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Research Article

Corona Virus ORF1ab-Derived Nsp7 and Nsp8 Small Non-Structural Proteins Including Previously Published Nsp9/10/13/16 Share Homologies to the Ribosomal Proteins as Well as rRNA Methyltransferases and Inhibit Host Protein Synthesis

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Abstract

Corona virus ORF1ab polyprotein-derived Nsp7 and Nsp8 are small proteins of 83aa and 198aa whose functions remain elusive as replication factors of Nsp12 RNA-dependent RNA polymerase. Using multi-alignment approaches we found such RNA binding proteins might have rRNA methyltransferase activities resembling Escherichia coli RlmB and RlmH like enzymes. Phylogenetic analysis suggested Nsp7 was cryptic MTase enzyme as compared to other methyltransferases but Nsp8 could be a vital methylating enzyme with similarity to S30 transposase. Moreover, Nsp8 has weak similarity to S2 ribosomal protein and L16 ribosomal proteins and Nsp7 to S8 ribosomal protein. We surprised to describe that Nsp7/8, Nsp9/10, Nsp13-16 and Nsp2 all have similarities to different ribosomal proteins (< 25%) which were also small RNA-binding proteins. We postulated both orf1a (4405aa) and ORF1ab (7096aa) large proteins act as rRNAs sequesters affecting ribosome turnover. Thus, methylation is a preference mechanism of Corona virus pathogenesis whereas COXI and COXII host mitochondrial enzymes synthesis may be affected causing low ATP synthesis followed by platelets aggregation and blood clotting in the lungs, heart and brain. Other words, we demonstrated new Corona virus targets that were never explored. Never-the-less the patients who were dying have weak immune-system and maximum load of virus occurred leading to coma and death but in maximum young patients the Corona virus was cleared by immune-system before the viruses could spread into other organs and the patients were recovered within 3 - 4 weeks.

Keywords: Nsp7 and Nsp8; Nsp Methyltransferase; Corona Virus Polyprotein; Clustal-Omega Software; Ribosomal Proteins Homologies

Introduction

Corona viruses (family Coronaviridae) are enveloped viruses with a 30 Kilobases single-stranded RNA genome and produces polyproteins in infected cells. ORF1ab polyprotein was processed into sixteen proteins including important C3/C5-proteases and a RNA-dependent RNA polymerase known as Nsp12 protein [1-6]. Further, Nsp13 was implicated as RNA helicase and Nsp16 as 2'-O-ribose rRNA methyltransferase. The Corona virus 1/3 of the 3'-terminal of the genome encodes the structural proteins like spike glycoprotein (S), envelope protein (E), membrane glycoprotein (M) and nucleocapsid protein (N) as well as few small transcripts like ORF2b, ORF7a and ORF2a etc. (see, accession no. DQ415908, KT779555). Biochemical and genetic analysis as well as immunologically, Coronaviruses were classified into three different groups: α-CoVs, β-CoVs, and γ-CoVs [7-10]. Coronaviruses primarily infect human, mammals and animals and responsible for variety of respiratory diseases similar to the common cold, such as pneumonia,

fever and severe acute respiratory syndrome (SARS) [11-13]. COVID-19 virus enters cells through ACE-2 receptor-mediated endocytosis in lungs alveolar epithelial cells but other epithelial cells of the intestine, heart and kidney may be affected during high virus load [14-16].

Recently Coronavirus outbreaks were rampant with more than million infections and > 300000 deaths worldwide. So, Coronavirus research has augmented considering its pandemic severe respiratory illnesses in human and every day thousand papers and Genbank data depositions have documented. Previously we demonstrated that unknown Nsp2 protein was a RNA topoisomerase and Nsp9/10. Nsp13-16 could be different rRNA methyltransferases which again had 20% similarities to the ribosomal proteins of *Escherichia coli* [17-23]. Similarities of Corona virus non-structural proteins to the ribosomal proteins were not known before and we astonishingly postulated that host protein ribosome assembly and rRNA methylation might be involved with the inhibition of protein synthesis and ATP synthesis at the mitochondria [18,23]. There were numerous rRNA methyltransferases known and also 30S and 50S ribosomal proteins were heterogeneous [24-33]. Those proteins were mostly RNA binding proteins and very essential in life. We have extended such studies and have found some similar homology streatches among Nsp7 and Nsp8 with rRNA methyltransferase and ribosomal proteins.

