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Targeting Critical Protein-Protein and Protein-RNA Interactions in SARS-CoV-2 as Therapeutic Strategies against COVID-19

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Abstract:

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the COVID-19 pandemic, relies on a complex interplay of protein-protein and protein-RNA interactions to facilitate its entry into host cells, viral replication, and assembly of infectious viral particles. This research work investigates the potential of disrupting these critical interactions as therapeutic strategies against COVID-19. Specifically, we explore the S1 domain interaction between the viral spike protein and the human ACE2 receptor, which mediates viral entry. Additionally, we examine the S2 domain-mediated membrane fusion process, essential for viral genome release into the host cell. Furthermore, we investigate the M-M and E-E interactions among the membrane and envelope proteins, respectively, crucial for viral assembly and envelope formation. Moreover, we focus on the N-RNA interactions between the nucleocapsid protein and the viral RNA genome, which are vital for viral genome packaging and replication. Disrupting these interactions could potentially inhibit the production of infectious viral particles and limit viral spread. Through a combination of computational approaches, biochemical assays, and in vitro experiments, we aim to identify and characterize small-molecule inhibitors, antibodies, or other therapeutic agents that can specifically target these critical interactions. By interfering with these essential processes, we seek to develop novel antiviral strategies that could impair SARS-CoV-2 replication, viral particle assembly, and infection, ultimately contributing to the development of effective treatments or preventive measures against COVID-19. This research provides insights into the molecular mechanisms underlying SARS-CoV-2 infection and highlights promising targets for therapeutic intervention, offering potential avenues for combating the ongoing COVID-19 pandemic and future emerging coronavirus threats.

Keywords: ***SARS-CoV-2, Protein-Protein Interactions, Protein-RNA Interactions, Therapeutic Targets, COVID-19 Antivirals***

Background

Viral surface glycoproteins play a pivotal role in the intricate interactions between viruses and their hosts. These proteins adorn the viral envelope and dictate crucial processes such as host cell recognition, entry, and immune evasion. Among the most extensively studied viruses, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of the ongoing COVID-19 pandemic, features a prominently important spike glycoprotein (S). (1) Understanding the significance of these glycoproteins and their roles in viral infection has far-reaching implications for scientific endeavors aimed at benefiting humanity. (2)

Spike Glycoprotein (S) and Host Cell Interaction: The SARS-CoV-2 spike protein,6 consisting of S1 and S2 subunits, interacts with the host cell receptor

2024 TPJ | VOLUME 2, ISSUE 2
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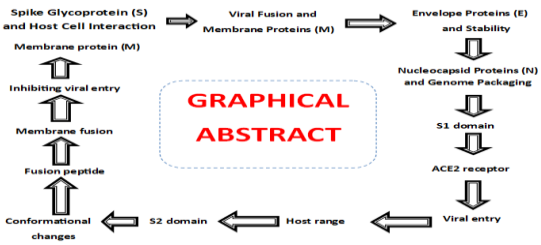
ACE2 in a process akin to a molecular handshake. (4) This interaction facilitates the virus's entry into respiratory epithelial cells, initiating the infection process. Extensive research has dissected the binding kinetics of this interaction, revealing its exquisite specificity. Unravelling the intricacies of the S-ACE2 interface provides valuable insights that inform drug design, vaccine development, and potential therapeutic interventions.

Viral Fusion and Membrane Proteins (M): Following the initial interaction with the host cell receptor, the S2 domain of the spike protein orchestrates the crucial process of membrane fusion. Upon receptor binding, the S2 (5) domain undergoes conformational changes that ultimately lead to viral entry into the host cell. Membrane proteins (M) play a supporting role in this process by interacting with each other during virus assembly and shaping the viral envelope. Disrupting these M-M interactions could potentially halt the virus assembly process, representing a promising therapeutic avenue. (6)

