

Unusual Enzymes in Corona Virus Genome and their Roles in Pathogenicity Control and Drug Design

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Received: July 08, 2020; **Published:** August 08, 2020

Abstract

Corona virus pandemic has threatened entire human population of this earth and within few months > 10 millions infections and > 600000 deaths and human activities have totally suffered due to lock down. Corona virus RNA-dependent RNA polymerase (nsp12), proteases (Nsp3 and nsp5) have been targeted for drug design as well as spike protein (S) and nucleocapsid protein (N). I predicted non-structural polyprotein-derived RNA topoisomerase (nsp2) and six rRNA methyltransferases (nsp8, nsp9, nsp10, nsp13, nsp14 and nsp16) as therapeutic targets. Previously nsp13 was published as RNA helicase, nsp16 as 2'-O-capping methyltransferase and nsp14/nsp15 as ribonucleases. We found all Corona virus enzymes have weak similarities to different *Escherichia coli* ribosomal proteins and might be inhibitory to host ribosome turnover, protein synthesis and oxidative phosphorylation. Nsp2 RNA topoisomerase might be important for drug design and vaccine development. However, antisense, ribozyme and nanotech drug designs could be beneficiary to those patients having very high virus load.

Keywords: Corona Virus; Pathogenicity Control; Drug Design

Introduction

Corona virus pandemic has created a horror worldwide with more than few million infections and > 600000 deaths [1,2]. Most importantly it has affected severely the United States of America and United Kingdom and now is rapidly spreading in Brazil, India and Russia. There are more than thirty thousand deaths in Italy, Spain, France as well as more than 15000 in India, Iran and Canada. No novel drug has been discovered against this ~30 kb ss-RNA virus (Figure 1) and clinical trials on vaccines have been initiated [3-6]. Most of the population in this Earth are suffering from lock down where school, college, cinema, restaurant are closed and travel industries like aeroplane, train, bus, metro are also suspended in most cities. In this situation rapid research efforts are ongoing to find a new target for drug design against this deadly respiratory disease.

I work on herbal drug design against multi-drug resistant bacteria and I have collected many MDR clones of *Escherichia coli* from Ganga River water as well as from human and animal sources [7-9]. I have also isolated many bioactive phyto-chemicals from household Indian medicinal plants [10]. During lock down, all my ingredients were lost as my research laboratory was 125KM away from my Kolkata residence. I was in panic and was very angry and hopeless. I thought that it was my death as I was 62 year old and no chance to recover work. I murmured myself, "I will kill Corona virus that is responsible for catastrophic situation worldwide"!

Astonishingly, I noticed that 2/3 of the RNA genome of Corona virus expressed two polyproteins in same reading frame (orf1a=4405aa and orf1ab=7096aa; accession no. MT049951) that later were cleaved into 16 polypeptides like very famous proteins, RNA-dependent

