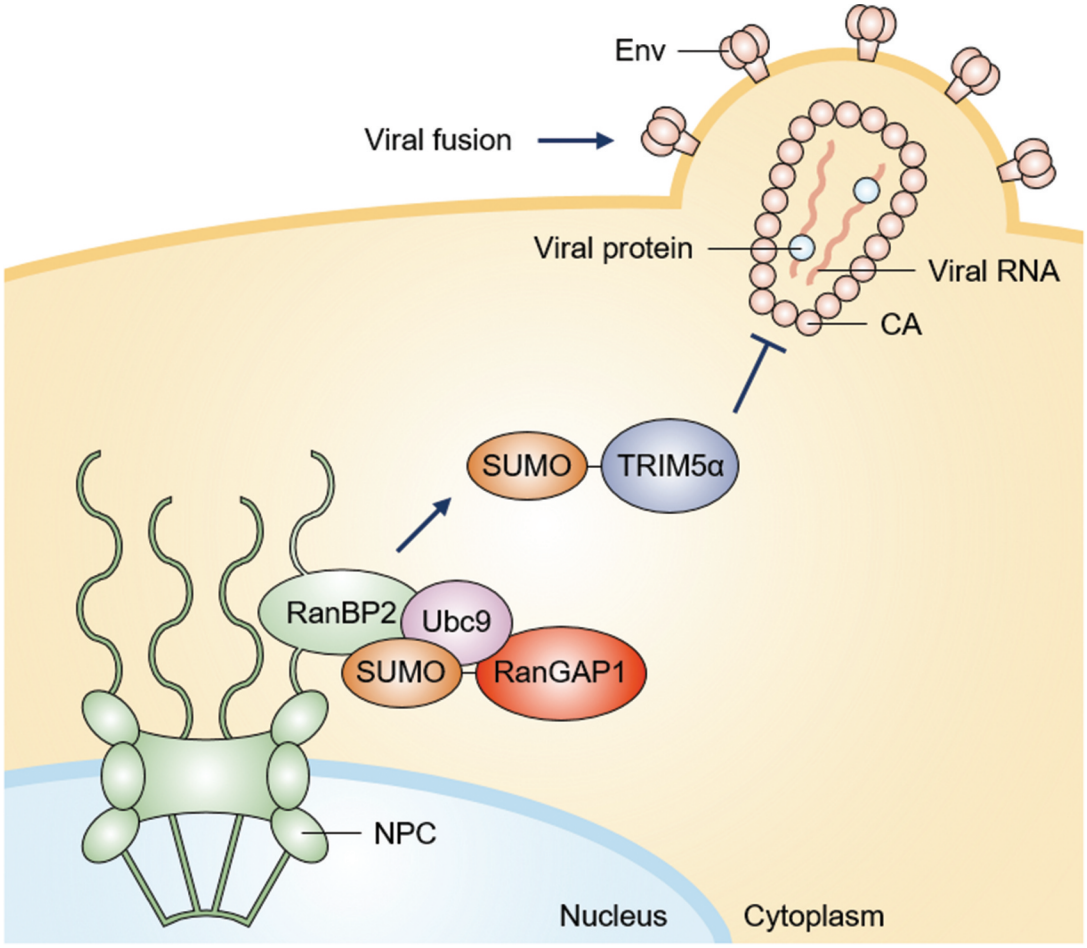

Figure 3. The function of RanBP2-mediated sumoylation of TRIM5α in HIV-1 infection. The envelope glycoprotein (Env) mediates human immunodeficiency virus type 1 (HIV-1) fusion with the cell membrane. The HIV-1 core consists of the capsid (CA) protein and its contents. The capsid proteins surround two copies of the RNA genome and proteins, such as reverse transcriptase and integrase. Before or during capsid disassembly, which is necessary for nuclear import of the viral genome, TRIM5α blocks retroviral infection by intercepting capsids before they reach the nucleus and promotes premature disassembly. The E3 SUMO ligase RanBP2 is associated with Ubc9 and sumoylated RanGAP1 at cytoplasmic filaments of nuclear pore complexes (NPCs). RanBP2 promotes sumoylation of TRIM5α in the cytoplasm, resulting in its proper localization and antiviral activity.

