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Structural similarity-based prediction of host factors associated with SARS-CoV-2 infection and pathogenesis

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ABSTRACT

The current pandemic resulted from SARS-CoV-2 still remains as the major public health concern globally. The precise mechanism of viral pathogenesis is not fully understood, which remains a major hurdle for medical intervention. Here we generated an interactome profile of protein-protein interactions based on host and viral protein structural similarities information. Further computational biological study combined with Gene enrichment analysis predicted key enriched pathways associated with viral pathogenesis. The results show that axon guidance, membrane trafficking, vesicle-mediated transport, apoptosis, clathrin-mediated endocytosis, Vpu mediated degradation of CD4 T cell, and interferon-gamma signaling are key events associated in SARS-CoV-2 life cycle. Further, degree centrality analysis reveals that IRF1/9/7, TP53, and CASP3, UBA52, and UBC are vital proteins for IFN-γ-mediated signaling, apoptosis, and proteasomal degradation of CD4, respectively. We crafted chronological events of the virus life cycle. The SARS-CoV-2 enters through clathrin-mediated endocytosis, and the genome is trafficked to the early endosomes in a RAB5-dependent manner. It is predicted to replicate in a double-membrane vesicle (DMV) composed of the endoplasmic reticulum, autophagosome, and ERAD machinery. The SARS-CoV-2 down-regulates host translational machinery by interacting with protein kinase R, PKR-like endoplasmic reticulum kinase, and heme-regulated inhibitor and can phosphorylate eIF2a. The virion assembly occurs in the ER-Golgi intermediate compartment (ERGIC) organized by the spike and matrix protein. Collectively, we have established a spatial link between viral entry, RNA synthesis, assembly, pathogenesis, and their associated diverse host factors, those could pave the way for therapeutic intervention.

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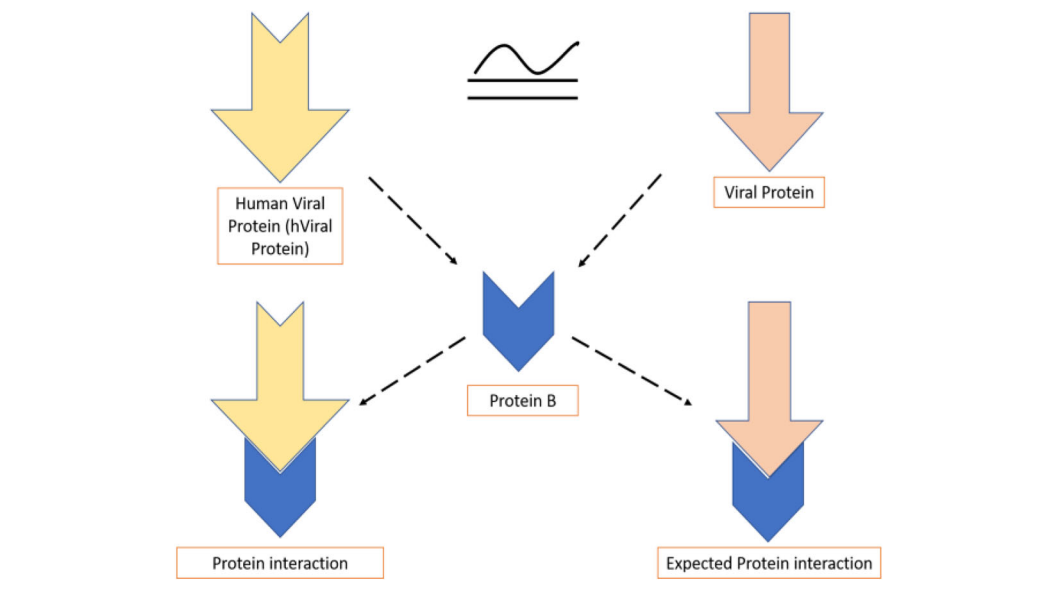

Abbreviations: ACE2: Angiotensin-converting enzyme 2; TMPRSS2: Transmembrane protease serine 2; CLTC: Clathrin heavy chain 1; DNM2: Dynamin-2; AP2A1: AP-2 complex subunit alpha-1; AP2A2: AP-2 complex subunit alpha-2; AP2B1: AP-2 complex subunit beta; AP2M1: AP-2 complex subunit mu; DAB2: Disabled homolog 2-interacting protein; ACTA1: Actin, alpha skeletal muscle; ATP2C1: Calcium-transporting ATPase type 2C member 1; ACTB: Actin, cytoplasmic 1; ACTC1: Actin, alpha cardiac muscle 1; DNM1: Dynamin-related protein DNM1; DNM3: Dynamin-3; DYNC1H1: Cytoplasmic dynein 1 heavy

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chain 1; RAB5: Ras-related protein Rab-5A; RAB5B: Ras-related protein Rab-5B; RAB5C: Ras-related protein Rab-5C; RAB11B: Ras-related protein Rab-11B; RAB13: Ras-related protein Rab-13; RAB7A: Ras-related protein Rab-7a; RAB7B: , Ras-related protein Rab-7b; RAB7L1: Ras-related protein Rab-7L1; VPS11: Vacuolar protein sorting-associated protein 11 homolog; VPS33A: Vacuolar protein sorting-associated protein 33A; SEC61: Protein transport protein Sec61 subunit alpha isoform 1; VAPB: Vesicle-associated membrane protein-associated protein B/C; COPA: Coatomer subunit alpha; ARCN1: Archain 1; COPB1: Coatomer subunit beta; GBF1: Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1; VAPA: VAMP Associated Protein A; STX17: Syntaxin-17; TMED1: Transmembrane P24 Trafficking Protein 1; ATG12: Ubiquitin-like protein ATG12; ATG16L1: Autophagy Related 16 Like 1; ATP13A2: ATPase Cation Transporting 13A2; BECN1: Beclin-1; C9ORF72: C9orf72-SMCR8 Complex Subunit; MAP1LC3A: Microtubule Associated Protein 1 Light Chain 3 Alpha; MAP1LC3B: Microtubule Associated Protein 1 Light Chain 3 Beta; EDEM1: ER Degradation Enhancing Alpha-Mannosidase Like Protein 1; OS-9: OS9 Endoplasmic Reticulum Lectin; DDX3Y: DEAD-Box Helicase 3 Y-Linked; IGF2BP1: Insulin Like Growth Factor 2 MRNA Binding Protein 1; GCN1L1: GCN1 Activator Of EIF2AK4; LARP: La Ribonucleoprotein 1, Translational Regulator; NCK1: NCK Adaptor Protein 1; ANPEP: Alanyl Aminopeptidase, Membrane; COPG1: COPI Coat Complex Subunit Gamma 1; COPG2: COPI Coat Complex Subunit Gamma 2; ERGIC1: Endoplasmic Reticulum-Golgi Intermediate Compartment 1

