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(REVIEW ARTICLE)

Inhibition of untranslated region, subgenomic RNA, and TMPRSS2 for SARS cov-2.

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Abstract

The SARS CoV-2 genome contains untranslated region (UTR) which regulates RNA replication and transcription, and subgenomic RNA (sgRNA) which encodes the 4 structural proteins where the virus cannot proliferate without them. The SARS CoV-2 host cell contains TMPRSS2 protease enzyme which activates the virus to enter the cell. The chemical compounds Peptide Conjugated Phosphorodiamidate morpholino Oligomer (PPMO) and Benzylesulfonyl-d-arginine-proline-4-amidinobenzylamide (BAPA) have prevented the TMPRSS2 expression in influenza host cell. PPMO also has inhibited the UTR of influenza genome and the sgRNA of porcine reproductive and respiratory syndrome virus (PRRSV), so PPMO and BAPA could inhibit the UTR and sgRNA of SARS CoV-2 and prevent the expression of TMPRSS2 in SARS CoV-2 host cell, that prevents virus replication. This may open the way for the development of anti SARS CoV-2 therapy.

Keywords: SARS CoV-2; Influenza virus; porcine reproductive and respiratory syndrome virus; Peptide Conjugated Phosphorodiamidate morpholino Oligomer; Benzylesulfonyl-d-arginine-proline-4-amidinobenzylamide; Drug

1. Introduction

In the last months of 2019 and the first months of 2020, several unknown pneumonia cases appeared in some hospitals in Wuhan city, and were infected via the exposure to the market of seafood in the same city [1]. After examination of these cases, it was found that, this is a novel coronavirus named Sever acute respiratory syndrome coronavirus (SARS CoV-2) by the International Committee on taxonomy of viruses. The disease that appeared by SARS CoV-2 called COVID-19 by World Health Organization [2]. This virus has spread all over the world, and became pandemic [1]. The classification of it according to virulence is the third, then SARS CoV, and MERS CoV [3]. Until now, the number of coronaviruses is seven and SARS CoV-2 is the 7th and latest one, and its genetic sequence is 70% similar to SARS CoV. It has infected animals and humans causing severe diseases such as pneumonia, gastrointestinal, hepatic, and neurological diseases, and symptoms such as fever, fatigue, dry cough, and dyspnea [1].

The SARS CoV-2 RNA genome has a nearly length of 30,000 nucleotides with a 5' cap structure and a 3' poly (A) tail [2] that represent a highly structured UTR which regulates RNA proliferation. It consists of 14 open reading frames (ORFs) [4] and from these ORFs one called ORF1ab that is the first of the 14 open reading frames and forms the most coronavirus genome length (two thirds). This ORF expresses two polyproteins (PP1a & PP1ab) that are cleaved and proceeded by enzymes like Chymotrypsin- like cysteine protease (3CL proteases)[2] EC [3.4.22.69] and papain-like protease (PL proteases)[2] EC [3.4.22.2] at 11 distinct sites to generate 16 nonstructural proteins (NSPs)[5] which formed the sgRNAs to encode the 4 main structural proteins; envelop protein (E protein), spike protein (S protein), membrane protein (M protein), nucleocapside protein (N protein), and other accessory proteins. Hence, this subgenomic RNA have enhanced the viral replication process and new viruses production [2].

The chemical compounds PPMO and BAPA have prevented the main protease expression in the influenza host cell where they have targeted TMPRSS2 that activates the hemagglutinin protein. As a result, it prevented the virus proliferation

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[6], [7]. PPMO also has inhibited the UTR of influenza genome [6] and the sgRNA of PRRSV [8]. So, these compounds could prevent TMPRSS2 expression in SARS CoV-2 host cell and inhibit the UTR and sgRNA of SARS CoV-2 genome,that prevents the virus replication. This may open the way for the development of anti SARS CoV-2 therapy.

2. SARS CoV-2 structure

The structures and proteins which are included in SARS CoV-2 are the following; Positive-sense, single-stranded genomic RNA, RNA-dependent RNA polymerase (RdRp), Chymotrypsin- like cysteine protease (3CL proteases), papain-like protease (PL proteases), Spike protein, Membrane protein, Envelop protein, Nucleocapsid protein, Hemagglutinin-esterase dimmer, and Nonstructural proteins.