Figure 1: Graphical Abstract of Current Review



The present review delves into the intricate web of interactions involving the surface glycoproteins of SARS-CoV-2, the virus responsible for the COVID-19 pandemic. These glycoproteins, particularly the spike (S) protein, play pivotal roles in the virus's life cycle and pathogenesis. The review dissects the molecular mechanisms underlying the S1 domain's interaction with the human ACE2 receptor, facilitating viral entry into host cells. Furthermore, it explores the S2 domain's role in mediating membrane fusion, a critical step for viral genome release. The review also sheds light on the M-M and E-E interactions, which govern viral assembly and envelope formation, respectively. Notably, the review examines the crucial N-RNA interactions between the nucleocapsid protein and the viral RNA genome, essential for genome packaging and replication. By unravelling these intricate interactions, the review provides insights into potential therapeutic targets that could disrupt the virus's ability to infect, replicate, and spread, ultimately contributing to the development of effective antiviral strategies against COVID-19. **(Figure 1)**

Envelope Proteins (E) and Stability:

Envelope proteins (E) are essential for the formation of the viral envelope. The interactions between these proteins, known as E-E interactions, contribute to the stabilization of the envelope structure. Targeting these E-E interactions could destabilize the virus, potentially rendering it less infectious. Insights into the dynamics of envelope proteins guide the development of novel antiviral strategies. (7)

Nucleocapsid Proteins (N) and Genome Packaging:

Nucleocapsid proteins (N) play a crucial role in binding to the viral RNA genome, ensuring its proper packaging and enabling viral replication. (8) Disrupting the interactions between these proteins and the viral RNA (N-RNA interactions) could halt the viral replication process. (9) Furthermore, nucleocapsid proteins serve as diagnostic markers, as they are targeted by antibody-based tests to detect SARS-CoV-2 infection. (10)

The significance and benefits of understanding viral surface glycoproteins extend far beyond the COVID-19 pandemic. First, insights into glycoprotein interactions aid in the design of effective vaccines, as exemplified by the success of mRNA-based

2024 TPJ | VOLUME 2, ISSUE 2
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vaccines targeting the SARS-CoV-2 spike protein. Second, the development of inhibitors targeting glycoprotein interactions, including small molecules, peptides, and neutralizing antibodies, holds promise for novel therapeutic interventions. Third, glycoproteins serve as diagnostic markers, enabling the detection of anti-SARS-CoV-2 antibodies and aiding in disease diagnosis and surveillance efforts. Finally, the knowledge gained from studying SARS-CoV-2 glycoproteins informs pandemic preparedness strategies, equipping us with valuable lessons to shape our responses to future outbreaks of emerging viral threats. (11)

S1 domain:

The spike protein on the surface of SARS-CoV-2 is a large protein that plays a crucial role in viral entry into host cells. (12) This spike protein has two subunits: S1 and S2. The S1 domain is responsible for binding to the host cell receptor.

ACE2 receptor:

For SARS-CoV-2, the host cell receptor that the S1 domain binds to is called ACE2 (angiotensin-converting enzyme 2). ACE2 is a protein found on the surface of many human cells, particularly in the lungs, heart, and blood vessels. (13)

Viral entry:

The interaction between the S1 domain of the spike protein and the ACE2 receptor is crucial for viral entry into the host cell. This binding allows the virus to attach to the cell and initiate the process of membrane fusion, which ultimately leads to the release of the viral genetic material into the host cell. (14)

Host range:

The binding affinity or the strength of the interaction, between the S1 domain and ACE2 determines the virus's host range. A higher binding affinity means that the virus can more effectively infect and replicate in cells expressing the ACE2 receptor. (15) This binding affinity plays a role in determining which species the virus can infect and how efficiently it can spread within a host population. S1 domain interaction refers to the crucial binding between the S1 subunit of the SARS-CoV-2 spike protein and the ACE2 receptor on human cells. This interaction is essential for viral entry and determines the virus's ability to infect and spread among humans, making it a crucial target for therapeutic interventions and vaccine development. (16)

The S2 domain interaction refers to the second crucial step in the viral entry process of SARS-CoV-2 after the S1 domain has bound to the ACE2 receptor. Here's an explanation of the key points:

S2 domain:

The S2 subunit of the spike protein is responsible for mediating the fusion of the viral membrane with the host cell membrane, enabling viral entry.

Conformational changes:

Upon binding of the S1 domain to the ACE2 receptor, the S2 domain undergoes structural changes, or conformational changes, which expose and activate specific functional regions within the S2 subunit. (17)

Fusion peptide:

Within the S2 domain, there is a region called the fusion peptide. This fusion peptide is a short sequence of amino acids that can interact with and insert into the host cell membrane.