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes a rainbow of diseases, ranging from flu-like symptoms to pneumonia, acute respiratory distress syndrome (ARDS), thrombosis, and fatal consequences (Tay et al., 2020). SARS-CoV-2 is a Coronaviridae family member that encapsulates a positive-sense, single-stranded RNA genome. Six human coronaviruses (HCoVs) are identified earlier; these are HCoV-229E and HCoV-NL63 belonging to alpha coronavirus group. The rest members, HCoV-HKU1, HCoV-OC43, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) belong to beta-coronavirus group (van der Hoek, 2007). However, over the past few years, highly pathogenic human coronaviruses have emerged. The appearance of SARS-CoV in 2002 exhibited 8,000 cases worldwide with mortality of ~10% and MERS-CoV in 2012, marked with 2,500 cases with a higher mortality rate of 36% (Graham et al., 2013; Ksiazek et al., 2003). Although the mortality rate of current SARS-CoV-2 is comparatively low, it is exceptionally contagious in nature. The SARS-CoV-2 mainly spread through aerosolized droplets, but also can be transmitted by direct contact and oral-fecal route (Liu et al., 2020). Lack of efficacious vaccine or antivirals is a measure concern for SARS-CoV-2 infection. The use of some antiviral drugs like remdesivir, lopinavir plus, and symptom-based management are currently available options for COVID patients (Wang et al., 2020). Therefore to develop new therapeutic interventions, a better understanding of the virus biology and host-pathogen interactions is necessary.

Our study involves implementing a computational method for predicting the interactions between SARS-CoV-2 and host proteins. The approach is based on protein structural similarity. At first, we determined the structural similarities between SARS-CoV-2 and human proteins using an established method by analyzing protein crystal structures. Further, we identified known interactions for these SARS-CoV-2 similar human proteins. We assumed that these interacting proteins of SARS-CoV-2 similar proteins would also interact with the SARS-CoV-2 proteins. This approach is also reported earlier for the prediction of HIV, Chandipura virus (CHPV), chikungunya virus (CHIKV), and dengue virus (DENV)-human interactions (Doolittle & Gomez, 2010, 2011; Rajasekharan et al., 2013; Rana et al., 2013). We predicted and shortlisted an interaction map for SARS-CoV-2 and host proteins using cellular co-localization information. We then validated these interactions by using previously published host factors datasets associated with coronaviruses and other RNA viruses (Gordon et al., 2020; Pfefferle et al., 2011; Xiong et al., 2020). The predicted interactions are highlighted based on their functional importance during SARS-CoV-2 infection and prioritized based on other related RNA viruses' available information. In the similar line we also predicted the host complement system driving the coagulation process in SARS-CoV-2 infection (Tiwari et al., 2020). While, in this study, these interacting proteins revealed that SARS-CoV-2 might use the clathrin-mediated endocytosis pathway for its entry. We also delineated the interplay of host proteins associated with the SARS-CoV-2 life cycle. We primarily focused on viral genome replication, translation, assembly, and predicted the pathways and the host factor requirements. Gene enrichment analysis of these interacting proteins reveals that apoptosis, IFN–γ signaling, and proteasomal degradation of CD4 T cells are highly associated with SARS-CoV-2 pathogenesis.

Material and method

Data sources

The PDB files for the crystal structures of SARS-CoV-2 were obtained from the two primary sources: RCSB PDB [main protease (PDB ID:5R7Y), spike glycoproteins (PDB ID: 6VSB, 6VXX), HR2 domain (PDB ID:6LVN), NSP15 (PDB ID:6VWW), NSP3 (PDB ID:6W02) and NSP9 (PDB ID: 6W4B)] and from Zhang Lab (modeled these structure by using I-TASSER) (Zhang et al., 2020). The PBD files were uploaded to the DaliLite v.5 webservers for structural similarities identification (Holm, 2019). The Dali server provides the PDB codes as an output file mapped to their corresponding Uniprot ID and gene name by DAVID Gene ID conversion or Uniprot ID mapping (Dennis et al., 2003; The Uniport Consortium, 2019). Reactome and g: Profiler database was used to perform the gene enrichment analysis, whereas the network interactions

were analyzed in Cytoscape software (Fabregat et al., 2017; Otasek et al., 2019).

Determination of structural similarity between SARS-CoV-2 and human proteins

The Dali server uses the alpha carbon distance matrix, allowing them for differences in domain order and produces a structural similarity score (z) to identify the structurally similar human protein (Holm, 2019). For the current study, we have used the cutoff z score of 2.0. The human protein showing the result above the cutoff score was selected and referred to as 'SARS-CoV-2 similar' proteins.

Interaction prediction

The SARS-CoV-2 proteins' interacting partners were determined by identifying the interaction partner for 'SARS-CoV-2 similar' proteins. We mined BIOGRID, HPRD, and MINT database to find the endogenous target proteins (Chatr-Aryamontri et al., 2007; Goel et al., 2012; Oughtred et al., 2019). These databases are sources of literature-curated interaction partners of human proteins. The assumption is that the human protein that interacts with 'SARS-CoV-2 similar' proteins will also interact with the SARS-CoV-2 viral proteins.

Cellular compartmentalization (CC) and GO enrichment analysis

The identified host protein interactors of the SARS-CoV-2 proteome were shortlisted based on their localization and associated functions. Therefore, the interactor host proteins must share at least one cellular compartment with the viral protein for direct communication. The information regarding cellular compartmentalization protein–protein interactions is available in UniProt database, curated by published literature. The host and viral protein sharing at least one cellular compartment were identified, and later, these proteins were submitted to the g:profiler for gene enrichment analysis (Fabregat et al., 2017). The Reactome functional annotation charts and human protein atlas organized as a tree structure. As the distance from root increased, the terms were supposed to be more specific. The Bonferroni procedure was used to obtain the corrected p-values, and these values were then plotted in -log10 terms for graphical representation of data. The identified proteins were imported to Cytoscape software for further analysis (Otasek et al., 2019). STRING and CytoNCA tool in Cytoscape was used for network generation and degree centrality calculation (von Mering et al., 2003).

Validation of predictions

The Dali server provides the PDB ID that was converted to the Uniport ID. We observed many duplicates in the datasets during the analysis, as multiple PDB structures are available for the same protein, hence leading to repetition in the interaction predictions. Therefore, we removed the duplicate copies by analyzing the prediction based on a single pair of individual Uniprot accessions and SARS CoV-2 protein names. For better understanding, a SARS-CoV-2 protein is labeled with the protein name, while Entrez Gene ID was assigned for the host protein. For further validation of the output data, we also compared the predicted datasets with published experimental results carried out for SARS-CoV-2 and other related RNA viruses.

