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Case Study

Comparative host–pathogen protein–protein interaction analysis of recent coronavirus outbreaks and important host targets identification

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Abstract

Last two decades have witnessed several global infectious outbreaks. Among these, coronavirus is identified as a prime culprit ranging from its involvement in severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) to COVID-19. These infections involved in huge healthcare and economic cost incurred globally. Every time, coronavirus improved its infection ability and surprised the medical practitioners and researchers. Currently, COVID-19 is also causing numerous infections and stalled global activities. Global efforts are underway to identify potential viral targets for management of these outbreaks, but significant progress in prevention of these outbreaks is not yet achieved. We explored host–pathogen protein–protein interactions of MERS, SARS and COVID-19, and identified host targets common among all recent coronavirus outbreaks. Further, we tried to understand their potential for management of coronavirus. The common proteins involved in coronavirus host–pathogen interactions indicate their indispensable role in the pathogenesis and therefore targeting these proteins can give strategies to prevent current and future coronavirus outbreaks. Viral variability necessitates development of new therapeutic modalities for every outbreak, in contrast targeting necessary human proteins required by all coronaviruses can provide us a clue to prevent current and future coronavirus outbreaks. We found that targeting FURIN and TMPRSS2 can provide good results due to their common involvement in current and previous outbreaks. We also listed some known molecules against these two targets for their potential drug repurposing evaluation. Although, several recent studies undergoing with targeting these proteins for management of coronavirus, but safety evaluation and risk assessment must be given prime importance while targeting human proteins.

Key words: coronavirus; host–pathogen interactions; FURIN; TMPRSS2; infection prevention

Introduction

During last two decades, *Betacoronavirus* genus gave us prominent pathogens leading to global public health emergency, which include Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS) and SARS-CoV-2 coronavirus [1]. Generally coronaviruses are involved in infections ranging from respiratory to enteric system, but recent outbreaks of SARS, MERS and current COVID-19 gave it a worldwide notoriety [2]. The cases of current coronavirus SARS-CoV-2 (causing COVID-19) overtook all previous coronavirus outbreaks in terms of incidence and mortalities. The symptoms of current outbreak range from mild illness, viral pneumonia with the power of affecting both lungs, acute respiratory distress syndrome to

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sepsis and septic shock, in addition to rapid infectivity and multiorgan involvement [3, 4]. Previous coronavirus outbreaks also created worldwide panic, but the current version of SARS-CoV-2 has affected all world activities in addition to worldwide panic and global public health emergency. Several researchers around the globe are working to contain the virus; unfortunately, the incidence and fatalities are out of control in the absence of proper treatment strategies. These all viruses are positive sense, single-stranded RNA virus with envelope and belongs to the *Coronaviridae* family. Coronaviruses generally emerge as zoonotic infections, where animal virus gains the ability to infect the human. The last three important coronaviruses are also suggested to be originated from the animal sources. Even the current SARS-CoV-2 is also considered to be a combination of bat and Malaysian pangolin coronavirus [5, 6].

Several factors are known to influence the ability of coronavirus severity, and it is considered that immune response subversion by viral or external factors is associated with its severity [7, 8]. Due to common involvement of three versions of betacoronavirus in recent outbreaks, several studies are going to understand the similarity between MERS, SARS and SARS2 coronavirus [9]. Some studies are finding common viral target to manage these infections, whereas some studies suggested strategies to develop broad-spectrum antivirals to manage all coronaviruses [10], but the virus is surprising researchers due to its remarkable variability and creating problems with development of antivirals [11].

Another strategy involved targeting of host as they are less susceptible to variation. During this study we performed comparative host–pathogen interaction (HPI) analysis of MERS, SARS and SARS-CoV-2 in order to identify common host targets necessary for current and previous outbreaks. We tried to understand about human proteins targeting which is likely to provide better response for the management of coronavirus outbreaks including the chances of their utilization in future coronavirus outbreaks due to their indispensable participation in all major coronavirus pathogenesis.