Materials and Methods

The BLAST search was done using NCBI web portal (www.ncbi.nlm.nih.gov/blast) and Coronaviruses cDNA sequences were analyzed using www.ncbi.nlm.nih.gov/protein. MEGA-X software was used for phylogenetic comparison. Promer design was done using NCBI Primer Design Software. The primers were further analyzed by Oligoanalyzer 3.2 software. Proteins were compared by Multalin protein homology software and CLUSTAL-Omega Software. NCBI BLAST seq-2 software was used to check the correct homology between two protein sequences. NCBI pubmed (www.ncbi.nlm.nih.gov/pubmed) used to retrieve papers [18,34,35]. The structure and localization of Corona proteins were demonstrated in figure 1A and different types of methyl transferases were shown in figure 1B.

There are many rRNA methyltransferases in bacteria as well as eukaryotes. We have previously shown that Rlm-type, Erm-type and Cfr-type rRNA methyl transferases have poor similarities to the Nsp9, Nsp10, Nsp13, nsp14 and Nsp16 [16-19]. The classification of *Escherichia coli* rRNA methyltransferases was shown in figure 1B. The 23S methyl transferases (Cfr and ErmB) are different than 16S methyltransferases (Rlm, ArmA) and methylations have occurred sequence specific [16]. Thus, possibility of five Coronavirus rRNA methyltransferases controlling 21S rRNA methylation in human cells may be significant controlling host protein synthesis. There are two ribosome subunits, 30S and 50S in bacteria where as 40S and 60S in human. Such subunits are made up 20 - 40 small RNA binding proteins and rRNAs were involved in the ribosome assembly process [17]. Thus, minor similarities to the Coronavirus proteins to the *Escherichia coli* ribosomal proteins may be important to find new control measure for coronavirus life cycle and pathogenicity.

Figure 1: Structure and position of corona virus and Nsp7/8 (A) and diversities of bacterial rRNA methyltransferases (B).

Results

BLAST and CLUSTAL-Omega software programmes have been shown very powerful to compare unknown DNA and protein sequences as vast number of gene sequences have deposited in the GenBank. Similarly, 3-D crystal structures and functional domains of protein sequences are known (www.pdb.com). Figure 2 disclosed the multialign-phylogenetic analysis of fifty DNA/RNA modifying genes with Nsp7 and Nsp8 non-structural proteins of Corona

virus and the functions of such proteins were still elusive. It was clearly indicated that Nsp7 share a homology with rlmB rRNA methyltransferase and Nsp8 with S30 transposase and bin recombinase. Figure 3 showed the multi-alignment and phylogenetic relation of Nsp7 and Nsp8 with fifty four *Escherichia coli* ribosomal proteins. It is clearly indicated that Nsp7 has a relative homology to S2 and Nsp8 to S8 ribosomal proteins of *Escherichia coli*. The homology described here is low but it was good to describe the unknown viral proteins relations to host important enzymes and such data may give new insight into drug design. Moreover, such similarities were disclosed by us for other non-structural proteins of Corona virus [16-19]. The frontline researcher will be utilized for biochemical research with COVID-19 transcription and translation for new drug design.

Figure 3: Multi-alignment phylogenetic relations among RNA/DNA modifying proteins and Nsp7/8 proteins of corona virus. Red arrows indicated the very homology positions.