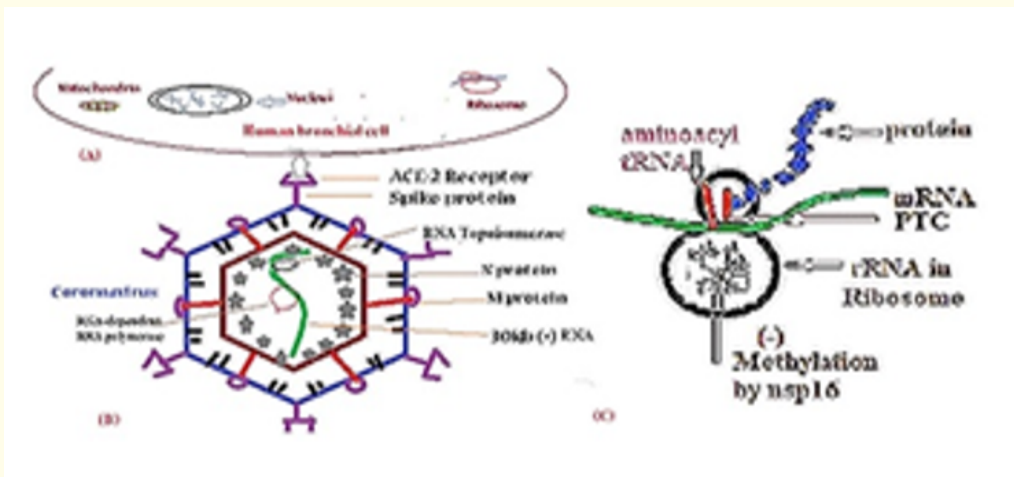


Figure 1: (A) Human bronchial cell and structure of 200 nm size Coronavirus (B). Coronavirus binds to ACE receptor causing abnormal synthesis of aldosterone lowering blood pressure and Na⁺ ion in cells. (C) The RlmE methyl transferase methylates 21S RRNA of human mitochondria causing the inhibition of mitochondrial protein synthesis and may stop oxidative phosphorylation [3].

RNA polymerase (nsp12), C3 protease (nsp3) and papain-like protease (nsp5) [11-13] (Figure 2). Rest 1/3 of the 3'-prime of the genome produced structural proteins like S, N, M, C and orf3A, orf8 etc [14-17]. Spike protein was important target as such protein bound to ACE-2 receptor of lungs that was the major target for Corona virus entry and pathogenesis [18]. Further I understood nsp16 designated as 2'-O-ribose methyltransferase and Nsp13 as RNA helicase. Nsp6 and Nsp7/8 suggested as assessor proteins of replication and Nsp14/15 as ribonucleases. But functions of 638aa long nsp2 was not known then! I will discuss in this minireview my efforts to know the functions of Coronavirus non-structural proteins like nsp2, nsp8-10 and nsp13-16 proteins by protein homology search [2,3].

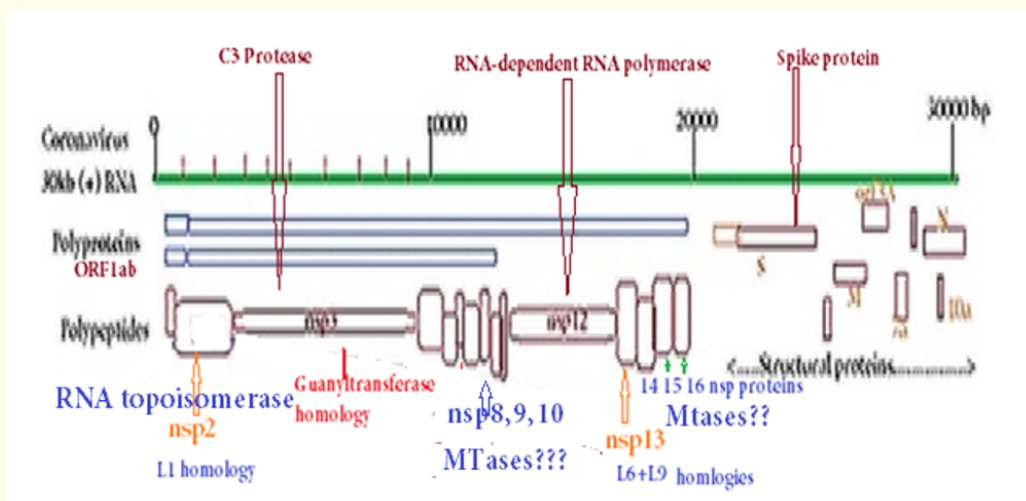


Figure 2: Corona virus genome and different non-structural proteins (nsp1-16) and structural proteins (S, N, M, C) [2].

Result

My research background was Kinetoplastida DNA topoisomerase (PhD thesis) and I have also worked on HIV/HasV retroviruses as well as HBV DNA virus genome's study [19-22]. Recently, I have written a review on methyl-transferases and also on plasmid-mediated recombinases-transposases (not published yet). So, from similarity search, I found nsp16 is a 2'-o-ribose uridine methyltransferase, not a capping enzyme [23,24]. Suddenly, it turned out nsp13 RNA helicase was also a rRNA methyl transferase (it resembles capping methyltransferase) and has similarities (< 25%) to the ribosomal proteins L6 and L9 [2,25] (Figure 3). I proposed that ribosomal turn over and protein synthesis might be inhibited by non-structural proteins those have slight similarities to the different ribosomal proteins of *E. coli* and thus mitochondrial protein synthesis and oxidative phosphorylation might be inhibited causing low ATP in blood and platelets aggregation as well as blood clotting [27].

