The Operating Principles, Escape Mechanisms and Applications in the Public Health System of SARS, MARS and COVID-19

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Abstract. In recent years, the outbreaks caused by coronavirus mutant strains such as SARS, MERS and COVID-19 have posed a serious threat to global public health security. As the virus continues to mutate, deeply understanding its infection mechanism and pathogenic patterns and transforming them into public health epidemic prevention and control has become an important research topic. This article systematically compares the receptor recognition mechanisms of the spike proteins of SARS-CoV, MERS-CoV and SARS-CoV-2, as well as their regulatory strategies on the host's innate immune signal transduction pathways. Studies have found that SARS-CoV and SARS-CoV-2 mainly enter cells through the ACE2 receptor, while MERS-CoV relies on the DPP4 receptor. Meanwhile, all three viruses can inhibit the RIG-I/MDA5-MAVS-TBK1-IRF3 interferon signaling pathway through non-structural proteins, but there are differences in the specific targets and mechanisms of action. In addition, this article also explores the application value of related molecular mechanisms in the development of broad-spectrum antiviral drugs and the genomic monitoring of sewage. In the future, structural biology and genomic monitoring technologies should be further integrated to conduct in-depth research on the adaptive evolution laws of new variant strains and establish a safety defense network system for epidemic prevention and control.

Keywords: Coronavirus; keywords covered; immune signal transduction.

1. Introduction

Over the past two decades, pandemics caused by novel respiratory pathogens have seriously threatened the global public health system. For instance, severe acute respiratory syndrome (SARS) has become rampant since 2003, affecting many countries and regions. A total of 8,096 cases and 774 deaths were reported, with a case fatality rate of approximately 9.6% [1]. There was also the Middle East Respiratory Syndrome (MERS) that emerged in 2012. Although its spread range is smaller than that of SARS, its fatality rate is almost as high as 34-35% [2]. The novel coronavirus pneumonia (COVID-19) that broke out at the end of 2019 and rapidly evolved into a global pandemic has caused hundreds of millions of confirmed infections and millions of deaths worldwide. Although these several coronaviruses vary in terms of the duration of their outbreaks, regional differences, and clinical manifestations, in-depth research has revealed that they all share a common feature - they are all caused by different types of coronaviruses [3]. This epidemic has caused severe morbidity and mortality rates and has had a significant impact on the global allocation of medical resources, economic growth and social operation.

The high pathogenicity and transmissibility of these coronaviruses are highly dependent on the unique molecular and cellular biological characteristics of these viruses. According to existing research, the first stage of coronavirus infection involves recognizing the surface spike protein of the virus, enabling it to bind to specific receptors in cells and enter cells through endocytosis. In addition, mutations at certain sites of the spike protein may increase the affinity for the interaction between the ligand and the receptor, thereby making the virus more contagious and capable of immune evasion. In vitro biochemical experiments have shown that when the virus enters, non-structural proteins such as NSP1 and ORF6 can inhibit the production of interferon. In terms of public health, research mainly focuses on assessing the impact of measures such as vaccination at the group level, contact tracing, mask-wearing and social distancing on the speed of epidemic spread [4].

However, a considerable amount of scientific work is dedicated to studying individual biological processes or public health intervention measures, and there are few systematic studies comparing the microevolutionary changes of coronaviruses from the aspects of "external invasion binding ability" and "intracellular immune interception efficiency". The aim of this study is to conduct a systematic comparison of the molecular processes of immune escape when representative coronaviruses (SARS-CoV, MERS-CoV, SARS-CoV-2) enter cells and the cytoplasm. Based on the results of this microevolution analysis, this study will explore the development of broad-spectrum antiviral drugs, early intervention strategies, and the practical implementation path of the "Early active Early warning System for molecular Epidemiology of sewage" from the perspective of public health.

2. Invasion: Research on the Molecular Mechanism of Spike-ACE2 Receptor Specific Binding

2.1. Structural Diversity of Spike Proteins in SARS-CoV, MERS-CoV, and SARS-CoV-2

The process by which viruses enter the human body depends on the spike proteins found in the envelope of virus particles, which are like small thorns. These spike proteins are the key to entering host cells. Although SARS, MERS and the novel coronavirus are all coronaviruses, there are considerable differences in the structure of their spike protein bonds, which leads to their binding to different receptors. The spike protein itself is composed of two main subunits - the extracellular S1 subunit mainly participates in receptor recognition, while the S2 subunit contains membrane fusion elements responsible for mediating the fusion of the viral envelope with the host cell membrane.