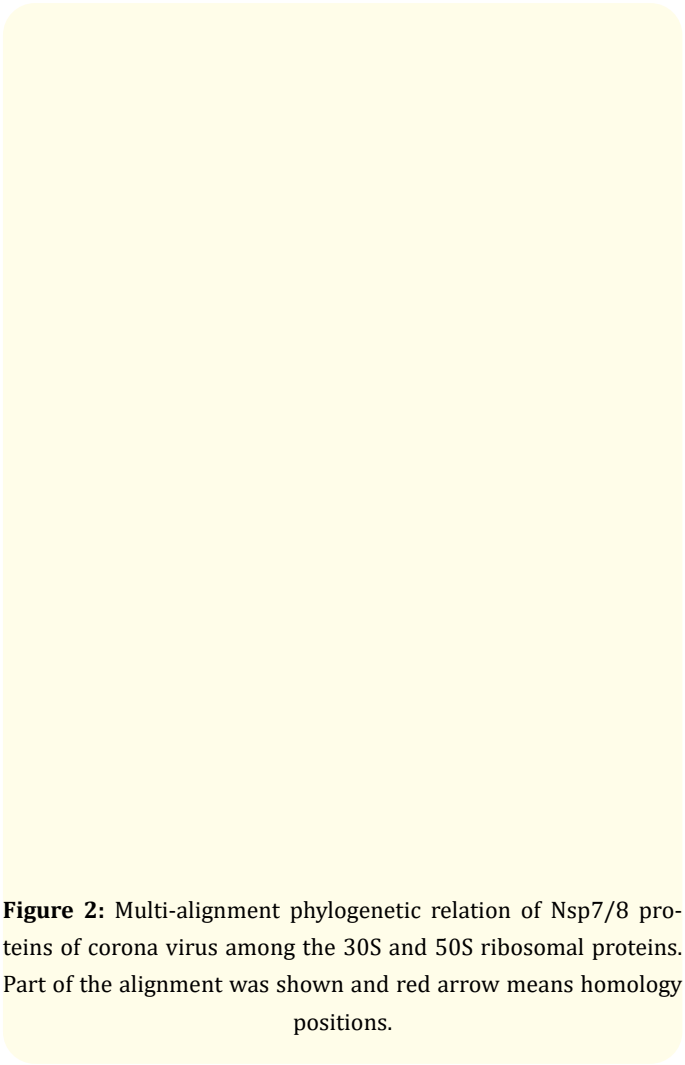

Figure 2: Multi-alignment phylogenetic relation of Nsp7/8 proteins of corona virus among the 30S and 50S ribosomal proteins. Part of the alignment was shown and red arrow means homology positions.

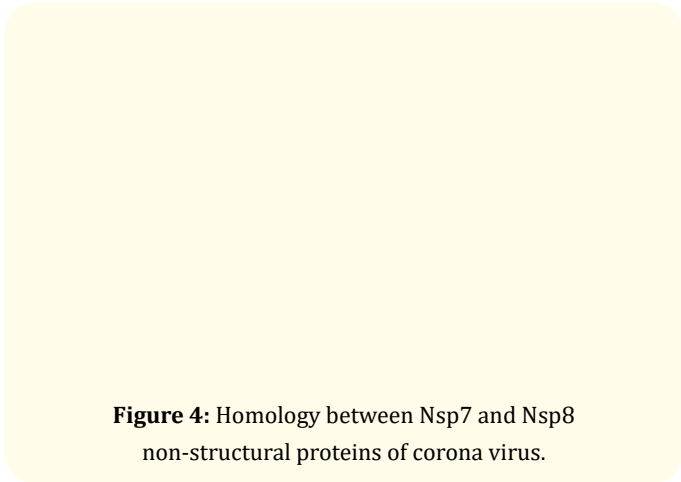

Figure 4: Homology between Nsp7 and Nsp8 non-structural proteins of corona virus.