Results

Identification of SARS-COV-2 host interactor proteins and gene enrichment analysis

The initial study identified 3735 human proteins showing similarity to 16 SARS-CoV-2 proteins. These SARS-CoV-2 similar proteins were predicted to interact with 12,872 (endogenous interaction) human proteins through 57,359 unique interactions. To filter out the noise and curate the data, we performed CC analysis. This resulted in finding the involvement of 6877 proteins through 19,047 unique interactions. Interestingly about 35% of resultant proteins are already reported to have some role in the pathogenesis of other coronavirus and RNA viruses. Next, we performed gene enrichment analysis by using g:GOSt tool of g:Profiler. For this, we uploaded the SARS-CoV-2 interactors protein lists (CC filtered) in g:GOSt tool and manually selected 'Reactome database' and 'Human protein atlas database' as data source option and set the threshold value at 0.05. The g:Profiler gave us a list of associated pathways and their adjusted p value for the input gene list. Subsequent Reactome dataset analysis revealed that SARS-CoV-2 interactors proteins enriched with the terms of axon guidance, membrane trafficking, vesicle-mediated transport, programmed cell death, apoptosis, etc. (Figure 1(a)). Similarly, the HPA database showed a higher expression of human cells for SARS-CoV-2 interacting human protein (Figure 1(b)).

SARS-CoV-2 use clathrin-mediated endocytosis for its cellular entry

The productive viral infection begins only after virions get access to a highly specific entry pathway. This allows the viral components to enter into the host cytoplasm for the following processes, virus uncoating, gene expression, genome replication, virion assembly, and nascent virion release. Most of the viruses accomplish this task via endocytosis. Recently, it was reported that host proteins ACE2 and TMPRSS2 are essential for SARS-CoV-2 entry. In the present study, we found that SARS-CoV-2 spike (S) interacts with several other host proteins for its entry through clathrin-coated pits. These include CLTC, DNM2, AP2A1, AP2A2, AP2B1, AP2M1, and DAB2. This result suggested that the SARS-CoV-2 internalization is dependent on clathrin-mediated endocytosis. Overall the SARS-CoV-2 entry process is very similar to that of SARS-CoV, HCV, and VSV (Blanchard et al., 2006; Inoue et al., 2007; Sun et al., 2005). The host proteins such as AP2A1, AP2A2, AP2B1, and AP2M1 are the subunits of the heterotetrameric adaptor protein complex AP2, while DAB2

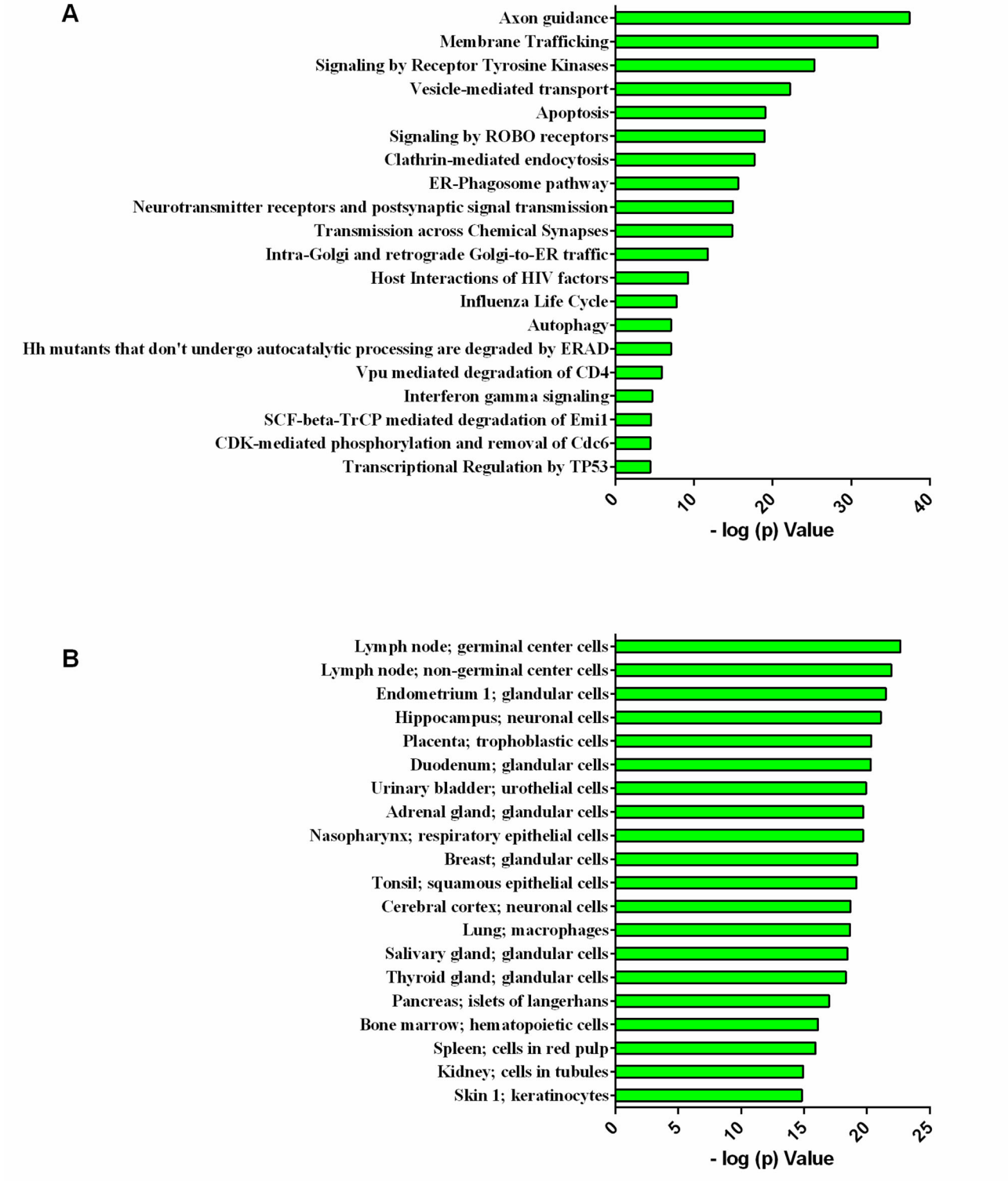

Figure 1. Gene enrichment analysis of SARS-CoV-2 interactor human proteins. (A). Enriched biological pathways obtained from reactome database (B). Cellular expression data of interacting protein obtained from HPA. Bonferroni corrected p-values were transformed by –log10. (The bar graphs were created by using GraphPad-Prism software.)