Materials and methods

Database

The Biological General Repository for Interaction Dataset (BioGrid) version 3.5.185 was used to download SARS-CoV-2 and other coronavirus-related interactions. The dataset was last modified up to 30 April 2020 at the time of analysis. All viral–viral, host–host and HPI derived from organisms other than human were filtered out. Only protein–protein interactions involving virus–human were selected for further analysis.

Comparative PPI map preparation and identification of common proteins

The Cytoscape v3.8.0 was used to create comparative protein–protein interaction network from known coronavirus interactions. The common human proteins known to interact with coronavirus were identified and filtered out for further analysis. These common interactors were ranked and categorized for their ability to interact with all or any two coronaviruses out of MERS, SARS-CoV and SARS-CoV-2.

Functional identification of common interactors

The Entrez gene IDs of common interactors were taken from BioGrid dataset and used to detect these proteins in Uniprot. The functional details of these common human proteins were collected from Uniprot and included for categorization of suitable host protein with therapeutic potential.

Identification of known inhibitors of selected common targets involved in coronavirus infection

The common human proteins detected in earlier steps with positive involvement in viral pathogenesis were screened at DrugBank, IUPHAR/BPS guide to Pharmacology and PubChem databases for finding known molecules targeting two proteins interacting with all three recent coronaviruses identified in earlier steps. In addition, literature was also searched for natural compounds involved in the inhibition of these two proteins activity.

Identification of metabolic pathways and biological processes affected by these common targets

The three common human proteins involved in all recent coronavirus outbreaks (identified in previous steps) were detected for their involvement in different pathways. Kyoto Encyclopedia of Genes and Genomes (KEGG) was considered for evaluation of their role in different pathways, whereas BioCyc database collection was used to detect gene ontology (biological process) to assess their role in different biological processes.

Results

We performed comparative HPI analysis of recent three coronavirus outbreaks and identified important human targets and their inhibitors in different databases. Figure 1 represents scheme for identification of important human proteins and their inhibitors. We used BioGrid version 3.5.185 for collecting HPIs of recent coronavirus outbreaks. BioGrid is a curated database and involve 72 164 literature references for 28 093 chemical association, 1814 182 protein and genetic interactions, and 874 796 posttranslational modification information at the time of data collection. The searching of database for SARS-CoV-2 and other related coronavirus-mediated interactions involved total 743 interactions including virus–human, human–human, virus–virus and interactions with host other than human. Filtering of all these interactions gave us 518 exclusive virus–human-mediated protein–protein interactions. These 518 interactions involved 63 redundant interaction derived from detection of same interactions by multiple approaches leaving 455 unique coronavirus HPIs. Table 1 represents detail about coronavirus-related HPI in BioGrid database. The details of interaction obtained from BioGrid version 3.5.185 is presented as Supplementary Table S1.

Construction of comparative HPI maps in Cytoscape 3.8.0 is presented in Figure 2. The common human proteins involved in different HPI maps are presented with different node sizes and colors. The detail about common interacting human proteins and their involvement in different HPI is presented in Table 2.

Human proteins commonly interacting with different coronaviruses and their functional role as per Uniprot is presented in Table 3. Proteins MASP2, FURIN and TMPRSS2 are involved in interaction with all three coronaviruses. Host protein MASP2 is known to interact with N protein, whereas FURIN and TMPRSS2 interact with S protein. These interactions are preserved in all three recent coronavirus outbreaks and indicate their importance in viral pathogenesis. MASP2 is involved in innate immune defense and therefore it was not considered for known inhibitors identification, in contrast FURIN and TMPRSS2 are involved in