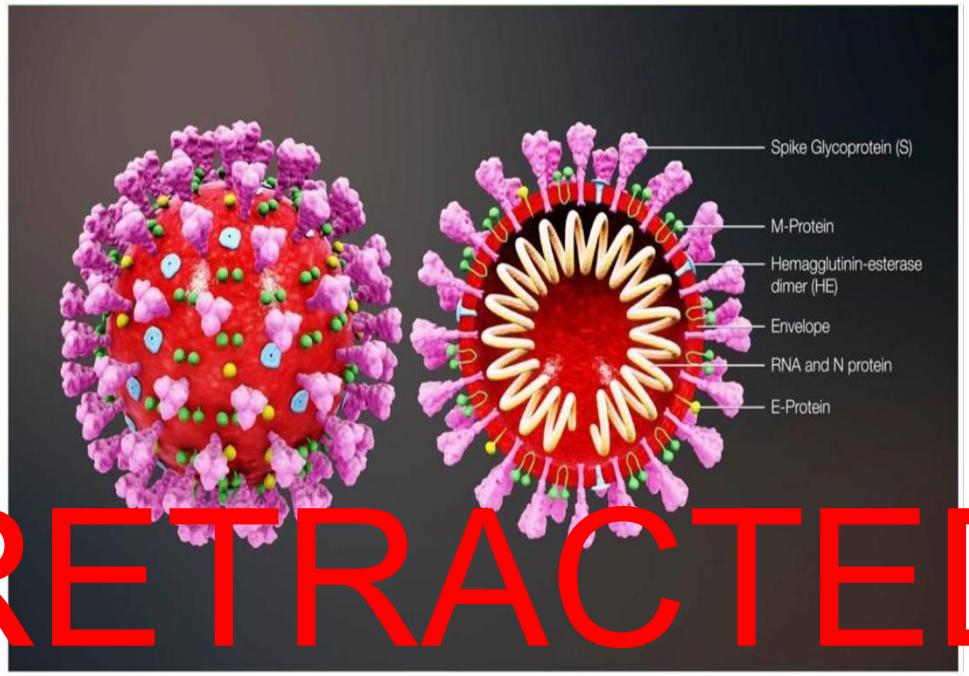

Figure 1 The image shows the main components in SARS CoV-2,that are the Spike (S protein), M protein, viral envelope (E protein), Nucleocapsid protein (N protein) and Hemagglutinin-esterase dimmer (HE protein). [9]

2.1. Positive-sense, single-stranded genomic RNA

The SARS-CoV-2 has a positive-sense, single-stranded genomic RNA, but this genome's length is about 30 KB. So, it is considered one of the longest known genomes of RNA. The genomic RNA (gRNA) has a 5'-cap and a 3'-poly (A) tail and can serve as mRNA for immediate viral polyprotein translation. Furthermore, both 50-and 30-ends of the gRNA represent a highly structured UTR which regulates RNA proliferation. The 50-UTR has seven stem-loop structures on it while the 30-UTR has a stem-loop and a pseudoknot. These two latter structures since their sequences overlap are mutually exclusive. A review hypothesized that the pseudoknot or stem-loop alternate formation participate in transcriptional regulation. The gRNA consists of 14 ORFs [4] and from these ORFs one called ORF1ab that is the first of the 14 ORFs and forms most coronavirus genome length (two thirds). This ORF expresses two polyproteins (PP1a & PP1ab), and these proteins have proceeded by SARS VoV-2 main proteases to form the structural proteins that are used for the formation of new viruses [2], [5].

2.2. RNA dependent RNA polymerase (RdRp)

The RdRp is an essential enzyme for coronaviruses and plays a significant role in coronavirus replication from RNA templates. The sequences of RdRp in SARS-CoV, MERS-CoV, and SARS-CoV-2 are similar [10], and the replication and transmission functions take place by a complex of multimeric RdRp with non-structural proteins [11] .