is present on the clathrin-coated vesicles. These host proteins continuously construct clathrin-coated pits and have a crucial role in the entry of SARS-CoV and MHV viruses (Eifart et al., 2007; Inoue et al., 2007). The cytoskeletal machinery containing actin and microtubule is essential for the entry process of the mouse hepatitis virus (MHV) (Burkard et al., 2014). This machinery is recruited through endocytosis after the coat formation, which triggers cell membrane deformation and virion internalization. The clathrin components, such as, actin, and microtubules complex, when associated with dynamin

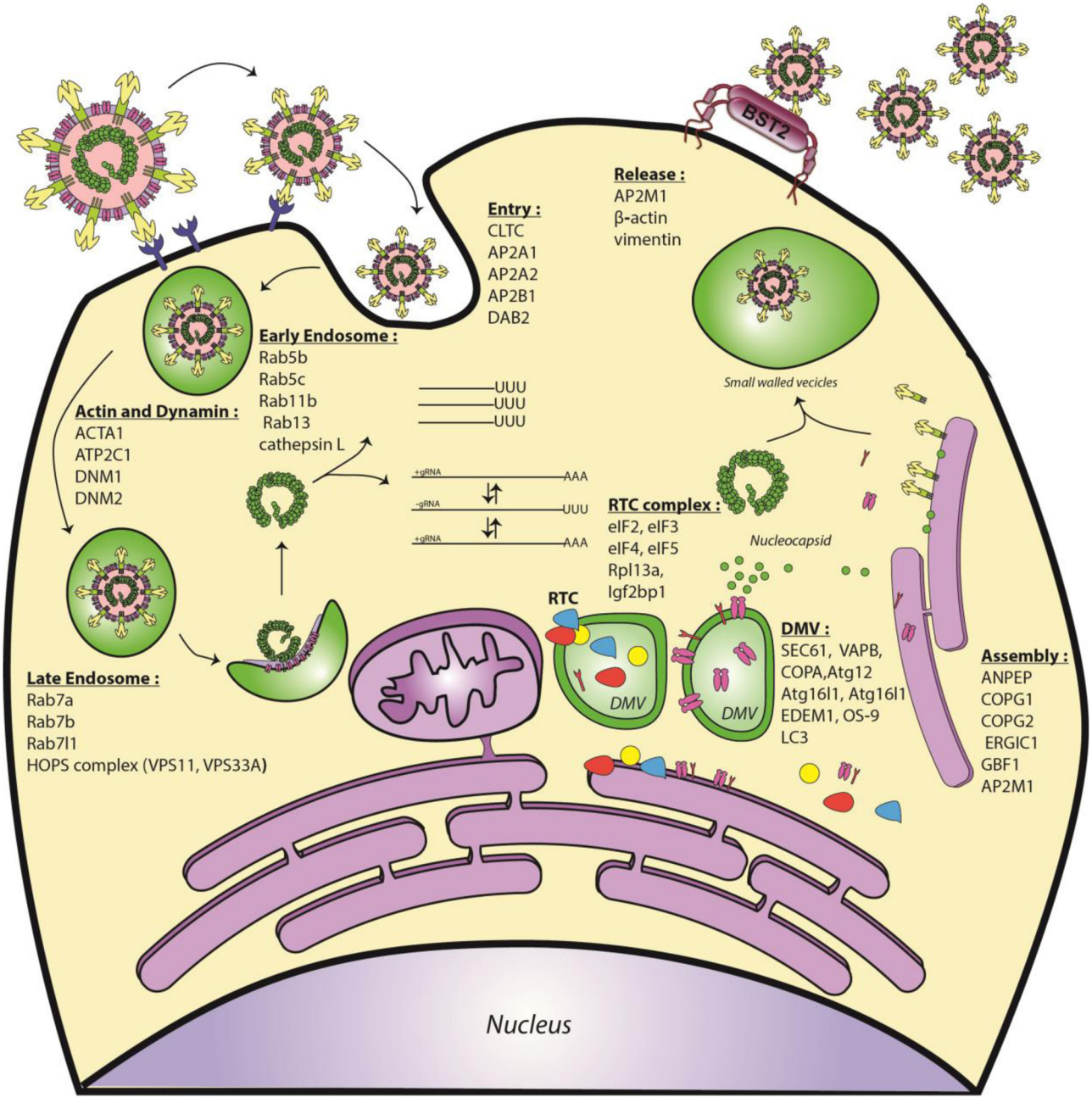

Figure 2. Schematic representation of the SARS-CoV-2 life cycle and its associated host proteins. This cartoon depicts endocytotic pathway for viral entry, followed by uncoating and release of viral genome to the host cell cytoplasm. Subsequently, viral RNA is associated with membrane of the replication and transcription complex (RTC) and double-membrane vesicles (DMVs) during the viral genome replication and transcriptions.

proteins, result in the activation of binding sites for several other proteins. For example, cortactin (CTTN) and intersectin 1 (ITSN1) can activate the Arp2/3 complex of the actin cytoskeleton (Uruno et al., 2001). This leads to enhanced localized polymerization of actin and microtubules, favoring virus internalization. Here, SARS-CoV-2 glycoprotein is predicted to interact with CTTN and ITSN1 along with various types of actin (ACTA1, ATP2C1, ACTB, and ACTC1), dynamin (DNM1, DNM2, and DNM3) and microtubules (DYNC1H1) molecules. Together, these components facilitate clathrin-mediated endocytosis of virion and also crucial for the intracellular trafficking of viral components during the virion assembly process (Lamason & Welch, 2017). Our data suggest that the interaction of SARS-CoV-2 spike (S) protein with Rab5b and Rab5c. It is established that upon clathrin-mediated uptake, SARS-CoV-2 is trafficked to the early endosomes in a RAB5-dependent manner (Mire et al., 2010). The S protein also interacts with Rab11b and Rab13, associated with the early endosome, and regulates the recycling of receptors and other ligands to the membrane (Urbe et al., 1993). After endocytosis, the lysosomal protease cathepsin L cleaves the S protein in the early endosome. Thus, initiating fusion of the viral envelope with the endosome membrane (Huang et al., 2006). The S protein interacts with the late endosomal proteins (RAB7A, RAB7B, and RAB7L1) for lysosome maturation (VPS11 and VPS33A) proteins. The HOPS complex regulates the late endosomes to the lysosomal maturation process (Figure 2) (Balderhaar & Ungermann, 2013). Although

the HOPS complex does not affect the infection process of other viruses such as VSV and IAV, viral entry is more dependent upon low pH conditions. While in the case of SARS-CoV-2, low pH is not sufficient to trigger virus entry. Hence, it appears that the HOPS complex could play a significant role in comparison to the pH factors during the SARS-CoV-2 entry process.