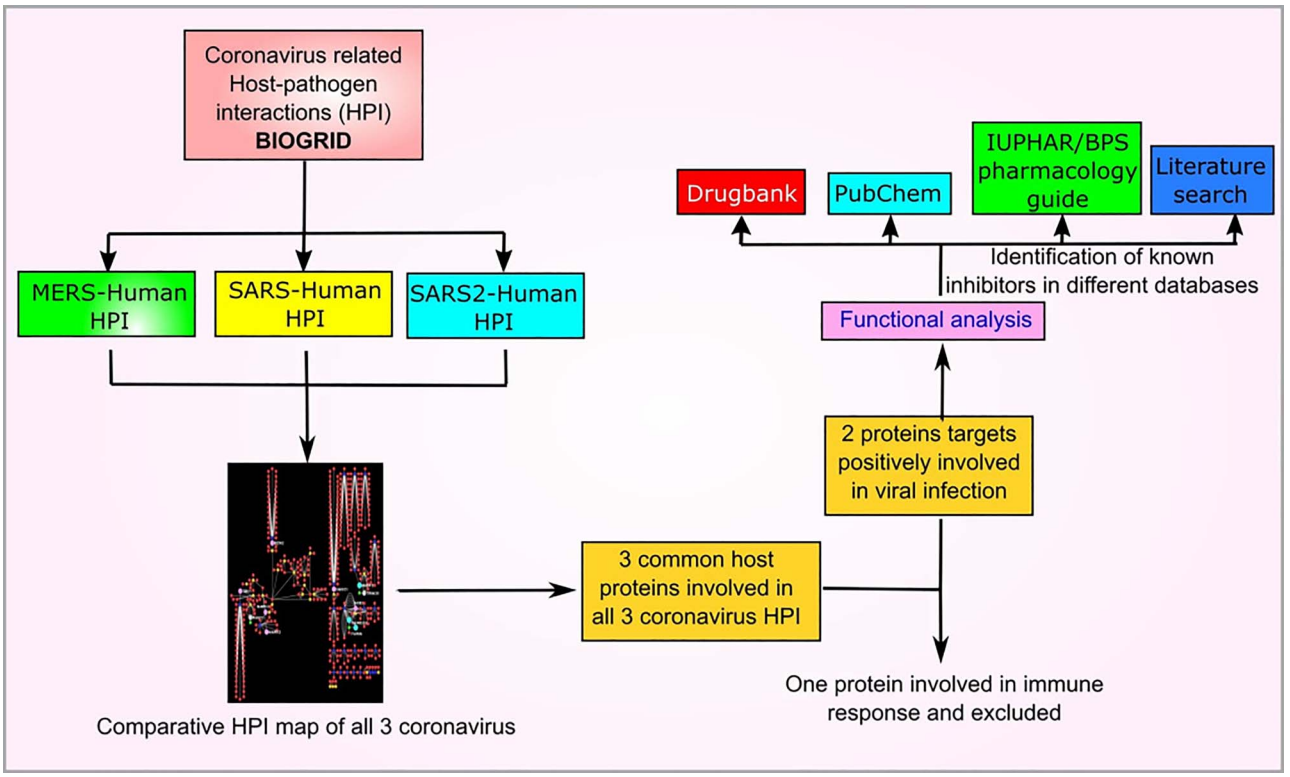

Figure 1. Work scheme representing identification of important human targets from comparative coronavirus HPI map.

Table 1. Details about coronavirus-related interactions in BioGrid

Interacting partners	Number of interactions
Total interactions	743
Human–Human	41
Mus musculus–SARS	01
Cricetulus griseus–SARS	01
MERS–MERS	03
SARS–SARS	165
SARS CoV2–SARS CoV2	14
MERS–human/human–MERS	02/04
SARS–human/human–SARS	142/17
SARS CoV2–human/human–SARS CoV2	340/13
Total HPIs (human specific)	**518**

For details please refer Table S1.

Table 2. Common human proteins found to interact with coronavirus of recent public health concern as per BioGrid database

Sr. No.	Virus	Common human interacting proteins
1	SARS, MERS and COVID	MASP2 FURIN TMPRSS2
2	SARS and COVID	ACE2 MASP2 FURIN TMPRSS2 BZW2 TBK1 SMOC1 MARK3 MARK2
3	MERS and COVID	MASP2 FURIN TMPRSS2
4	SARS and MERS	MASP2 TRIM25 FURIN TMPRSS2 RCHY1

viral infection progression and therefore considered for further analysis. The results are presented in Table 3.