According to the cryo-electron microscopy data obtained in previous studies, the folding patterns of the spike protein S1 of the SARS coronavirus and SARS-CoV-2 are very similar, and they both bind to the ACE2 receptor on human cells. Although the receptors are the same, the novel coronavirus has undergone some conformational changes in the receptor-binding pocket through mutations. For instance, in the Delta and Omicron variants, the local charge distribution on their surfaces has undergone key amino acid displacement with the hydrophobic side chains, thereby generating a more stable hydrogen bond network and enhancing the stability of ACE2 binding [5]. The MERS coronavirus utilizes a completely different receptor binding mechanism, featuring a unique amino acid sequence and a distinctive S1-RBD three-dimensional folding. The MERS-CoV RBD receptor does not bind to ACE2 on the surface of human cells but binds to the DPP4 receptor.

2.2. A Comparison of the Ways in Which Three Coronaviruses Recognize Different Human Cell Receptors and Their Binding Capabilities

In addition to receptor differences, the stability of ligand-receptor interactions must be evaluated by measuring the ligand-receptor binding affinity, which is expressed by Kd (dissociation constant) or binding free energy. The Kd value essentially reflects the magnitude of the dissociation tendency of the complex in physical and chemical aspects. The spike protein serves as the ligand L, while the specific human cell receptor is labeled as R. Complex LR is formed by a reversible reaction between the two. From kd = [L][R]/[LR], it can be known that the smaller the kd, the larger the LR, and the more stable the complex, which means that the chance of virus attachment and infection initiation is greater [6].

Both SARS-CoV and SARS-CoV-2 use ACE2 as the receptor. The Kd of SARS-CoV binding to ACE2 is within tens of nanometers (15-50 nM). SARS-CoV-2 RBD can form additional hydrogen bonds with ACE2, resulting in Kd in the single-digit nanomolar range (1-10 nM), and thus has a higher binding affinity. The research of Lan et al. shows that the binding mode of SARS-CoV-2 to ACE2 is almost the same as that of SARS-CoV, except that the key binding sites have been optimized [7]. The interaction between MERS-CoV and DPP4 has affinity in the nanomolar range (15-45 nM). Although the entire receptor system is significantly different from the SARS spectrum, it utilizes

precise conformational complementarity to provide stable anchoring for human lower respiratory tract and kidney tissues, a feature confirmed by low Kd values.

Due to the evolution of the pandemic, the Delta variant and its Omicron lineages (such as BA.1, BA.5, XBB) have accumulated a dense array of key mutation sites in the RBD region of the S protein (N501Y, L452R, etc.). N501Y is referred to as a receptor-adaptive mutation because, according to research, a single N501Y substitution increases the binding affinity of RBD-ACE2 by 4.3 times ($\Delta - \log Kd \approx 0.64$), thereby significantly enhancing the infectivity and transmissibility of the virus. The Omicron variant has accumulated more than 15 mutations in the RBD region of the S protein (such as N501Y, Q498R and Q493R). The synergistic effect of Q498R and N501Y mutations leads to an increase in ACE2 binding affinity while maintaining immune escape characteristics [8].

3. Defense: Pathway Analysis of Viral non-structural Proteins blocking interferon signal transduction

3.1. The Normal Alarm Process of Cells

When the SARS-CoV-2 virus penetrates the host cell membrane through endocytosis and releases the genome, the host cells will activate the intracellular innate immune defense mechanism to identify and eliminate the threat from the pathogen. The interferon signaling pathway is one of the main defense mechanisms of cells and serves as their alarm system. The basic steps include capturing viral nucleic acid signals through cytoplasmic pattern recognition receptors (PRRs); Issue an alarm signal before the virus replicates widely; Trigger and synthesize type I interferons (IFN-α and IFN-β) through cascade amplification reactions; Finally, send antiviral warning signals to the surrounding healthy cells to prevent the spread of viral infection [9].