Intracellular replication and assembly of SARS-CoV-2

Host proteins involved in SARS-CoV-2 translation

Upon cellular entry and uncoating, the viral RNA serves as a transcript for the cap-dependent translation of ORF1a, ORF1b, and produce polyproteins (pp1a and pp1ab). The autoproteolytic cleavage of both polyproteins generates 15–16 nonstructural proteins (NSPs) associated with various functions. Simultaneously, the host mounts innate immune response by augmenting antiviral protein synthesis primarily orchestrated by type-I interferon (IFN-I) signaling mechanism. To counter this, viruses (including + ve sense RNA viruses) modulate host protein synthesis by restricting the host mRNAs translation and favoring viral protein synthesis.

Host mRNA translation is initiated by forming the heterotrimeric eIF2 complex comprising eIF2α, eIF2β, and eIF2γ. The complex recruits Met-tRNAi to start ribosomal translation in a GTP-dependent manner. Once the initiation process is completed, eIF2-GDP gets discharged from the ribosome, and GTP substitutes GDP to form active eIF2-GTP complex to participate in another round of translation initiation (Jackson et al., 2010). The eIF2 complex can be inactivated by phosphorylation at a single serine (Ser51) of its alpha subunit (eIF2a) by one of the four mammalian kinases in response to various stimuli. In this study, we found that three mammalian kinases interact with SARS-CoV-2 proteins, protein kinase R (PKR), PKR-like endoplasmic reticulum kinase (PERK), and heme-regulated inhibitor (HRI) can phosphorylate eIF2a and shut off the host cell's translation machinery (Table 1).

Host proteins involved in double-membrane vesicle (DMV) formation during SARS-CoV-2 infection

Positive-sense RNA viruses perform RNA replication in the cytoplasm. Genome replication is associated with virus-induced structures derived from cellular endomembranes (Romero-Brey & Bartenschlager, 2016). In the case of coronaviruses, replication takes place in double-membrane vesicles (DMVs). The mechanism by which these DMVs are formed is not fully understood. In our study, we predict that the viral NSP4 interacts with the host proteins; namely, SEC61, VAPB, COPA, ARCN1, COPB1, GBF1, VAPA, VAPB, STX17, and TMED1, while NSP15 interacts with protein disulfide isomerase (PDI) associated with the endoplasmic reticulum (ER). Earlier studies have reported that SEC 61A and PDI are present on the inner surface of DMV (Hagemeijer et al., 2014). Vesicle-associated membrane protein-associated protein A (VAP-A) and VAP-B are also crucial for viral RNA replication and present in the DMV. Electron microscope tomography studies earlier confirmed that DMVs are part of a reticular network of modified ER membranes. The inner surface of DMVs also contains double-stranded RNA (dsRNA) derived from viral replication intermediate (Knoops et al., 2008). The outer layer of SARS-CoV-2 induced DMVs can be continuous with ER cisternae suggesting that the secretory pathway is also essential for virus replication. The depletion of COPB1 and GBF1, associated with the same path, affected SARS-CoV replication, intensifying this phenomenon (de Wilde et al., 2015).

Previous studies suggested that coronaviruses take advantage of the cellular autophagy system for DMV biogenesis (de Wilde et al., 2015). We observed that NSP4 interacts with several factors associated with autophagy, namely, ATG12, ATG16L1, ATP13A2, BECN1, C9ORF72, MAP1LC3A, MAP1LC3B, etc. suggesting that autophagy could play a crucial role in SARS-CoV-2 mediated DMV formation. Interestingly, NSP13 shows interaction with ERDA regulators EDEM1 and OS-9. Both EDEM1 and OS-9 are associated with the ERAD tuning pathway. It is observed that the prototypic coronavirus, MHV interferes with ERAD. This directs to intracellular accumulation of EDEM1 and OS-9, leading to relocalization and limitation of these chaperones, and enhance MHV-induced DMV formation (Figure 2) (Cali et al., 2008). Our study suggests that SARS-CoV-2 mediated DMV formation is associated with the endoplasmic reticulum, autophagosome, and ERAD machinery.

Host proteins involved in SARS-CoV-2 replication

The + ve sense RNA genome of coronavirus serves as a template for the replicase that synthesizes a full-length negative-sense RNA, which serves as a template for the further synthesis of nascent genomic RNA. This process occurs in virus replication/transcription complex (RTC), a complex of both viral and host proteins. The viral proteins (NSP3-NSP16) having various enzymatic activities are considered to be part of the RTC. These enzymes' actions include deubiquitination, protease, helicase, polymerase, Exo and endonuclease, and N7- and 2′O-methyltransferases. Interestingly, we observed that many host interactors of RTC complex are associated with RNA processing, [ELAVL like protein 1 (ELAVL1), ribosomal proteins (40S ribosomal protein S6 (RPS6), 40S ribosomal protein S4, X isoform (RPS4X), 40S ribosomal protein S3a (RPS3A), polyadenylate-binding protein 1 (PABPC1)]. Protein involved in translation initiation, especially multiple subunits of eukaryotic translation initiation factors 2, 3, 4, 5(eIF2, eIF3, eIF4, eIF5) and DDX3Y helicase. The viral proteins also showed interactions with the 60S ribosomal protein L13A (RPL13A) and its regulatory elements, such as IGF2BP1, GCN1L1, LARP, and NCK1 (Figure 2). These findings indicate that the host cell translation machinery is present near the RTC complex.

Host proteins involved in SARS-CoV-2 assembly and release

Coronavirus assembly occurs in the ER-Golgi intermediate compartment (ERGIC) and organized by the viral matrix (M) protein (Klumperman et al., 1994; Masters, 2006). The M-S and M-nucleoprotein (N) interactions facilitate the

Table 1. List of human protein kinase and their interactor SARS-CoV-2 proteins.

Serial number	Host protein	Protein name	Interacting viral protein
1	EIF2AK1/HRI	Eukaryotic Translation Initiation Factor 2 Alpha Kinase 1/Heme-Regulated Inhibitor	NSP4, NSP7, NSP8, NSP14
2	EIF2AK2/PKR	Eukaryotic Translation Initiation Factor 2 Alpha Kinase 2/Protein kinase R	NSP3, NSP4, NSP7, NSP8
3	EIF2AK3/PERK	Eukaryotic Translation Initiation Factor 2 Alpha Kinase 3/PKR-like endoplasmic reticulum kinase	NSP4, NSP7, NSP8

recruitment of structural components to the assembly site. Contrastingly our analysis did not pick any hSARS-CoV-2 for N protein interactions. SARS-CoV-2 S and M proteins interact with 43 host proteins (i.e. ANPEP, COPG1, COPG2, ERGIC1, and GBF1) associated with the ERGIC inter-organelle compartment. This result shows SARS-CoV-2 also uses a similar mechanism as other coronaviruses. This mechanism is also conserved with other viruses such as HIV and VSV and can be inhibited by Brefeldin A (BFA) (Pal et al., 1991). BFA is an antiviral, antibiotic drug containing a 13-member macrocyclic lactone ring (Misumi et al., 1986). It causes disassembly of the Golgi complex and inhibits lipid vesicle secretion (Fujiwara et al., 1988). Besides ERGIC associated proteins; SARS-CoV-2 also interacts with β-actin, vimentin (an intermediate filament protein), and AP2M1, which are essential for virus assembly and release (Neveu et al., 2012). The viral S protein also interacts with a host antiviral protein; bone marrow stromal antigen 2 (BST-2), which is known to inhibit release of nascent virion from the cell. The BST-2 is a well-defined antiviral protein that tethers many enveloped viruses release like HIV1/2, Dengue, HCV, VSV, etc (Tiwari et al., 2019).

