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The Role of nsp5 in SARS-CoV-2

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Abstract. SARS-CoV-2, the causative agent of the COVID-19 pandemic, is a single-stranded positive-sense RNA virus belonging to the coronavirus family. It emerged at the end of 2019 and has swiftly disseminated globally, presenting an unparalleled threat to public health worldwide. The non-structural protein 5 (nsp5) is a key component in the SARS-CoV-2 replication process, also known as the main protease (Mpro or 3CLpro), which is essential for cleaving viral polyproteins into functional proteins required for replication and transcription. The conserved characteristics and essential role of nsp5 render it an attractive target for antiviral pharmacological development. Recent developments in nsp5-targeted treatments, like Paxlovid, exhibit significant effectiveness in decreasing COVID-19-associated hospitalizations and fatalities. However, the development of nsp5 inhibitors continues to face challenges due to viral mutations and specific administration requirements. This review explores the structural and biochemical properties of nsp5, with particular attention to its catalytic dyad, substrate recognition sites, and three-domain architecture that underpin its role in viral replication. It highlights the potential impact of nsp5 research on advancing antiviral strategies, aiding in the fight against current SARS-CoV-2 infections, and laying the groundwork for future coronavirus outbreak preparedness.

Keywords: SARS-CoV-2, nsp5, nsp5 inhibitors

1. Introduction

SARS-CoV2 is a member of the nested virus and belongs to the coronavirus family. It is a single-stranded positive-stranded RNA virus [1, 2]. The COVID-19 pandemic erupted in 2019 and swiftly expanded worldwide, leading to an unparalleled public health emergency [3]. The virus is mainly transmitted through droplets and contact with both symptomatic and asymptomatic individuals. As of the end of 2020, it has caused over 670000 infections and over 15000 deaths across China [4], with SARS-CoV2 having a higher infection rate and lower mortality rate. The lifecycle of SARS-CoV-2 is divided into three main stages: invasion, replication, and packaging. This process involves both structural and non-structural proteins. Structural proteins, including the spike (S), envelope (E), membrane (M), and nucleocapsid (N)—play key roles in virus assembly, infection, replication, and transmission during the invasion stage. Conversely, non-structural proteins, comprising 16 different types, are

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predominantly involved in processes such as transcription, replication, translation, and other vital functions during the replication stage [3, 5].

NSP5 is a nonstructural protein of the virus, also known as the main protease. It plays a crucial role in the replication process of the virus lifecycle. Firstly, the structure of nsp5 is roughly heart-shaped, characterized by its multiple enzyme cleavage sites that can recognize specific sequences of various linkers and short peptides [6-8]. Research teams from Mexico, the United States, and China have confirmed that nsp5 possesses numerous recognition sites and strong specificity [9-11]. It can self-cleave from polyproteins and facilitate the sequential cleavage of spike, membrane proteins, and polymerase from polyproteins, thereby producing functional structural or non-structural proteins [12,13]. Additionally, due to its distinctive properties, nsp5 can cleave TRMT, an essential modifying protein for tRNA formation in host cells, effectively inhibiting host cell translation. Beyond human systems, nsp5 also plays a significant role in animals. In 2024, a Chinese research team studied the emerging G4P porcine rotavirus and discovered that the main protease nsp5 contains a unique 344-nucleotide repeat sequence, further confirming its specific sequence recognition and essential role in the replication of all positive-stranded RNA viruses [14,15]. During the COVID-19 pandemic, a research team from Mexico discovered that the main protease nsp5 played a crucial role in the replication phase of the virus. Additionally, they found that nsp5 enhanced the activity of signaling pathways involving logic gates such as NF-κB and IL-6, which are associated with inflammatory responses. In 2021, a research team from the United States identified eight common mutations in nsp5 of SARS-CoV-2 and conducted an in-depth analysis to evaluate their impact on vaccine design strategies [16, 17]. Their findings highlighted the significant potential of nsp5 as a target, establishing it as an important factor in the selection and development of SARS-CoV-2 vaccines. Therefore, NSP 5 can be an ideal target for the development of drugs to inhibit the SARS-CoV-2 replication. In this paper, we explored the function and role of nsp5 in viral replication and discussed its potential as a target for antiviral therapy. This approach not only supports efforts to combat current SARS-CoV-2 infections but also lays the groundwork for preparedness against future coronavirus outbreaks [18].

2. Structure and Function of nsp5 in SARS-CoV-2

SARS-CoV-2 nsp5 is a highly conserved cysteine protease with three distinct domains crucial to its enzymatic activity and stability [6]. Domains I and II of nsp5 exhibit a chymotrypsin-like fold, forming the active center, while domain III is critical for dimerization [7]. Dimerization is crucial for nsp5's catalytic activity, since it facilitates the structural stability required for substrate binding and catalysis [8]. The active region contains a catalytic dyad comprised of histidine (H41) and cysteine (C145), which is common among proteases in the coronavirus family [9]. This dyad is responsible for the proteolytic cleavage of polyproteins, which is a crucial step in the viral replication cycle. The conserved active site pocket of nsp5 enables it to selectively identify cleavage sites in viral polyproteins characterized by hydrophobic residues at P2 and glutamine at P1 [10]. The nsp5 protein from SARS-CoV and SARS-CoV-2 has 96.1% sequence homology, indicating substantial structural conservation that facilitates similar substrate recognition and catalysis [11]. The conserved nature of nsp5 renders it an attractive therapeutic target, as inhibitors may effectively impede replication in several coronaviruses [12]. NSP5 primarily functions to cleave the polyproteins pp1a and pp1ab, thereby releasing essential non-structural proteins required for viral replication and transcription [13]. By specifically recognizing cleavage sites within these polyproteins, nsp5