Intermediate products are produced during the replication and transcription of coronaviruses within cells. The innate immune system sensors in the cytoplasm, namely retinoic acid-induced gene I (RIG-I) and melanoma differentiation-related gene 5 (MDA5) proteins, precisely recognize and bind to viral double-stranded Rnas (dsRNAs), leading to structural changes and exposing the special domain (CARD) at the N-terminal [10]. The CARD domain rapidly moves to the outer mitochondrial membrane, where it binds to the linker molecule MAVS (mitochondrial antiviral signaling protein) present at that location. This combination facilitates the polymerization and assembly of MAVS molecules on the mitochondrial surface, forming a supramolecular complex that serves as an important station for signal transduction. The aggregated MAVS complex then recruits downstream cytoplasmic kinases, especially Tank-binding kinase 1 (TBK1). Activated TBK1 leads to the phosphorylation of the transcription factor IRF3 (interferon regulatory factor 3). Subsequently, IRF3 dimerizes to form a homodimer, which then translocate through the nuclear pore into the nucleus, activating IRF3 to bind with the promoter of type I interferon genes, leading to transcription and expression. The large amount of type I interferons produced thereby is secreted and attached to the receptors on the cell membranes of adjacent healthy cells, inducing the expression of hundreds of antiviral effector proteins (ISGs), forming a broad-spectrum antiviral state, and effectively reducing viral replication and transmission.

3.2. Counter-detection Methods for Viruses

Facing the sophisticated defense network developed by the host's continuous evolution, SARS-CoV, MERS-CoV and SARS-CoV-2 have developed a variety of non-structural and helper proteins on this basis to attack different nodes of the innate immune signaling pathway and interfere with the interferon pathway, thereby achieving immune evasion.

In SARS-CoV, the non-structural protein NSP1 specifically interacts with the 40S ribosomal subunit of the host, blocking the mRNA entry channel and promoting mRNA degradation. Inhibit the translation of host proteins and the synthesis of interferons and related antiviral proteins [11]. In addition, the auxiliary proteins ORF3b and ORF6 are located on the nuclear membrane and interact

with the nuclear transport receptor complex KPNA2/KPNB1, blocking the nuclear pore channels and preventing the nuclear translocation of the phosphorylated IRF3 dimer, thereby interfering with the transcriptional activation of interferons. MERS-CoV shows a strong antagonistic effect on interferon. In particular, the ORF4a protein can directly bind to the viral dsRNA and hinder the recognition of the host receptor RIG-I/MDA5 through steric hindrance effects. In addition, the ORF4b protein has functional nuclear localization signals that compete for nuclear transport channels, thereby preventing the activation and translocation of downstream transcription factors and allowing inflammatory factors to be transcribed into the nucleus.

Compared with the above-mentioned viruses, although the SARS-CoV-2 virus has a lower clinical mortality rate, it has a more complex intracellular immune evasion strategy. Lei et al. discovered a variety of viral proteins that can inhibit interferon production, including NSP1, NSP13, NSP14, etc. NSP1 can bind to the host ribosome and block the translation of host proteins. NSP13 helicase inhibits the phosphorylation and activation of TBK1, while NSP14 exonuclease activity accelerates the degradation of IFNAR receptors [12].

4. Translational Public Health Applications Based on Molecular Targets

4.1. The Normal Alarm Process of Cells

The continuous in-depth research on the mechanism of coronavirus infection has gradually clarified the key molecular targets in the process of virus invasion of host cells and immune escape, providing an important theoretical basis for the development of broad-spectrum antiviral drugs and clinical treatment strategies.

At the extracellular physical blocking level, although the S1-RBD region of SARS-CoV-2 (especially the Omicron variant) is highly mutated, studies have shown that the S2 subunit region responsible for mediating membrane fusion and some Cryptic Pockets of RBD remain largely unchanged in evolution. Clinical applications of peptide inhibitors (such as EK1, etc.): Additionally, based on the essential mechanism of transmembrane fusion that the interaction between the HR1 and HR2 domains in the S2 subunit forms a six-helix bundle (6-HB), researchers have designed peptides like EK1 and their derived lipopeptides (such as EK1C4) [13]. This physical blocker can act as a "fake key" to competitively bind to the HR1 region, physically locking the fusion process between the virus and the cell membrane. In public health practice, this type of polypeptide can be directly prepared into broad-spectrum nasal sprays or nebulized inhalers, establishing a physical barrier on the surface of the respiratory tract mucosa to achieve immediate and broad-spectrum pre-exposure and post-exposure prophylaxis.