The screening of FURIN and TMPRSS2 in different databases for known inhibitory molecules is presented in Table 4. The DrugBank database was found to have two molecules for FURIN and one molecule for TMPRSS2. Identification of targets in IUPHAR/BPS guide to Pharmacology and Pubchem database revealed several molecules for FURIN and TMPRSS2, which indicated about several RNAi molecules. Some natural compounds known for these proteins inhibitory activity are also presented in Table 4.

The role of common human proteins in metabolic pathways according to KEGG is presented in Supplementary Figures S1–S3, whereas the role of these common human proteins in biological processes as determined by BioCyc database is presented in Table S2.

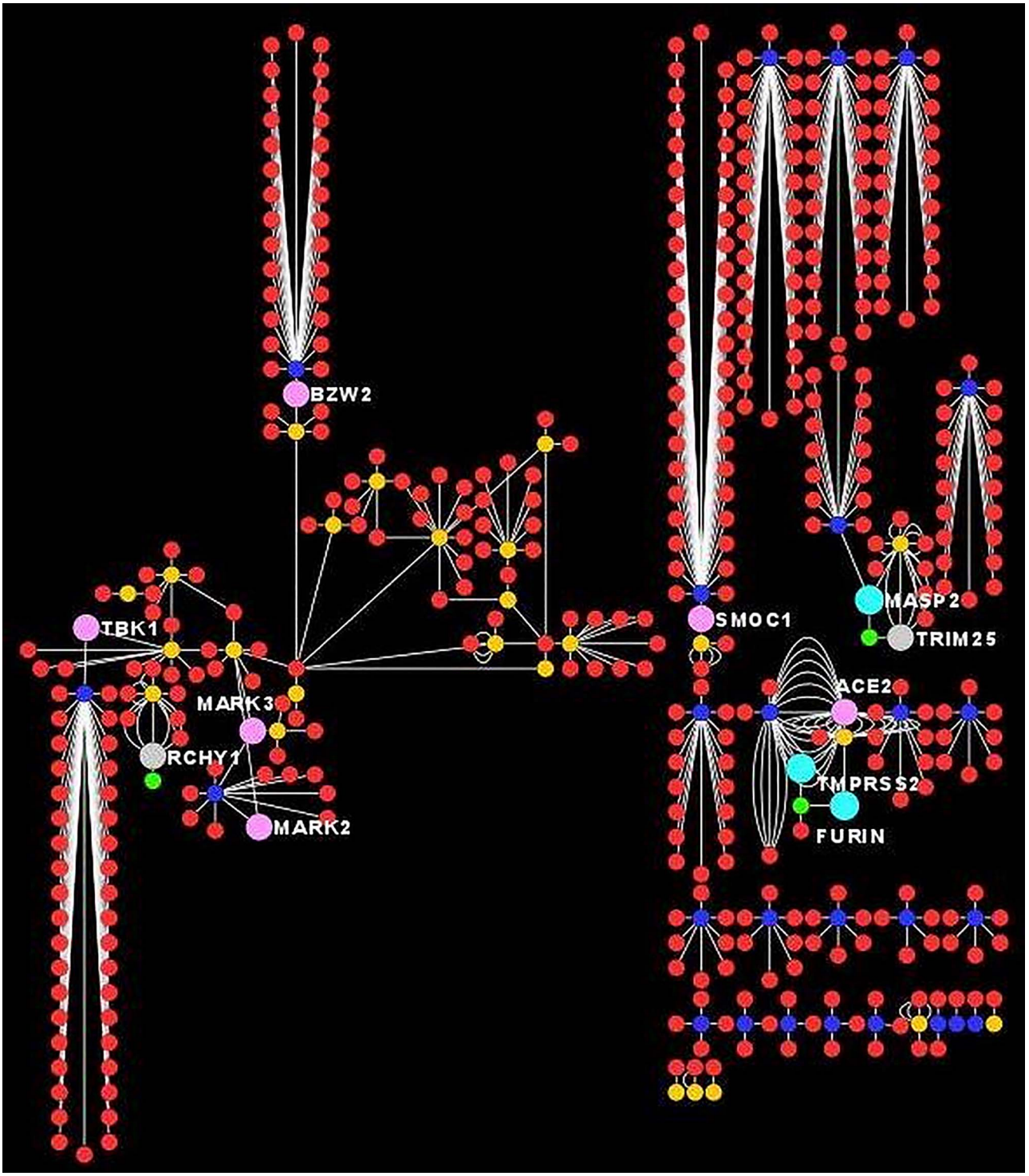

Figure 2. Comparative host–pathogen protein–protein interactions of MERS, SARS and SARS2. Viral proteins are indicated with different node colors (MERS: fluorescent green, SARS: yellow, SARS2: blue). Human proteins are generally indicated with red colors but common human proteins are shown with large size nodes. In addition, the proteins common among all coronaviruses are shown with sky blue color, whereas human proteins common among other pathogens are also shown with different colors. Multiple edges indicate about detection of same interaction through multiple experimental evidences.

Discussion

The HPI plays an important role in the pathogenesis of any infectious organisms, but their role is more prominent in viral pathogenesis due to their reliance on effective utilization of host machinery for establishing the infection. The role of HPIs in coronavirus infections is also discussed earlier [12], but computational study of host–pathogen protein–protein interactions is adding more value for understanding the pathogenesis of coronavirus [13]. BioGrid is a well-known database of protein, genetic and chemical interactions between various organisms

Table 3. Details of common interactors and their interaction with different coronaviruses

Common human protein interactor	Primary function according to Uniprot	Viral interactor proteins		
		MERS	SARS	SARS2