Membrane fusion:

The interaction between the fusion peptide of the S2 domain and the host cell membrane initiates the process of membrane fusion. This fusion process brings the viral membrane and

2024 TPJ | VOLUME 2, ISSUE 2
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the host cell membrane together, creating a single continuous membrane and allowing the viral genetic material to be released into the host cell cytoplasm. (18)

Inhibiting viral entry:

Since the S2 domain-mediated membrane fusion is a critical step for viral entry, inhibiting this interaction could potentially prevent SARS-CoV-2 from entering and infecting host cells. Developing drugs or antibodies that block or interfere with the conformational changes, fusion peptide interactions, or membrane fusion process could be a therapeutic strategy to combat COVID-19. (19) S2 domain interaction involves the structural changes and fusion machinery within the S2 subunit of the SARS-CoV-2 spike protein, which facilitates the fusion of the viral and host cell membranes, enabling viral entry. Disrupting this interaction could be a target for preventing viral infection and disease progression. (20)

Membrane protein (M):

The membrane protein (M) of SARS-CoV-2 plays a pivotal role in the virus assembly process through interactions known as M-M interactions. During virus assembly, these membrane proteins interact with each other via specific protein-protein interactions. These M-M interactions are essential for the proper organization and shaping of the viral envelope, which is a lipid bilayer derived from the host cell membrane. The M-M interactions facilitate the assembly of the viral components, including the viral genome, nucleocapsid proteins, and envelope proteins, into a complete virus particle. They act as a scaffold, providing a platform for the assembly of the other viral components and the formation of the viral envelope. Importantly, the M-M interactions help to curvature the viral envelope, giving the virus its characteristic spherical shape, which is crucial for the stability and infectivity of the virus particle. Disrupting these M-M interactions could potentially impair the virus assembly process and the formation of the viral envelope, thereby preventing the virus from efficiently packaging its genetic material and producing infectious particles. As a result, targeting the M-M interactions with small molecules or other therapeutic agents could be an approach to inhibit viral replication and spread, making them a potential target for therapeutic interventions against COVID-19. (21)

Envelope protein (E):

The envelope protein (E) of SARS-CoV-2 plays a vital role in the formation and stabilization of the viral envelope through specific protein-protein interactions known as E-E interactions. During the virus assembly process, these envelope proteins interact with each other via E-E interactions, facilitating the proper assembly and organization of the envelope proteins within the viral envelope, which is a lipid bilayer derived from the host cell membrane. These E-E interactions are essential for the proper formation and shaping of the viral envelope, as the envelope proteins act as building blocks, coming together and interacting through E-E interactions to form the complete envelope structure that encapsulates the viral genome and other components. Moreover, the E-E interactions contribute significantly to the structural stability of the envelope, helping to maintain its integrity and rigidity, ensuring that the virus particle remains intact during its life cycle and transmission. (22) Disrupting these E-E interactions could potentially destabilize the viral envelope structure, making the virus more susceptible to environmental factors or host defenses. As a result, targeting these interactions with small molecules or other therapeutic agents could be an approach to impair viral stability and infectivity, thereby inhibiting viral replication and spread, making the E-E interactions a potential target for therapeutic interventions against COVID-19.

Nucleocapsid protein (N):

The nucleocapsid protein (N) of SARS-CoV-2 plays a pivotal role in the viral life cycle

through its interactions with the viral RNA genome, known as N-RNA interactions. These interactions are essential for the proper packaging and organization of the viral genetic material. During virus assembly, the N-RNA interactions facilitate the encapsidation of the viral RNA genome into a compact structure called the nucleocapsid, which is a complex consisting of the viral RNA tightly bound to multiple nucleocapsid proteins, forming a protective and organized structure. (23) Proper packaging of the viral genome is crucial for the subsequent steps of virus assembly and the production of infectious viral particles. Additionally, the N-RNA interactions also play a role in the viral replication process, as the nucleocapsid proteins interact with the viral RNA genome during the transcription and replication stages, facilitating the synthesis of new viral RNA molecules.(24) Disrupting these N-RNA interactions could potentially inhibit the viral replication machinery, preventing the virus from producing new copies of its genetic material. Consequently, targeting the N-RNA interactions with small molecules, antibodies, or other therapeutic agents could potentially disrupt the packaging and replication of the viral genome, thereby inhibiting the production of infectious viral particles and limiting viral spread and disease progression. (25) Thus, the N-RNA interactions represent a promising target for antiviral interventions against COVID-19, as disrupting these interactions could be an effective strategy for inhibiting SARS-CoV-2 replication and spread.