2.3. Chymotrypsin- like cysteine protease (3CL protease)

The 3CL proteases is one of the two main proteases of SARS CoV-2 and participates in the formation of structural proteins which used in the production of new viruses. It is composed of the three Domains; Domain I, II, and III. Domain

I consists of (8-101) amino acid remains, and domain II consists of (102-184) amino acid remains, and both domains I and Domain II are essentially beta barrels (transmembrane proteins). Domain III consists of (201-306) amino acid remains, and it is alpha helices. The substrate binding region of the 3CL protease enzyme locates at the cleft of Domain I and II and composed of conserved His 41 and Cys 145 catalytic dyad. The SARS contains other buried subsites (S1 and S2) in the main protease [3], [11]. S1 subsite composed of amino acid remains named His163, Cys145, Glu166, His172, Gly143, and Phe140 and S2 subsite composed of amino acid remains named Cys145, His41, and Thr25. The SARS also contains other 3 shallow subsites (S3, S4, S5) composed of amino acid remains named Met49, His41, Met165, Glu166, and Gln189. Due to the function of the 3CL protease and PL protease in the life cycle of the virus, maturation, and SARS CoV-2 replication; they became an important target for the development of effective anti SARS CoV-2 therapy [3].

2.4. papain-like protease (PL protease)

The PLpro plays a significant role in the viral replication where it hydrolyzes the peptide and isopeptide bonds in viral and the cellular substrate because it processes the viral polyproteins and proteins of the host cell to produce structural proteins and enhance viral replication. It cleaves the viral polyprotein into its 16 viral proteins that participate in the formation of a membrane-associated cytoplasmic enzyme complex (the replicase complex) which modulates the replication and transcription of viral genome. The PLpro helps SARS CoV-2 to overcome the innate immunity during the viral evasion [12].

2.5. Spike protein (S protein)

The SARS CoV-2 envelope contains a glycoprotein transmembrane spike protein (S protein) that enables the virus to invade the cell [13]. The S glycoproteins of SARS-CoV, MERS-CoV, and SARSCoV- 2 have amino acids range between 1104 to 1273, an amino (N)- terminal S1 subunit, and a carboxyl (C)-terminal S2 subunit and each of them has a specific function[14]. The first subunit is S1 and the second is S2. The first subunit has a Receptor Binging Region (RBD) that mainly targets the Angiotensin-converting enzyme-2 (ACE2) that locates on the host cell surface (ACE2 is SARS CoV-2 receptor)[13]. Previously, a crystal structures chain of the SARS-CoV RBD was determined from various strains in complex with SARS CoV-2 host cell receptor (ACE2). These stru ctures have shown that there are a core and receptor-binding m tif (RBM) in the RBD of SARS-CoV and the RBM enhances the viral attachment to ACE2 [15]. The second subunit is S2 and enhances the viral invasion. For several CoVs, S is cleaved at the borders between the subunits S1 and S2. The S is further activated for fusion by the host cell proteases at S2 site [13].

2.6. Membrane protein (M protein)

The M protein is a standard transmembrane glycoprotein composed of a triple membrane domain and contains 80 amino acids that account for around one-third of all protein (221 residues in total). It is considered the dominant of the structural proteins in the SARS CoV-2 and determines the viral envelope shape and organizes CoV assembly [16] where it interacts with all other large structural coronavirus proteins [17]. It is also virus-specific humoral response and able to neutralize antibodies in the patients with SARS CoV-2[16].

2.7. Envelope protein (E protein)

The CoV E protein is a small protein in CoV membrane. It consists of amino acids range between76–109, and it ranges in size between 8.4– 12 kDa [17]. It is a structural protein participates in different phases of the infection with the virus. The CoV contains about 20 copies of this protein [18]. Thanks to envelope protein structures (primary and secondary structure), it became known that the E protein consists of small hydrophilic amino terminus (consists of 7–12 amino acids), large hydrophobic transmembrane domain TMD (consists of 25 amino acids), and long hydrophilic carboxyl terminus (consists of most proteins)[17] . In particular, the virions that lack E protein have no ability to invade the cells or has very low pathogenicity. So, the work on these proteins is very important [18].