Interestingly, we found some homology between Nsp7 and Nsp8 (Figure 5) and we also showed homology of Nsp7 with 144-226aa of RlmD rRNA methyltransferase (A) as well as with 136-218aa of RlmB (B) (Figure 6). Similarly, we found some minor homology of Nsp7 to Is2 transposase indicating its progenitor (Figure 7). Figure 8 demonstrated similar minor homology of Nsp8 with RlmH methyltransferase of Escherichia coli. Further we studied the similarity index of S2 30S ribosomal protein and L1 50S ribosomal protein with Nsp8 protein of Corona virus (Figure 9) and also a similarity index observed for IS30 transposase with Nsp8 protein (Figure 10). Such similarities of Coronavirus proteins with ribosomal proteins as well as rRNA methyltransferases were not documented in the literature. Previously we documented new findings into the Preprint Servers [17,18] and also have published in Journals [16,19].

Figure 5: Homology between Nsp7 protein of corona virus and S8 ribosomal protein of *E. coli.*

Figure 6: Homology of Nsp7 protei with rRNA methyltransferases. (A) with 144-226aa of RlmD rRNA methyltransferase, (B) with 136-218aa of RlmB (COOH-terminas).

Figure 7: Minor homology of nsp7 protein with 44-127aa of IS2 transposase.

Figure 8: Minor homology between nsp8 protein and RlmH rRNA methyltransferase of *E. coli.*

Figure 9: Minor homologies of Nsp8 protein with L1 and S2 ribosomal proteins of *E. coli.*

Figure 10: Homology between Nsp8 protein and IS30 transposase of *E. coli.* N mean direct homology or similar type amino acids.

Discussion

Thus, we have shown by bioinformatics approach that Nsp7 and Nsp8 may be RlmB/H-like rRNA methyltransferases. RlmB methyltransferase (protein id. BAI33654) modifies G2251 of 23S rRNA and RlmH performs the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to the nucleotide at position m3ψ1915 of *Escherichia coli* 23S rRNA [36,37]. Therefore, *in vitro* biochemical tests needed to confirm our hypothesis. Eukaryotic translation is complex and ribosomal assembly is a complex association of rRNAs and 25 - 35 ribosomal proteins. We predicted that host mitochondrial protein assembly might be affected by Corona virus infections where such proteins (Nsp8, Nsp9/10, Nsp13, and Nsp16) drastically methylated the 21S rRNA at different positions to inhibit host protein synthesis like Cox-1 and CoxII ETC proteins inhibiting further oxidative phosphorylation [17-19]. We argue that although our hypothesis has weak similarities but genuinely, we found multiple rRNA methyltransferases which must have effect on rRNA methylation which might favour Corona virus structural protein synthesis [3,5,38,39]. Many methyltransferases have multiple sites specificities implicating RlmA-N methyltransferases have role in protein synthesis and drug resistance [30]. Many RNA viruses have capping methyltransferases that favours viral protein synthesis over host protein synthesis [29].

Conclusion

Bioinformatics analysis is a modern research to find similarities among unknown protein specifically viral origin. COVID-19 has created a problem in society that never was anticipated before challenging our scientific endeavour. It is true 7000 millions peoples in this Earth need some remedy for Corona virus infections but vaccine was far away. India is in grief now as Corona virus infections are increasing steadily even strong long down for months. Worldwide millions infections and > 300000 deaths means huge loss of society. Few millions have lost jobs during lock down and most school and colleges were closed impacting social reforms and prosperity. Humanity greatly suffers due to lock down, musk in mouth and nose and no medicine approved yet. Thus, we agree our present report is bioinformatics work but has some merit to the front line worker who has no time for rigorous analysis of databases. We need drug against Corona virus and need now as > 300000 deaths with > 4 millions infections and hospitalization creating a worldwide cry for PPE appliances. Genetic drugs, herbal products and antibiotics must be discovered to control Corona virus pandemic [40].

Acknowledgement

I thank WHO and CNN for updating the fear of Corona virus today and also thank our Prime Minister Sir Narendra Modi to inspire doctors and scientists to work on Coronavirus of any kind to save mankind.

Ethical Issues

No patient was used in the study.

Conflict of Interest

Author has no conflict of interest.

Funding

Lock down research from Kolkata home using my own computer.

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