IRF1/9/7 suggested to have a regulatory role in IFN-γ mediated signaling pathway during SARS-CoV-2 infection

The innate immune response is a conserved defense strategy of the host; this is critical for the first detection and localized restriction of pathogens. Various case reports indicate that SARS and SARS-CoV-2 downregulate IFN-I, but at the same time, type II interferon (IFN-γ) present in higher concentration in SARS and SARS-CoV-2 patients (Wong et al., 2004; Yao et al., 2020). Hence, it is highly possible that IFN-γ to be a critical player in SARS-CoV-2 mediated lung injury and could be a potential biomarker for disease severity. Our results also suggest a significant role of IFN-γ pathways associated with SARS-CoV-2 infection. We show that 62 candidates (associated with IFN-γ) interact with SARS-CoV-2 proteins (Supplementary Table 1). The degree centrality analysis suggests that IRF1, IRF9, IRF7, and HLA-DRB1 protein (HLA-DRB1) have crucial and central roles in cytokines IFN-γ signaling pathway (Figure 3, Table 2).

TP53 and CASP3 are the critical players of SARS-CoV-2 mediated apoptosis

Apoptosis is a sort of programmed cell death characterized by the highly controlled disassembling of cellular structures released in membrane-bound vesicles engulfed by neighboring cells or phagocytes (Elmore, 2007). Apoptosis occurs by two pathways; extrinsic and intrinsic, regulated by a preformed cascade of proteases called caspases. Individually, these pathways stimulate downstream caspases that initiate morphological and biochemical changes in the cell that eventually begin apoptosis (Galluzzi et al., 2018). Many viruses encode specific proteins that modulate the apoptosis pathway. In coronavirus infected patients, one of the common abnormalities manifested is lymphopenia resulting from the depletion of the T cell population (Tavakolpour et al., 2020). Autopsy findings of SARS-CoV-2 and SARS cases show T cells apoptosis in various infected tissues, such as lungs, liver, and thyroid (Zhao et al., 2020). In line with this, we observed apoptosis pathways to be highly enriched in gene enrichment analysis. Here 137 host proteins (associated with apoptosis) showed interaction with SARS-CoV-2 proteins (Supplementary Table 1). The degree centrality analysis of these genes suggests that cellular tumor antigen p53 (TP53), followed by caspase-3 (CASP3), catenin beta-1 (CTNNB1), and ubiquitin-60S ribosomal protein L40 (UBA52) are having crucial roles in the apoptosis pathway (Figure 4, Table 3).

SARS-CoV-2 protein-induced proteasomal degradation of CD4 T cell

In eukaryotes, the majority of intracellular proteins are diminished by the ubiquitin (Ub)–proteasome pathway (UPP) (Huang & Figueiredo-Pereira, 2010). Ubiquitin is a 76-amino acid polypeptide present and conserved in every eukaryotic cell (Li & Ye, 2008). The covalent modification of proteins with ubiquitin chains forms a strong targeting signal driving recognition and destruction by the 26S proteasomes. The covalent interaction of ubiquitin to lysine residues requires action of at least three ubiquitin enzymes: the ubiquitin-activating enzyme E1, one of several ubiquitin-conjugating enzymes, E2, and one of the multiple ubiquitin ligases, E3 (Wilkinson, 1995).

Some viruses use ubiquitin (Ub)–proteasome pathway for cellular protein degradation, and the best example for this is HIV-I. Vpu of HIV-I targets CD4 protein for degradation. CD4 is a class I integral membrane glycoprotein expressed on the surface of a subset of T lymphocytes that recognize MHC-II associated peptides. This process is vital for construction and maintenance of the immune system. The HIV-Vpu interacts with the CD4 in the endoplasmic reticulum and triggers its proteolytic degradation. At first, Vpu interacts with the cytoplasmic domain of CD4 and connect it to βTrCP (a member of the F-box protein family first characterized as components of ubiquitin-ligase complexes). Following this the, N terminal of βTrCP interact with SKP1, a targeting factor for ubiquitin-mediated proteolysis (Schubert et al., 1998). Interestingly in our data highlights 43 candidates out of 52 known proteins associated with Vpu mediated degradation of CD4 T cells. Degree centrality analysis reveals that ubiquitin-60S