MASP2	Activation of complement by its serum protease activity via mannose-binding lectin. Cleaves and activates C2 and C4, resulting in formation of C3 convertase	N	N	N
FURIN	Endoprotease with the ability to cleave at the RX(K/R)R consensus motif. Required for H7N1 and H5N1 influenza virus infection by hemagglutinin cleavage [32]	S	S	S
TMPRSS2	Promotes SARS-CoV and SARS-CoV-2 infections via proteolytic cleavage of Angiotensin-converting enzyme 2 (ACE2) receptor promoting viral uptake, and cleavage of spike glycoproteins of coronavirus and its activation for host cell entry [33, 34]	S	S	S
ACE2	Receptor for entry of SARS-CoV and SARS-CoV-2, NL63/HCoV-NL63	–	S	S
BZW2	May be involved in neuronal differentiation	–	nsp8ab	M
TBK1	Serine/threonine kinase playing an important role in inflammatory responses against foreign substances	–	nsp5ab	nsp13
SMOC1	Important roles in eye and limb development. Probably regulates differentiation of osteoblast	–	ORF7a	ORF8
MARK3	It is a serine/threonine-protein kinase [35], which is involved in the specific phosphorylation of microtubule-associated proteins for MAP2 and MAP4 and phosphorylate MAPT/TAU. It negatively regulates hippo signaling pathway	–	ORF9b,nsp13ab	ORF9b
MARK2	It is a serine/threonine protein kinase [35], which is involved in cell polarity and microtubule dynamics regulation	–	nsp13ab	ORF9b
TRIM25	Ubiquitin E3 ligase and ISG15 E3 ligase [36], which is implicated in innate immune response for viruses [37, 38]. Mediates signal transduction leading to the production of interferons against viral infection [37, 39]	N	N	–
RCHY1	E3-dependent ubiquitination and proteasomal degradation of target proteins. Involved in the cell cycle progression regulation and enhance androgen receptor (AR) transcription factor activity	nsp3ab	nsp3ab	–

and host. We downloaded interactions from BioGrid with published experimental evidences and this provides reliability of high-quality interaction data [14]. Among these, few interactions are verified by multiple experimental approaches as evidenced in Figure 2 and Supplementary Table S1 and indicate about additional reliability of source data.