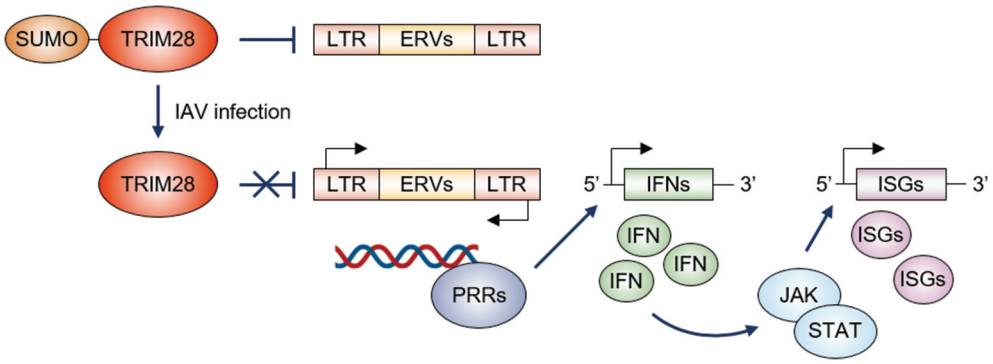

Figure 4. The role of sumoylation of TRIM28 in influenza A virus infection. The sumoylation of TRIM28 typically suppresses the transcription of endogenous retroviral (ERV) genes. Upon the infection of the influenza A virus (IAV), the decreased level of SUMO-modified TRIM28 derepresses the expression of ERV-encoded double-stranded RNAs that are recognized as non-self by pattern recognition receptors (PRRs), and then PRRs trigger the expression and secretion of interferons (IFNs). The IFN-mediated stimulation of the JAK/STAT signaling pathway subsequently induces the activation of hundreds of IFN-stimulated genes (ISGs) encoding), which encode proteins with diverse antiviral functions.

ERVs transcription induces the subsequent activation of IFN-mediated antiviral response via the RIG-I-, MAVS-, TBK1-, and JAK1-dependent pathway [76]. TRIM28 may play a critical role in SARS-CoV-2's entry into human cells [78]. It was recently reported that angiotensin-converting enzyme 2 (ACE2), which is co-expressed with TRIM28 in type II pneumocytes, is the cellular receptor protein for SARS-CoV-2 [79,80]. Also, the knockdown of TRIM28

stimulates *ACE2* expression via IFN-γ dependent immune response [78]. Although the SUMO modification of TRIM28 has not been studied in coronavirus-infected cells, studying SUMO function in coronaviruses may provide clues to developing new therapies and life-saving vaccines for coronavirus diseases.

Conclusions and perspectives

Coronaviruses are well-known zoonotic pathogens that infect many vertebrate species, including humans, and can cause respiratory, gastrointestinal, hepatic, and neurological disorders [81]. Coronaviruses possess unstable RNA genomes that mutate continuously and undergo genetic reassortments, result in high-frequency polymerase error and RNA recombination. Therefore, developing therapeutic agents against coronavirus proteins is considerably more difficult due to the changes in viral proteins resulting from the genome's mutations [82]. Three different coronaviruses, SARS-CoV, MERS-CoV, and SARS-CoV-2, have accounted for considerable outbreaks, in the last two decades, with SARS-CoV-2 currently causing a pandemic that has taken a toll on human life and the global economy [1]. As of now, we do not know when this pandemic will end, and furthermore, future SARS-CoV-2 outbreaks may trigger pronounced crises. Therefore, there is an impetus to understand coronavirus infection and spread and develop antiviral therapies. Herein, we have described recent observations of how the SUMO system may be involved in coronavirus replication and pathogenesis. Protein sumoylation contributes to certain pathological conditions and has the capability to both benefit and harm in viral infections [16]. Studying SUMO function in coronaviruses may provide clues for developing new therapies and life-saving vaccines for the current and upcoming coronavirus diseases.

Disclosure statement

The author declares no conflict of interest.

Funding

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (no. 2020R1C1C1009367). English language editing was provided by Enago (https://www.enago.com/)

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