Intracellular, however, the gene sequences of non-structural proteins (NSPs) necessary for viral replication exhibit extremely high evolutionary conservation. Therefore, immune escape proteins have become the core direction in the development of Direct-Acting antiviral drugs (DAAs) for oral small molecule antiviral drugs. Nirmatrelvir (the core component of Paxlovid), which has been widely used in clinical practice, covalently binds to the highly conserved main protease of the coronavirus, cutting off the normal cleavage and processing of polyprotein precursors including the aforementioned NSP1 and NSP13, fundamentally blocking the intracellular assembly of the virus [14]. Nucleoside analogues such as Remdesivir and Azvudine forcibly terminate the replication and extension of the viral genome by targeting RNA-dependent RNA polymerase (RdRp) [15]

4.2. Wastewater Genomic Surveillance and Molecular Early Warning Systems

Combining the microscopic patterns of gene mutations with macroscopic epidemiology to establish epidemiological public health Surveillance based on Wastewater Genomic Surveillance is a core means for the early identification of infectious disease epidemics. In the early stage of infection, coronaviruses (especially SARS-CoV-2) show no symptoms but are excreted through feces and upper respiratory tract secretions and eventually accumulate in urban sewage networks. By conducting

nucleic acid testing and genomic monitoring on urban sewage, researchers can detect signs of virus transmission before a large number of clinical cases emerge, achieving early warning of the epidemic.

Due to the severe dilution of household and industrial wastewater, the viral nucleic acids in the sewage are usually in an ultra-microstate, accompanied by a large amount of impurity interference. The first step in conducting monitoring is to utilize polyethylene glycol (PEG) precipitation method, ultrafiltration membrane filtration method or charged membrane adsorption method [16]. Efficient enrichment of virus particles in large-volume sewage samples. After the extraction of viral RNA was completed, Targeted Next-Generation Sequencing technology was used to specifically conduct ultra-deep Sequencing on the core mutation region of the receptor binding domain (RBD) of the spike protein (S protein). This micro-level sequencing can precisely analyze the relative abundance of nucleic acid sequences of different variant strains in sewage samples [17]. Clinical medical statistics show that due to the existence of asymptomatic cases and the lag in residents' medical treatment and testing, the increase in clinical confirmed data often lags behind the actual group infection by 7 to 14 days. Smyth et al. discovered "cryptic lineages" that have long existed but are rarely detected in clinical samples after conducting long-term high-depth targeted sequencing on multiple sewage treatment plants in New York City, USA [18], help the local area issue early warnings. The successful monitoring of wastewater genomes has broken down the disciplinary barriers between micro-virology and macro-public health management. It not only enhances the agile response capability of the public health system but also sets a model of translational medicine for the global routine monitoring of future mutant strains.

5. Conclusion

This paper takes three representative coronaviruses, namely SARS-CoV, MERS-CoV and SARS-CoV-2, as the research objects, and systematically compares the receptor recognition of different spike proteins and the immune escape mechanism of viruses to the host immune signal transduction pathway. Research has found that all three viruses rely on spike proteins to bind to host receptors to achieve cell invasion, but there are significant differences in receptor selection, binding affinity Kd values, and key mutation sites. Meanwhile, they can all interfere with the interferon signaling pathway through non-structural proteins and helper proteins, thereby inhibiting the host's antiviral response and enhancing the efficiency of viral replication. Meanwhile, this paper further explores the application of physical blockers and the realization of sewage gene monitoring, completing the deep transformation of knowledge into macro public health early warning.

By comparing the molecular mechanisms of different coronaviruses, this paper further reveals the biological basis on which viral invasion and immunosuppression jointly drive the occurrence and development of diseases and explains the important application value of related research in the development of broad-spectrum antiviral drugs, as well as in the genomic monitoring of sewage and the construction of public health early warning systems. However, this article mainly conducts a review and analysis based on existing literature and lacks experimental data for verification. Meanwhile, the coronavirus is still evolving, and the molecular characteristics of newly emerged variants may differ from those of existing research results. Future research should further integrate biological theoretical knowledge with monitoring techniques, deeply analyze the receptor adaptation mechanism and immune escape rules of new variants, and promote the transformation of basic research results into clinical drug development, vaccine design and public health monitoring practices, providing more reliable theoretical basis and technical support for responding to possible new infectious diseases and subsequent epidemics in the future.

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