During identification of common human interactors in SARS, MERS and SARS-2 protein–protein HPI, three proteins were found to be involved in HPI with all three coronaviruses (Table 2). In addition, their host–pathogen interacting partners were also common among all three coronavirus HPI indicating indispensable nature of these interactions in coronavirus infection (Table 3). These three proteins, namely MASP2, FURIN and TMPRSS2 were detected for their function in human using Uniprot. Uniprot is a universal protein knowledgebase providing comprehensive information about major aspects of proteins [15]. We found that MASP2 is involved in complement activation and innate immune response, which is important for protection against pathogen. In contrast, FURIN and TMPRSS2 are positively involved in viral infection (Tables 3 and S2). This activity is also indicated while analyzing their role in metabolic pathways and biological processes through KEGG [16] and BioCyc [17] database (Figures S1–S3, Table S2). Therefore, we selected FURIN and TMPRSS2 for further analysis.

We detected the relevant databases for identification of known inhibitors of these two proteins. It included DrugBank v 5.1.6 [18], IUPHAR/BPS guide to Pharmacology [19] and PubChem [20] databases. These databases are renowned and provide extensive coverage of known and experimental molecules targeting particular proteins. Under certain situations, the references providing detail about these activities are also mentioned (Table 4). In addition, literature search for natural molecules inhibiting these two proteins also revealed some important findings. This cataloging is supposed to provide some hits about potential of these molecules in prevention of coronavirus outbreaks.

Table 4. Known molecule targeting of important human interactors and their possible reactions

Database	Human protein								
	FURIN					TMPRSS2			
DrugBank	DRUGBANK ID	DB04951	DB03600			DB13729			
	Name	Pirfenidone (NCT04282902)	Capric acid			Camostat (NCT04353284, NCT04338906, NCT04321096, NCT04455815)			
	Drug group	Approved, investigational	Experimental			Experimental inhibitor			
IUPHAR/BPS guide to Pharmacology	Ligand	MI-1148 [32]	Phenylacetyl-Arg-Val-Arg-4-amidinobenzylamide [40]	Peptide 18 [41]	Furin inhibitor peptide [42]	I-432 [43]	Compound 5 [44]	Nafamostat (NCT04418128, NCT04352400) [45]	Camostat (mentioned above)
	Action	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition
PubChem	CID/SID	16760442	44584947	57358476		85101385		85108608	70 siRNA with reagent vendors
	Name	Furin inhibitor I	Furin inhibitor peptide	Hexa-D-arginine (furin inhibitor II)		RNAi of TMPRSS2 (R & D)		RNAi of TMPRSS2 (R & D)	
Literature search	Luteolin	Found in several food, inhibit viral replication through furin inhibition [46]				Geniposide (NPC306344)		A major iridoid glycosides of gardenia fruit predicted to inhibit TMPRSS2 during docking analysis [47]	
	Baicalein, oroxylin A and chrysin	Isolated from stem bark of medicinally active plant *Oroxylum indicum* shoes modest to strong furin inhibitory activity [48]				Nafamostat (mentioned above)		Known to inhibit MERS [45] and proposed for COVID-2019 inhibition	

The few clinical trials (identifier) of certain molecule for COVID-19 are also mentioned in brackets.

In addition to our results, some studies have also indicated the use of these proteins separately for management of these infections. For example, it has been proposed that targeting TMPRSS2 can help in the management of influenza and coronavirus infections [21]. In addition, TMPRSS2 inhibitor camostat is efficient to prevent entry and growth of SARS-CoV during *in vitro* experiments [22]. However, ACE2 proteins is known to interact with both SARS and SARS2 spike (S) proteins, and therefore several studies are undergoing to find S protein inhibitory molecules in addition to ACE2 inhibitors. It has been observed that emodin and promazine have ability to prevent interaction of S proteins with ACE2 and thereby inhibit coronavirus infection. These two molecules are also suggested as alternative choice for management of COVID-19 [23]. As per the previous information, the coronavirus entry into human cells depends on proteolytic cleavage of S protein through FURIN and TMPRSS2. The viral spike (S) protein is cleaved to S1 and S2 subunits through FURIN where S1 subunit binds to ACE2 receptor present on host cells, whereas S2 protein is further cleaved by TMPRSS2 resulting in membrane fusion and subsequent entry of virus into host cell, and therefore inhibition of FURIN and TMPRSS2 has been also suggested to control SARS-CoV-2 infection [24–27]. Indeed, several known FURIN and TMPRSS2 inhibitors are undergoing through clinical trials for COVID-19, including FURIN inhibitor pirfenidone (NCT04282902) and TMPRSS2 inhibitors camostat (NCT04353284, NCT04338906, NCT04321096, NCT04455815) and nafamostat (NCT04352400) (Table 4). While some other molecules need further investigation for their potential to control coronavirus infection. For example, luteolin is a flavonoid present in several edible plants; it is also proposed to have ability to manage COVID-19 and need detailed clinical investigations [28]. In addition, natural compound baicalein is also found to inhibit SARS-CoV-2 protease during *in vitro* study and draw special attention for development of antivirals against SARS-CoV-2 [29]. Our results validate these findings and indicate about preferential assessment of these two common human proteins due to their indispensable nature in all recently important coronavirus pathogenesis. The targeting of FURIN and TMPRSS2 is also suggested in several other studies and therefore supports our findings [25].