References:

1. Amanat, F., & Krammer, F. (2020). SARS-CoV-2 vaccines: Status report. Immunity, 52(4), 583-589. https://doi.org/10.1016/j.immuni.2020.03.007
2. Banerjee, A., Santra, D., & Das, S. (2021). Therapeutic targeting of the SARS-CoV-2 spike protein. Molecular Therapy, 29(4), 1164-1182. https://doi.org/10.1016/j.ymthe.2021.03.008
3. Cai, Y., Zhang, J., Xiao, T., Peng, H., Sterling, S. M., Walsh, R. M., Jr., Rawson, S., Rits-Volloch, S., & Chen, B. (2020). Distinct conformational states of SARS-CoV-2 spike protein. Science, 369(6511), 1586-1592. https://doi.org/10.1126/science.abd4251
4. Chen, Y., Liu, Q., & Guo, D. (2020). Emerging coronaviruses: Genome structure, replication, and pathogenesis. Journal of Medical Virology, 92(4), 418-423. https://doi.org/10.1002/jmv.25681
5. Cheng, V. C., Lau, S. K., Woo, P. C., & Yuen, K. Y. (2007). Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clinical Microbiology Reviews, 20(4), 660-694. https://doi.org/10.1128/CMR.00023-07
6. Corbett, K. S., Flynn, B., Foulds, K. E., Francica, J. R., Boyoglu-Barnum, S., Werner, A. P., Flach, B., O'Connell, S., Bock, K. W., Minai, M., Nagata, B. M., Andersen, H., Martinez, D. R., Noe, A. T., Douek, N., Donaldson, M. M., Nji, N. N., Alvarado, G. S., Edwards, D. K., ... Graham, B. S. (2020). Evaluation of the mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. New England Journal of Medicine, 383(16), 1544-1555. https://doi.org/10.1056/NEJMoa2024671
7. Finkel, Y., Mizrahi, O., Nachshon, A., Weingarten-Gaharu, S., Morgenstern, D., Yahalom-Ronen, Y., Tamir, H., Ephros, H., Tabib, A., Ekman, E., Milrot, E., Levin, S., Weissman, I., Cohen, I., Perahya, Y., Champion, L., Ahar, A., Juso, S., Rothman, A., ... Stern, A. (2021). The coding capacity of SARS-CoV-2. Nature, 589(7840), 125-130. https://doi.org/10.1038/s41586-020-2739-1
8. Gao, Y., Yan, L., Huang, Y., Liu, F., Zhao, Y., Cao, L., Wang, T., Sun, Q., Ming, Z., Zhang, L., Ge, J., Zheng, L., Zhang, Y., Wang, H., Zhu, Y., Zhu, C., Hu, T., Hua, T., Zhang, B., ... Wang, R. (2020). Structure of the RNA-dependent RNA polymerase from COVID-19 virus. Science,