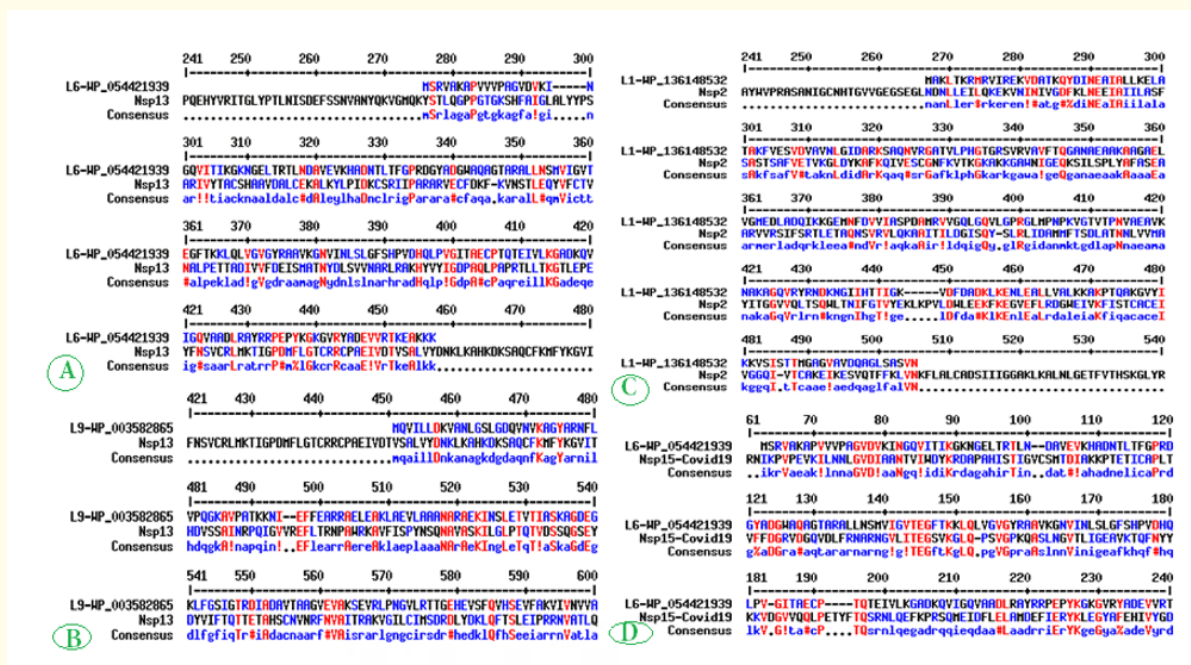


Figure 3: (A) Homology between ribosomal L6 protein and 275aa-453aa of Nsp13 protein of Coronavirus. (B) Homology between ribosomal L9 protein and 457aa-5596aa of Nsp13 protein of Coronavirus. (C). Homology between ribosomal L1 protein and Nsp2 protein (RNA Topoisomerase) of Coronavirus. (D). Homology between ribosomal L6 protein and Nsp15 protein of Coronavirus [2].

Nice hypothesis but how I will kill the Corona virus? Well, this is hard part and leading scientists are working on that. But I devised few RT-PCR primers and few peptides for vaccine development. Who will believe the hypotheses as have published in low priority journals! In my opinion, there are six rRNA methyltransferases (Nsp8, nsp9, nsp10, nsp13, nsp14, nsp16) and drugs could be designed like antisense, ribozyme or gene therapeutics to retard the Corona virus life cycle (Figure 2)!