In addition, comparative HPI analysis also revealed that SARS-CoV-2 is having more known HPI than SARS and MERS. It may be due to extensive global efforts to understand SARS-CoV-2 pathogenesis because of its more intensity and global coverage. Otherwise the results indicate that SARS-CoV-2 is able to affect host in better way and these viruses are increasing their overall infection ability without altering common HPIs. It can be assumed that targeting these common interactors may be more superior and must be given preferential importance for management of these outbreaks.

Although, our study indicates targeting of FURIN and TMPRSS2 for management of coronavirus outbreaks, but this study has certain limitations. For example, our study is based on interactions known up to 30 April 2020 in BioGrid Database, but future studies may uncover some other common interactions with coronavirus management potential. In addition, sometimes the proteins can interact without physical contact, like transcription factors can affect expression of several proteins without interacting with them. Therefore, much wider picture of HPIs may arise while considering viral influence on host cell without any physical HPIs. In addition, the targeting of human proteins can have widespread clinical implications in addition to their effects on viral pathogenesis. Although the role of protease inhibitors are widely discussed in the management of COVID-19, but using protease inhibitors can lead to several associated adverse events including their effects on cardiovascular, renal system etc. [30, 31]. These all associated adverse events can undermine the importance of these two proteins in management of coronavirus. However, we are optimistic with the global efforts that we will be able to find certain molecules targeting FURIN and/or TMPRSS2 without causing anticipated adverse events. Considering present situation, where virus is causing significant mortality with global economic impact, and improving its infection ability, we must consider these options not only for present situation but also as our preparation for future similar outbreaks.

Conclusion

Identification of FURIN and TMPRSS2 as common host proteins positively involved in all recent coronavirus outbreaks indicates toward therapeutic potential of these proteins in the management of these viral infections. Screening of several databases for identification of potential inhibitors of these proteins and subsequent identification of clinical studies going toward coronavirus management potential of few inhibitors supports our findings. Natural compounds known to inhibit these proteins may also be an important alternative and needs clinical investigations for management of coronavirus outbreaks. Targeting FURIN and TMPRSS2 can give us valuable insights about management of coronavirus outbreaks provided that these protease inhibitors must satisfy safety standards.

Key Points

- Recently, the world has witnessed several outbreaks caused due to coronavirus. It includes SARS, MERS and COVID-19 that are posing challenges to healthcare workers and researchers.
- We performed comparative host–pathogen interaction analysis of coronavirus involved in MERS, SARS and COVID-19, and identified common interactions among all outbreaks.
- The common interactions among all recent coronavirus outbreaks indicate their indispensable role in pathogenesis.
- Targeting common interactors can give clue to prevent recent and future coronavirus outbreaks, but viral variability is another challenge and targeting of host proteins can provide better response.
- Several studies are undergoing that target human proteins interacting with coronavirus, but preferential assessment of common human interactors FURIN and TMPRSS2 can give important molecules with the potential to manage current and future outbreaks.

Supplementary data

Supplementary data are available online at https://academic.oup.com/bib.

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None.

Conflict of Interest

The authors declare no conflict of interest.

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