368(6492),779-782. https://doi.org/10.1126/science.abb7498

9. Grifoni, A., Sidney, J., Zhang, Y., Scheuermann, R. H., Peters, B., & Sette, A. (2020). A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. Cell Host & Microbe, 27(4), 671-680.e2. https://doi.org/10.1016/j.chom.2020.03.002
10. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N.-H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell, 181(2), 271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052
11. Jackson, C. B., Farzan, M., Chen, B., & Kuhn, J. H. (2022). Functional significance of the SARS-CoV-2 virus' life cycle protein interactions with host membranes. Viruses, 14(2), 344. https://doi.org/10.3390/v14020344
12. Ke, Z., Oton, J., Qu, K., Cortese, M., Zila, V., McKeane, L., Nakane, T., Zivanov, J., Neufeldt, C. J., Cerikan, B., Lu, J. M., Peuchen, E. H., Xiong, X., Kräusslich, H.-G., Scheres, S. H. W., Bartenschlager, R., & Briggs, J. A. G. (2021). Structures and distributions of SARS-CoV-2 spike proteins on intact virions. Nature, 588(7838), 498–502. https://doi.org/10.1038/s41586-020-2665-2
13. Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., & Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature, 581(7807), 215–220. https://doi.org/10.1038/s41586-020-2180-5
14. Li, F. (2016). Structure, function, and evolution of coronavirus spike proteins. Annual Review of Virology, 3(1), 237–261. https://doi.org/10.1146/annurev-virology-110615-042301
15. Li, M., Li, Y., Zeng, R., Guo, Q., & Lin, C. (2021). The potential of targeting host-virus protein-protein interactions for developing broad-spectrum antiviral drugs. Viruses, 13(8),1497. https://doi.org/10.3390/v13081497
16. Liu, C., Zhou, Q., Li, Y., Garner, L. V., Watkins, S. P., Carter, L. J., Smoot, J., Gregg, A. C., Daniels, A. D., Jervey, S., & Albaiu, D. (2020). Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. ACS Central Science, 6(3), 315–331. https://doi.org/10.1021/acscentsci.0c00272
17. Lu, M., Uchil, P. D., Fu, W., Sesaki, H., Hakiem, H., Cho, N. J., Liang, C., John, B., Ramos, J. V., Chuang, P. K., Clark, B. M., Savis, H., Abrahamyan, L., Meckler, J. F., Venev, S. V., Anand, S. P., Bowman, S. A., Budzisz, R., Richardson, L., ... Grobler, J. A. (2022). Inhibition of SARS-CoV-2 entry utilizing lectins from the Narcissus species. Viruses, 14(1), 94. https://doi.org/10.3390/v14010094
18. Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., Guo, L., Guo, R., Chen, T., Hu, J., Xiang, Z., Mu, Z., Chen, X., Chen, J., Hu, K., Jin, Q., Wang, J., & Qian, Z. (2020). Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature Communications, 11(1), 1620. https://doi.org/10.1038/s41467-020-15562-9
19. Padhi, A. K., Tripathi, T., Raviprakash, N., Sangar, V. C., Rezousova, O., Moiseeva, E. V., Liu, X. L., Umarova, E. J., Meng, K., Sharezhov, M., Allam, M. F., Eltaher, W. A., Kamel, M. S., Hassan, M. S., El-Husseiny, A., El-Esnawy, M., Mohammed, N., Alamri, M. A., Singh, A., ... Al-Misned, F. (2022). Novel small molecules inhibit SARS-CoV-2 entry by targeting Spike-ACE2

interaction. Viruses, 14(4), 701. https://doi.org/10.3390/v14040701

20. Pillay, T. S. (2020). Gene of the issue: The SARS-CoV-2 spike glycoprotein. Gene, 761, 145038. https://doi.org/10.1016/j.gene.2020.145038
21. Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., & Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. Nature, 581(7807), 221–224. https://doi.org/10.1038/s41586-020-2179-y
22. Sloan, D. D., Sassano, M. F., Arevalo-Solis, J., Staas, W. C., Nidley, N., Arbaiza, J. M., Smith, M., Sanders, J., Hirka, G., Maichin, K., & Strunic, A. (2022). Targeting the SARS-CoV-2 spike protein: Small molecule inhibitors of the spike protein-human ACE2 protein-protein interaction. Chem Med Chem, 17(10), e202101136. https://doi.org/10.1002/cmdc.202101136
23. Wang, Y., Huang, Y., Xu, Z., Gao, C., Zentner, I., Geng, Z., Yang, D., Zhang, G., Ye, Y., Liu, C., Zhou, Y., Chen, Q., Gu, J., Ding, J., Lei, J., & Sun, S. (2020). Functional analysis of SARS-CoV-2 spike and envelope glycoproteins using gemini virus-derived vectors. Molecular Plant, 13(12),1732–1739. https://doi.org/10.1016/j.molp.2020.10.014
24. Walls, A. C., Park, Y.-J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell, 181(2), 281-292.e6. https://doi.org/10.1016/j.cell.2020.02.058
25. Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science, 367(6485), 1444–1448. https://doi.org/10.1126/science.abb2762