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catalyses reactions at P2-P1-P1' sequences, characterized by hydrophobic residues at P2 and glutamine at P1, resulting in the production of mature, active proteins [14]. The catalytic mechanism utilized by nsp5 is essential for viral viability. Inhibition or compromise of nsp5 function prevents SARS-CoV-2 from completing polyprotein maturation, hence halting replication [15]. As a cysteine protease, nsp5 is optimized for activity under physiological conditions, including neutral pH and body temperature [16]. Studies indicate that nsp5 functions independently of cofactors, although its activity is significantly enhanced when associated with the viral replication complex [17]. Additionally, pH and temperature modulate nsp5 activity, providing adaptability to the virus in various host environments [18].

3. Nsp5 as a Drug Target

NSp5 is crucial for viral replication. Moreover, unlike similar proteases in human cells, nsp5 has limited homologs inside the human body, thus diminishing the probability of off-target effects [19]. Consequently, nsp5 is an ideal target for antiviral therapies. Antiviral medicines can inhibit viral replication by targeting nsp5, therefore diminishing infection severity and transmission [20]. Protease inhibitors have been successfully used against viruses such as HIV and hepatitis C virus (HCV), highlighting the therapeutic potential of targeting viral enzymes [21]. For example, HIV protease inhibitors effectively reduce viral load by inhibiting key viral enzymes. Similarly, nsp5 inhibitors, such as Paxlovid, have shown significant efficacy in treating COVID-19, leading to marked reductions in hospitalizations and mortality rates [22, 23]. Currently, various nsp5 inhibitors are under development, spanning small molecules and peptide-based inhibitors. These inhibitors are engineered to bin d nsp5's active site, preventing substrate interaction and catalysis [24]. Paxlovid, a notable nsp5 inhibitor, has demonstrated clinical efficacy in patients with mild to moderate COVID-19, showcasing the broad clinical potential of nsp5- targeted therapies [25]. Paxlovid's success has set a precedent for nsp5-targeted therapies and highlights the broader potential of protease- targeted antivirals [26]. However, mutations in nsp5, such as those observed in emerging variants, may reduce the efficacy of single-target inhibitors. Consequently, researchers are investigating multi-target inhibitors and combination therapies as potential solutions to address this challenge [27, 28]. Developing nsp5 inhibitors still presents some challenges, particularly in specificity and delivery. To achieve therapeutic efficacy, nsp5 inhibitors must ensure stability and bioavailability inside host cells, necessitating meticulous optimization [29]. Efficient delivery systems—such as oral and injectable formulations—are essential for ensuring that inhibitors get at the viral replication sites without degradation [30]. This has led to research into novel delivery methods, including nanoparticle- based formulations, aimed at enhancing cellular uptake and maintaining inhibitor stability [31]. The presence of nsp5 mutations poses an additional hurdle for drug development, as structural changes can influence binding affinity and inhibitor effectiveness [32]. Thus, inhibitor design must incorporate considerations for variant adaptability, ensuring efficacy across emerging strains. Combination therapies targeting multiple viral proteins or pathways could enhance treatment effectiveness while reducing resistance risks [33, 34].

4. Summary

SARS-CoV-2 represents a highly transmissible and resilient virus that has caused an unprecedented global health crisis, emphasizing the urgent need for effective and targeted

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therapeutic strategies. The viral protease nsp5 is central to the replication machinery of SARS-CoV2, essential for the virus's replication, transcription, and survival. Progress in the structural and functional comprehension of nsp5 has enabled the creation of potential inhibitors, several of them are presently undergoing clinical studies. However, the rapid emergence of viral mutations and potential resistance necessitates the design of adaptable and robust drug formulations to ensure ongoing efficacy. A comprehensive understanding of nsp5, from its structural dynamics to inhibition mechanisms, will be instrumental in enhancing antiviral strategies and improving treatment outcomes for SARS-CoV-2. Furthermore, multi-target approaches, combined with optimized drug delivery systems, are crucial for establishing therapies that are both safe and effective, especially in the face of an evolving viral landscape. As SARS-CoV-2 continues to adapt, future research must focus on designing nsp5 inhibitors that can maintain efficacy against a broad spectrum of viral variants. Additionally, further exploration of nsp5's interactions with host cell mechanisms may yield insights into minimizing adverse effects and improving specificity, making antiviral therapies more viable for clinical use. Global collaboration among research institutions will also be essential in tracking the evolution of SARS-CoV-2 and assessing the potential for cross-species transmission, which poses the risk of new outbreaks. Such a collaborative approach not only strengthens the response to the current pandemic but also establishes a robust foundation for addressing future coronavirus threats. By continuously refining therapeutic strategies and enhancing our understanding of nsp5, the insights gained from this research will have a lasting impact on virology and antiviral drug development, aiding in the mitigation of similar viral threats in the future.

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