2.8. Nucleocapsid protein (N protein)

The SARS CoV nucleocapsid protein (N protein) participates in the viral infection because it synthesizes viral RNA, helps the genomic RNA transcription, and participates in viral protein translation. There are three distinct domains that form coronavirus nucleocapsid protein structure; N-terminal domain (~130 residues), central domain (~120 residues) [They are supposed to recognize the RNA], and the C-terminal domain [It motivates the protein replication][11]. The N protein facilitates the viral genome package into a helical ribonucleocapsid and virion assembly and interacts with the membrane proteins [11]. It also modulates the infected cell metabolism and regulates the interactions of the host cell. These interactions such as reorganization of actin, progression of host cell cycle, and apoptosis [19]. During the infection, N protein immunogenicity and expression become high. So, it is a target for vaccine production and serological assays [20], but its accurate structure is not available[11].

2.9. Hemagglutinin-esterase dimmer

Many virus membrane including the coronavirus have a Receptor destroying enzyme (RDE). This enzyme contains acetylesterase and receptor-binding function. So, it is called hemagglutinin- esterase (HE)[21], and this activity is believed to improve and help the S protein in the viral invasion and spread through the mucosa[22]. COVID-19 lacks the same gene of HE as SARS-CoV and MERS-CoV[23][9].

2.10. Nonstructural proteins (accessory proteins)

The coronavirus genome contains a 50 cap structure and 30 poly (A) tail that participate in the replicase polyproteins translation where they work as an mRNA. The replicas genes encode the nonstructural proteins; nsps 1–16. The nsp16 forms a heterodimer with its cofactor nsp-10 which stimulates the activity of 20-O-methyltransferase (20-O-MTase). Besides 20-O-MTase activity, the nsp-16 modifies the virus genetic material and makes it similar to human RNA and protects viral RNA from the recognition by MDA5 helicas enzyme and innate immune response [24]. The genes of the NSPs lie between the genes of the structural proteins. The number and sequence of these accessory genes differ among different coronaviruses. The specific function of the nonstructural proteins is unclear, but several studies suggested that they enhance the replication and pathogenicity of the virus [25] because they participate in the formation of the structural proteins that participate in the new viruses formation [2].

3. Inhibition of untranslated region, subgenomic RNA, and TMPRSS2

Phosphorodiamidate morpholino oligomers (PMOs) prevented influenza virus replication where they have blocked the complementary RNA, that prevents the storage of the information in single strand, have acted against the 5' UTR and/or the start codon of translation (AUG) in mRNA that prevents the translation process, have interfered with spliceosome protein that mediates mRNA maturation reactions, and have targeted the TMPRSS2 pre-mRNA or RNA which cleaves the HA to suppress influenza viruses spread. The locations in TMPRSS2 mRNA that were targeted by different PPMOs are explained as followed; scramble sequence TGCTCTGTCTACAGTAGTGTCA has targeted the nonsense sequence control, T-AUG sequence CAAAGCCATCTTGCTGTTATCAAC has targeted the nt 42-65 (initiator AUG), T-ex4 sequence TGATGC[illegible]CTT[illegible]TAGTC[illegible] has targeted the nt 295-318 (5_ end of exon 4 adjacent to splice site), and T-ex5 sequence CAGAG[illegible]TG[illegible]AGCACTTGC[illegible]GCCCA has targeted the nt 38[illegible]-405 (5_ end of exon 5, adjacent to splice site) [6]. Other PP[illegible]OS have targeted regions in some positive RNA strands. PPMO 5UP2 has targeted the 5' terminal region of the PRRS[illegible], PPM[illegible]5H[illegible] has targeted the 5' UTR of genomic RNA. PPMO 6P1 has targeted ORF6, PPMO 7P1 has targeted ORF7, and dozen PPMO has targeted variable sites in PRRSV genome and the sgRNA [8]. BAPA has prevented influenza TMPRSS2 expression. Hence, it has prevented the lysis of influenza virus hemagglutinin (HA),that reduced viral titers [7].

4. Conclusion

PPMO and BAPA could prevent SARS CoV-2 replication and open the way for the development of anti SARS CoV-2 therapy.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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