SARS Coronavirus: Minireview

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The infection of SARS Coronavirus (CoV) was first observed in 2002. Since 2004, there have not been any new cases of this disease. However, new coronaviruses able to infect humans were found in bats and during the outbreak of Middle East Respiratory Syndrome Coronavirus. To effectively fight the coronavirus infections, the properties of SARS-CoV are still studied. In this review, we describe the genome, replication and transcription and important proteins of SARS--CoV. Also the ability of SARS-CoV to evade the innate immune system of host is described. The second part of this review discusses the methods for detection and treatment of SARS-CoV.

Keywords: coronavirus; neutralizing antibodies; severe acute respiratory syndrome

1. Introduction

In 2002, an outbreak of respiratory illness emerged from China, later named as a Severe Acute Respiratory Syndrome [1,2]. It was the first previously unknown pandemic transmissible disease of 21st century [3]. The disease spread quickly throughout the world (affecting 32 countries) with more than 8500 individuals infected and 900 dead (case fatality rate of 10%). It can be transmitted via respiratory droplets, direct contact or airborne processes [4].

The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) is a virus belonging to the Betacoronavirus genus from family *Coronaviridae* [5]. Coronaviruses are closely related to influenza viruses and both SARS and MERS (Middle East Respiratory Syndrome) are more lethal than other human coronaviruses [2]. SARS-CoV nucleocapsid has a helical symmetry; it is enveloped with a positive sense, non--segmented, capped and poly-adenylated RNA genome the size of 30 kb [2].

SARS-CoV is a zoonotic virus, it was transmitted to humans from its natural reservoir horseshoe bats (of the Rhinolophus genus) after still undefined mutations, possibly through intermediate hosts such as palm civets (Paguna larvata) from live animal market [3]. Since 2004, there were no new cases of SARS-CoV in humans, although a SARS-like coronavirus strain able to infect humans can still be found in Chinese bats (named as a SL-CoV-W1V1) [6]. It uses the human angiotensin converting enzyme II receptor to enter the cells, thus it does not require any mutations or intermediate hosts to infect human cells [7]. Moreover, the recent emergence of MERS in Middle East shows that coronaviruses are still a threat to worldwide population.

2. Molecular biology of SARS Coronavirus

The genome of SARS Coronavirus contains 9 mRNA transcripts with 28 open reading frames (ORFs). Coronaviruses in general have a unique position in the RNA virus world because of their RNA-synthesizing machinery [8]. ORF1a and ORF1b form about 75% of the SARS-CoV genome and they are translated in a cap-dependent manner into large polyproteins [9], pp1a and pp1ab. Thiel et al. showed that pp1a and pp1b create 16 non-structural

proteins (nsp) via the proteolytic cleavage [10]. The replication and transcription of coronavirus RNA are mediated by these nsps. Snijder et al. found out that the nonstructural proteins encoded in ORF1b (nsp12-16) are expressed at significantly lower levels than those in ORF1a (nsp1-11) [11]. The nonstructural proteins associate into replicative/transcription complex after the maturation of polyproteins. This complex is responsible for the synthesis of new viral genomes and subgenomic mRNA encoding structural and accessory proteins [12]. The nsp12 encodes the RNA-dependent RNA polymerase, responsible for RNA replication and transcription of the viral RNA. SARS-CoV also possess helicase and capping enzymes, 3´-5´ exonuclease, involved in proofreading, as was described by Minskaia et al. [13]. Ivanov et al. have also found endoribonuclease in SARS Coronavirus [14].

SARS Coronavirus has four major structural proteins – spike (S), envelope (E), nucleocapsid (N) and membrane (M) - encoded by the remaining two thirds of the genome [9]. The spike, envelope and membrane proteins were found to be translated on the membrane-bound polysomes on endoplasmic reticulum [15].

The spike protein is glycosylated, situated on the surface of SARS-CoV particle and it is responsible for receptor binding and virus-cell fusion [16]. Li et al. observed that it initiates the entry of SARS Coronavirus into host cells by binding to receptors for angiotensin-converting enzyme 2 [17]. The minimal receptor-binding domain was found to be a 193-amino acid fragment in the S1 subunit of spike protein [18]. After the binding and some structural changes, the membranes fuse and the viral genome is delivered to the cytoplasm. Aydin et al. have determined that the membrane fusion is mediated by S2 subunit of spike protein [19]. Even minor changes in S protein can affect the tissue and species tropism and the virulence of coronaviruses, as was described by Li et al. [17].

The envelope protein is very small, containing only 76 amino acids [20]. It is not essential for genome replication or subgenomic mRNA synthesis; however, it was found, that it affects viral morphogenesis [21], budding [22], assembly, intracellular trafficking [23] and virulence. The alterations in E protein showed a high impact on SARS-CoV pathogenesis due to the induction of stress and unfolded protein responses and changes in the cellular ion concentrations [24]. Jimenez-Guardeno et al. suggested that the PDZ domain-binding motif of E protein targets PDZ domains present in cellular proteins [24].

The nucleocapsid protein is translated on free polysomes, it is highly basic and forms helical ribonucleoprotein complexes with a newly synthesized viral RNA [15], thus comprising the core structure of the SARS-CoV virion [9]. Chen et al. determined that these complexes are also involved in transcription, replication and virus packaging [25].