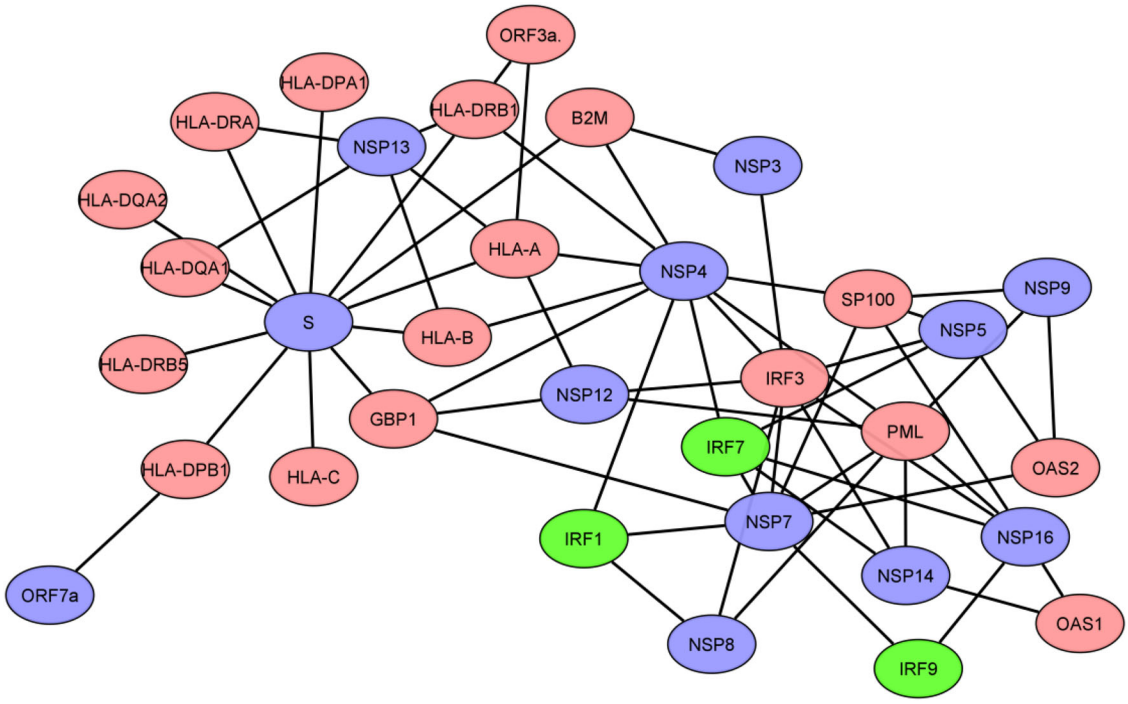

Figure 3. A predicted interaction map of the top 20 IFN-γ associated human proteins and their interacting SARS-CoV-2 protein. Blue color represents the virus proteins, and brown color represents the human interactor proteins. Twelve SARS-CoV-2 proteins interact with human proteins related to IFN-γ signaling pathway. IRF1, IRF7, and IRF9 (represented in green color) are highly weighted proteins and may regulate IFN-γ signaling cascade. (Interaction map was created using Cytoscape.)

Table 2. Degree centrality analysis of proteins associated with IFN-γ signaling pathway.

Serial number	Host protein	Ensemble gene ID	Degree centrality (weight)
1	IRF1	ENSP00000245414	46.187
2	IRF9	ENSP00000380073	45.519
3	IRF7	ENSP00000380697	44.485
4	HLA-DRB1	ENSP00000353099	44.311
5	HLA-DRA	ENSP00000378786	44.263
6	HLA-DQA1	ENSP00000339398	44.217
7	HLA-DPA1	ENSP00000393566	44.048
8	IRF3	ENSP00000471896	44.038
9	HLA-DQA2	ENSP00000364076	44.028
10	PML	ENSP00000268058	43.965
11	HLA-DPB1	ENSP00000408146	43.961
12	HLA-DRB5	ENSP00000364114	43.878
13	B2M	ENSP00000452780	43.652
14	HLA-A	ENSP00000379873	43.531
15	OAS1	ENSP00000388001	43.358
16	SP100	ENSP00000343023	43.092
17	HLA-C	ENSP00000365402	42.578
18	HLA-B	ENSP00000399168	42.534
19	GBP1	ENSP00000359504	42.492
20	OAS2	ENSP00000342278	42.453

ribosomal protein L40 (UBA52), followed by ubiquitin C (UBC), and 26S proteasome regulatory subunit 8 (PSMC5) having crucial roles in the proteasomal degradation of CD4 expressing T cells (Figure 5, Table 4). We found that core elements of the 26S and 20S proteasome complex (Psmd1, Psmc2, and Psmb3) interact with SARS-CoV-2 proteins. These findings suggest that SARS-CoV-2 could also use a similar pathway to degrade CD4 expressing cells. A recent case study on COVID19 patients having lower CD4 and CD8 T cell counts corroborates this notion. We hypothesize that SARS-CoV-2 proteins directly bind to CD4 and direct its degradation by using ubiquitin (Ub)–proteasome pathway.

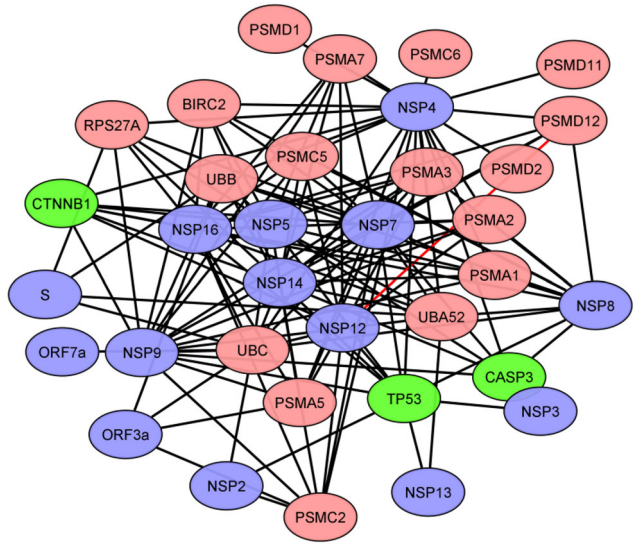

Figure 4. A predicted interaction map of the top 20 apoptosis associated human proteins and their interacting SARS-CoV-2 protein. Blue color represents the virus proteins, and brown color represents the human interactor proteins. Fourteen SARS-CoV-2 proteins interact with human proteins connected with apoptosis. Degree centrality analysis revels that TP53, CASP3, and CTNNB1 (represented in green color) are the principal players in the SARS-CoV-2 mediated apoptosis. (Interaction map was created using Cytoscape.)

Discussion

A successful viral infection depends on the virus's ability to manipulate host biological pathways to evade the host immune system during the pathogenesis. In the present study, we constructed a network of the protein–protein interactome of the host and SARS-CoV-2 proteins by applying a structure-based computational approach. This computational

Table 3. Degree centrality analysis of proteins associated with apoptosis pathway.

Serial number	Host protein	Ensemble gene ID	Degree centrality (weight)
1	TP53	ENSP00000269305	73.562
2	CASP3	ENSP00000311032	57.312
3	CTNNB1	ENSP00000344456	53.542
4	UBA52	ENSP00000388107	52.511
5	UBB	ENSP00000304697	52.417
6	BIRC2	ENSP00000477613	52.298
7	UBC	ENSP00000441543	51.775
8	RPS27A	ENSP00000272317	51.714
9	PSMD1	ENSP00000309474	47.781
10	PSMC6	ENSP00000401802	46.648
11	PSMA5	ENSP00000271308	46.036
12	PSMC5	ENSP00000310572	46.01
13	PSMA3	ENSP00000216455	45.966
14	PSMD11	ENSP00000261712	45.945
15	PSMD12	ENSP00000348442	45.92
16	PSMD2	ENSP00000310129	45.898
17	PSMA1	ENSP00000414359	45.89
18	PSMA2	ENSP00000223321	45.848
19	PSMA7	ENSP00000359910	45.761
20	PSMC2	ENSP00000391211	45.214

Table 4. Degree centrality analysis of proteins associated with proteasomal degradation of CD4 T cells.