My second story was however, more interesting! I found nsp2 protein had similarities to the *Vibrio haemolytica* DNA topoisomerase I and DNA topoisomerase IV whereas chimera of topoI and topoIV could align better [28]. As the homology % is low, I searched database and it was appeared *Marinobacter* and *Desulfococcus* genus topoisomeraseI might be more precedent. Again, I compared the gyrA and gyrB, transposase and DNA primase etc and I got few homologies which predicted the different domains of predicted RNA topoisomerase in Corona virus [29] (Figure 4). So, it could be a target and now I have ordered a company for nsp2 protein but sadly it has not

made yet! Well, unique bi-subunit Kinetoplastida DNA topoisomerase IB has some similarities to small subunit and large subunit. I knew anti-leishmanial drug sodium stibogluconate inhibits *Leishmania* topoisomerase I. Thus, I proposed that pentostam might be also inhibit Corona virus replication as also camptothecin drug which was inhibitory to Trypanosoma topoisomerase IB [20] (Figure 4). Interestingly, bacterial or viral TopoI inhibitors were not available but recently I found a phyto-chemical (NU-2) from *Suregada multiflora* inhibited *E. coli* Topoisomerase I but others data were lacking like gyrase or human topoisomerase data due to lack of funding (unpublished)! So in my opinion NU-2 could be studied further for Corona virus control if it might be inhibited nsp2 RNA topoisomerase [10]!

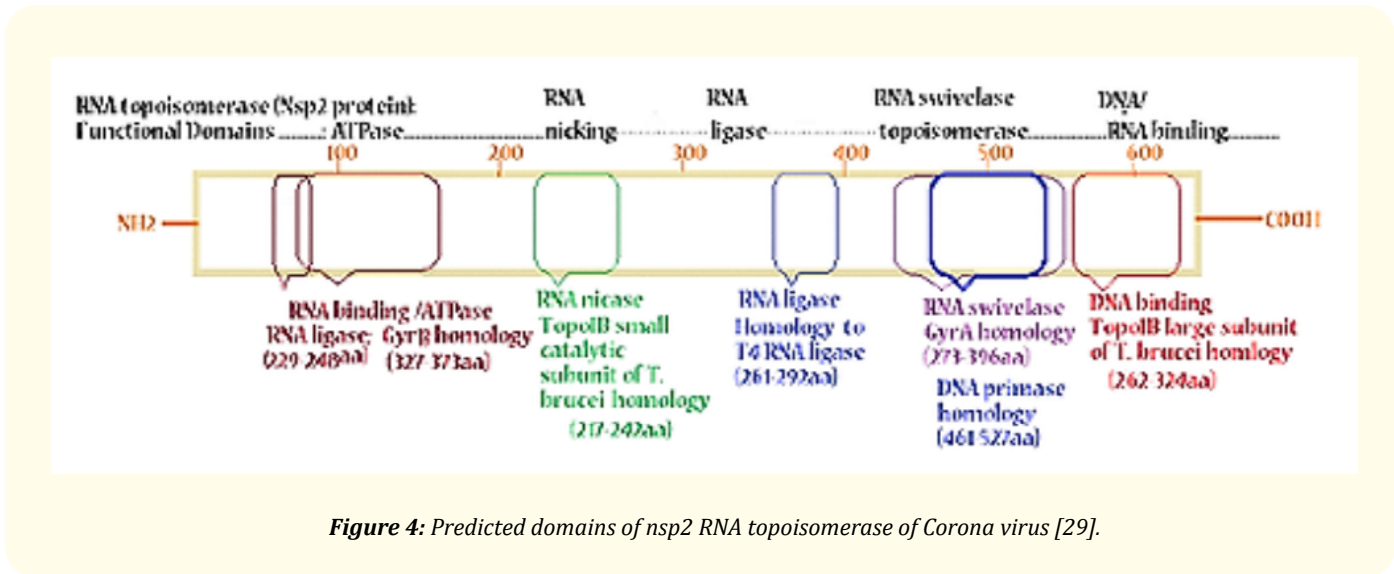


Figure 4: Predicted domains of nsp2 RNA topoisomerase of Corona virus [29].

Discussion and Conclusion

So far vaccine development was projected for corona control. Some researcher predicted hydroxyl-chloroquine and others erythromycin or dexamethasone or remdesivir against Corona virus pathogenesis. My lock down bioinformatics research may through some high lights for Corona virus new drug discovery. Why Corona virus non-structural proteins have similarities (< 25%) to bacterial ribosomal proteins was not known at this point? However, such information may be valuable for drug design [30]. Many rRNA methyltransferase in Corona virus is very important but biological assays on purified enzymes must be done. Sadly, I am unable to study live Corona virus to test my hypotheses based on protein homology study and computer-based model study may be beneficial.

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Volume 4 Issue 9 September 2020

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