The membrane protein triggers virus budding after interaction with the envelope protein in ER-Golgi intermediate compartment [26]. Membrane protein then interacts with spike and nucleocapsid proteins and the formed virions accumulate in large vesicles. Theses vesicles are released from the host cell after the fusion with the cell membrane [27]. Hsieh et al. observed that the membrane protein together with envelope or nucleocapsid protein can form virus-like particles [28].

SARS Coronavirus also contains eight ORFs encoding accessory proteins unique for SARS-CoV and SL-CoV-W1V1 – p3a, p3b, p6, p7a, p7b, p8a, p8b and p9b [29]. These accessory proteins were found to be nonessential for SARS-CoV replication, but contribute to its pathogenesis [30], they are involved in cell proliferation, DNA synthesis, induction of caspase-dependent apoptosis, activation of stress response pathways and pro-inflammatory cytokine production [31].

The 3a protein, containing 274 amino acids [32], contributes to the increased virulence of SARS-CoV. It can form homo- and heterotetramers in infected cells [33] and ion channels selective to potassium ions [34], important in the virus life cycle and its pro-apoptotic function [35]. Minakshi et al. determined that calcium can serve as a ligand to the gated channel, causing conformational changes in the protein [36]. The 3a protein also assists in the internalization of viral spike protein from cell surface to

the intracellular sites [37] and Minakshi et al. confirmed a tyrosine-based sorting motif and a di-acidic motif present in this protein [38].

SARS Coronavirus has developed a mechanism to evade the antiviral activities of innate immune signaling pathway [39]. It contains a papain-like protease with deubiquitination activity, serving as an antagonist to the interferon [40] by the disruption of the signaling required for its induction [41]. Ratia el al. determined that the papain-like protease prefers the ubiquitin chains in comparison with mono--ubiquinated molecules [42]. It also blocks the induction of the interferon regulatory factor-3 after its phosphorylation [43], but does not inhibit its dimerization, nuclear localization or DNA binding [44].

3. Detection and treatment of SARS Coronavirus

The presence of SARS-CoV in patients can be detected using serological tests. However, Mahony et al. showed that seroconversion usually occurs 2 or 3 weeks after the infection [45], the development of methods for early detection of SARS-CoV is therefore necessary. Molecular methods can be employed for this early diagnosis. Peiris et al. used real-time RT-PCR method for the amplification of p1b gene [46]. The group of Hadjinicolaou et al. have described a real-time RT-PCR assay for the detection of spike, envelope, membrane and nucleocapsid genes, using molecular beacons with tolerance to mismatches. This method can be also used for the detection of generically non-identical strains that can emerge in the future [47].

However, traditional real-time RT-PCR suffers from varying levels of sensitivity to different RNA viruses [48]. Therefore, Keightley et al. used the real-time nucleic acid sequence-based amplification for the detection of polymerase and nucleocapsid genes and this method demonstrated equivalent sensitivity to SARS-CoV in comparison with traditional real-time RT--PCR [49]. Another molecular method employable for the early detection and epidemiological surveillance of SARS-CoV can be the single nucleotide polymorphism DNA microarray. The gene encoding spike protein contains 27 single nucleotide polymorphisms, closely related to the virus phylogenicity, virulence and pathogenesis, as was described by Kan et al. [50]. Guo et al. suggested a microarray able to detect and genotype samples with 100% accuracy [51].

Sunwoo et al. used antibody-based sandwich enzyme-linked immunoassay (ELISA) with monoclonal antibodies for the detection of spike protein. Using a bi-specific monoclonal antibody for the detection, they were able to increase significantly the sensitivity of their method [52]. The group of Roh et al. developed a method using RNA aptamer conjugated with quantum dots for the detection of SARS-CoV nucleocapsid protein, which can be easily used as a basis for a chip [53].

Nowadays, several vaccine strategies are being studied. Protective and neutralizing antibodies against coronavirus infection are often engineered to target spike proteins [54], especially its receptor binding domain on S1 subunit [55,56]. The possibility of post-infection passive immunization was studied by Yasui et al. with the increased elimination efficacy of homologous neutralizing antibodies in comparison with heterologous, caused by the cooperation of anti-SARS antibodies and phagocytic cells (monocyte-derived infiltrating macrophages and partially alveolar macrophages) [57].

However, vaccination against one type of coronavirus can significantly increase the probability of infection with other coronaviruses [58]. Moreover, Wang et al. determined that using antibodies against SARS-CoV spike protein might trigger the antibody dependent enhancement effect, thus undesirably increasing the virus yields [59]. This effect is caused by infection of large numbers of susceptible cells, mediated by immunoglobulin receptors such as Fc that facilitate the uptake of virus-antibody complexes in phagocytes and enhance the target cell infections [60].

The deletion or modification of E protein PDZ domain-binding motif and internal regions in the carboxy terminus of E protein, resulting in attenuated virus, can be another effective way of vaccine preparation [61]. Wong et al. also described peptidomimetic inhibitors for the main protease of SARS Coronavirus [62].

4. Conclusions

The recent emergence of MERS Coronavirus showed that there still are unknown coronavirus strains with high virulency in humans. The first dangerous strain observed was SARS Coronavirus in 2002 with 900 casualties. The molecular attributes of coronaviruses are very similar. SARS Coronavirus is probably the most studied. Due to the new insights into molecular biology of SARS, novel strategies for the detection and treatment of coronaviruses were proposed.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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