Serial number	Host protein	Ensemble gene ID	Degree centrality (weight)
1	UBA52	ENSP00000388107	40.771
2	UBC	ENSP00000441543	40.754
3	PSMC5	ENSP00000310572	40.751
4	UBB	ENSP00000304697	40.748
5	PSMC2	ENSP00000391211	40.719
6	PSMB2	ENSP00000362334	40.717
7	PSMA3	ENSP00000216455	40.716
8	PSMD1	ENSP00000309474	40.703
9	PSMA5	ENSP00000271308	40.686
10	PSMA6	ENSP00000261479	40.684
11	PSMA4	ENSP00000044462	40.684
12	PSMB1	ENSP00000262193	40.68
13	PSMB4	ENSP00000290541	40.675
14	PSMC4	ENSP00000157812	40.673
15	PSMA1	ENSP00000414359	40.662
16	PSMD12	ENSP00000348442	40.653
17	PSMB3	ENSP00000483688	40.643
18	PSMC1	ENSP00000261303	40.641
19	PSMD7	ENSP00000219313	40.64
20	PSMC6	ENSP00000401802	40.63

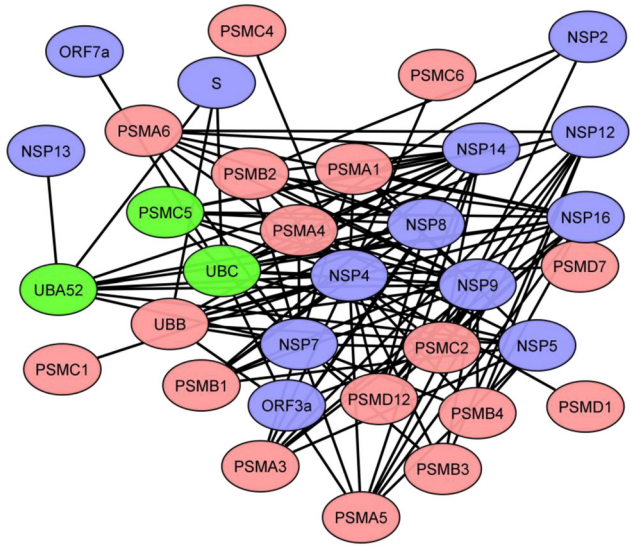

Figure 5. A predicted interaction map of the top 20 proteasomal degradation of CD4 associated human proteins and their interacting SARS-CoV-2 protein. Blue color represents the viral proteins, and brown color represents the human interactor proteins. Thirteen SARS-CoV-2 proteins interact with human proteins connected with proteasomal degradation of CD4 T cells. Degree centrality analysis revels that UBA52, UBC, and PSMC5 (represented in green color) are the key player in the process of proteasomal degradation of CD4 T cells. (Interaction map was created using Cytoscape.)

method's principle is based on the assumption that proteins with similar structures would share related interaction partners.

Based on this analysis, we craft possible chronological events associated with SARS-CoV-2 pathogenesis and brought light into the molecular partners and their possible role in host-pathogen interactions. The GO analysis of interacting proteins reveals that SARS-CoV-2 may use clathrin-mediated endocytosis for its entry. Among the viral proteins, the spike protein was found to interact with a series of host proteins. These include actin and microtubule cytoskeletal proteins (CTTN and ITSN1), early endosome (RAB5, RAB11B, and RAB13), late endosome (RAB7A, RAB7B, and RAB7L1), and HOPS complex (VPS11, VPS33A) proteins. These factors are known for their association with the clathrin-mediated virus entry (Inoue et al., 2007). We also predict role of several host factors essential for SARS-CoV-2 replication and assembly. Hence, therapeutic modalities could be targeted to restrict these factors (if affordable) to mitigate viral infection. Most of the positive-sense RNA viruses replicate at virus-induced endomembranes (Wolff et al., 2020). While in the case of coronaviruses, genome replication occurs at the DMV sites (V'kovski et al., 2020; Wolff et al., 2020). Various cellular organelles reorganized and form DMV after virus infection. Here we report that the ER, the autophagosome, and ERAD machinery are associated with DMV formation. After the DMV formation, SARS-CoV-2 non-structural proteins (NSP3-NSP16) interact with the host proteins and make an RTC complex. Among these host proteins, ELAVL1, RPS6, RPS4X, PABPC1, eIF2/3/4/5, and 60S ribosomal protein contribute for RTC complex formation, which is essential for virus replication. Similarly, these viral proteins interact with candidates associated with ERGIC, where virus assembly takes place (V'kovski et al., 2019).

Further, we elaborate significantly enriched pathways associated with SARS-CoV-2 pathogenesis. These include SARS-CoV-2 mediated apoptosis; IFN-γ signaling pathway and proteasomal degradation of CD4 pathway playing an important role in viral pathogenesis. Various clinical reports show elevated blood levels of IFN-γ, associated with higher degree of SARS-CoV-2 pathogenesis (Gadotti et al., 2020). The degree centrality analysis of IFN-γ signaling suggests that IRF1/9 and IRF7 are the key players of this pathway. Previously it was observed that coronavirus induced apoptosis in host cells. However, this is not required for virus replication and infection (Bordi et al., 2006). It might be a possible mechanism by which viruses degrade immune cells and cause leukocytopenia (Tavakolpour et al., 2020). In this case, the degree centrality analysis reveals TP53 and CASP3 are the most weighted protein in SARS-CoV-2 mediated apoptosis process. Apart from core apoptosis, we uncovered one more interesting pathway, 'Vpu mediated degradation of CD4 cells'. Similar to HIV-I Vpu causing proteasomal

degradation of CD4 T cells (Binette et al., 2007; Schubert et al., 1998), we learn that core elements of the 26S and 20S proteasome complex (Psmd1, Psmc2, and Psmb3) interact with SARS-CoV-2 proteins. Considering these pieces of evidence, we postulate that SARS-CoV-2 induces ubiquitination-mediated proteasomal degradation of CD4 T cells and other immune cells in the affected host. Lower CD4 T cell counts in severe SARS-CoV-2 patients support this hypothesis. Overall, we bring crucial mechanistic insight to SARS-CoV-2 pathogenesis in this study. These observations could be the foundation for future clinical and in vivo studies.

Associated content

Supplementary Table 1: Represents the list of SARS-CoV-2 similar proteins and CC qualified interacting partners. They were downloaded from HPRD, BIOGRID, and MINT database. Supplementary Table 2: Gene enrichment analysis obtained from Reactome database and HPA database by using g: profiler tool for pathway analysis. Supplementary Table 3: Degree centrality analysis.

Authors' contributions

R. T., A. R. M., and D. N. conceptualized the study. R. T. and A. R. M. performed data mining, bioinformatics studies, and computational biological work. R. T., A. R. M., and D. N. wrote the manuscript.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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