Drug Repositioning Suggests a Role for the Heat Shock Protein 90 Inhibitor Geldanamycin in Treating COVID-19 Infection

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Abstract

Drug repositioning offers an unmatched opportunity to offer novel therapeutics to treat SARS family of coronaviruses (SARS-FCoVs); an issue that became extremely urgent with the spreading of a novel virus with potential to threaten the lives of millions of people. Hereby, we analyzed a dataset of patients who presented with SARS during the 2003 outbreak. We established a gene signature that defines differential gene expression in patients who were sick with SARS vs. healthy controls and convalescent patients. We used a robust platform to conduct drug repositioning based on clustered gene expression and pathway enrichment to identify best matching drugs. We identified 55 agents of potential benefit. In most of these drugs we were able to establish a link to previous related research, use as antiviral, or at least a hypothetical role in treating SARS-FCoVs. Most notably, the heat shock protein 90 (hsp90) emerged as a major component that enables viruses to hijack infected cells through the process of autophagy. Almost half of the drugs identified could be linked to hsp90. As such, we propose using hsp90 inhibitors, mainly geldanamycin and its derivatives, to treat COVID-19.

Introduction

A new outbreak of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which causes Coronavirus Disease 2019 (COVID-19), was initially recognized in January 2020. Its rapid spread poses a potential threat to the security, health and economy of the world.[1] While researchers struggle to produce new therapeutics in timely fashion; the rapid spread of the disease calls for immediate interventions to protect exposed individuals and treat those affected. In the best-case scenario, new therapeutics require months to develop and manufacture, a luxury the global health community can ill afford given the urgency of the situation. To date, WHO reports more than 90,000 affected individuals, 3,000 deaths, and 70 affected countries, statistics that increase daily. At the time of writing this manuscript, more than 80 clinical trials are being launched in China related to treatment of COVID-19. These trials include studies involving stem cells, herbal remedies, novel antivirals (e.g. remdisivir, a nucleotide analog), anti-retroviral drugs (e.g. Kaletra, a combination of ritonavir and ipinavir), antimalarial drugs (e.g. chloroquine) and serotherapy (using convalescent plasma). [2-5]

Previous experience with coronaviruses proved how difficult their treatment can be. Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome (MERS) viruses had high case fatality rates despite attempts to identify effective therapy.[6] Vaccine development by multiple biopharmaceutical companies is ongoing; with the most promising approach being nucleic acid-based vaccines. [7] Biologically, SARS- CoV-2 shares many similarities to previous viruses. All three viruses are enveloped, positive- sense, single-stranded RNA Beta-coronaviruses. Additionally, the spike glycoprotein of these viruses use the angiotensin-converting enzyme 2 (ACE2) surface protein as a receptor, making this interaction the focus of intensive research in different animal species.[8] Due to these similarities and for simplification, we will refer to the three viruses (SARS- CoV, MERS and SARS- CoV2) as the SARS family of coronaviruses (SARS-FCoVs).

Drug repositioning, defined as the use of approved drugs for new indications, often unrelated to their primary use, can identify already available drugs that may effectively treat emerging diseases like COVID-19 immediately, even while more specific therapies and vaccines are being developed. In-silico testing offers immediate insight into pathways and drugs that interact with these pathways to promote or inhibit their activity. Eleven studies have been published about previous drug-repositioning efforts for SARS-FCoVs [9–19]. These studies were identified using the keywords (“Drug Repurposing” OR “Drug Repositioning”) AND (SARS OR MERS OR coronavirus OR COVID-19 OR SARS-CoV-2). With most of these publications being review and insight papers, we were looking for studies that used gene signature generated by comparing sick patients with SARS-FCoVs to controls, in order to identify modifiable host factors that may help to combat the disease. This model is at the core of drug repositioning/repurposing strategy.[20] This approach was highlighted by the recent study of Amemiya et al for Dengue hemorrhagic fever, which identified 8 candidate drugs through drug repositioning. Notably, 3 of these drugs appeared in our identified drugs (sirolimus, valproic acid and estradiol) as shown in our results below. We felt there is a need for a study that includes sufficient number of patients to perform our analysis. Due to time- constraints, we chose to study previously deposited data. We hope this work will help researches identify new agents of potential use to treat COVID-19 and related illnesses.

Results

GSE5972 provides logarithmically transformed gene expression values for a group of patients who presented to Toronto area hospitals. They had had positive PCR and/or seroconversion as evidence of their SARS-CoV infection.[21] In total, there were 70 sets; 10 for healthy controls (median age, 28.5 years; Females, 50%), 5 patients had samples during convalescence (6 samples total, median age, 42 years, Females 100%, median days since onset, 27 day), and 35 patients with samples labelled as pre-O2-nadir (26 samples) and post-O2-nadir (28 samples). We selected samples labelled as “pre-O2-nadir” and labelled them as “sick”. There were 19 individuals in this group (26 Samples, median age, 49.9 years, Females, 79%, median days since onset, 4 days).

We studied 4 analysis sets of gene expression: 1- top differentially expressed genes in sick vs. convalescent samples (N = 100), 2- Genes with absolute LFC>1 and FDR<0.05 that were differentially expressed in sick vs. convalescent samples (N = 601), 3- top differentially expressed genes in sick vs. healthy samples (N = 100), and 4- Genes with absolute LFC>1 and FDR<0.05 that were differentially expressed in sick vs. healthy individuals (N = 272).

Heat maps with 5 gene clusters were generated using cogen functions. These clusters were subjected to KEGG pathway enrichment. As expected, sets with lower number of genes were less likely to show significant enrichment. Gene enrichment results for the 4 analysis sets are shown in table 1. Subsequent drug repositioning search focused on all up- regulated genes (analysis set 1), cluster 1 (analysis set 2), all down-regulated genes (analysis set 3) and clusters 1 (analysis set 4). Identified drugs were then used for Pubmed search and the number of hits was recorded. Drugs with identified hits were then searched manually and synopsis of their role as antiviral medications is mentioned in the discussion below.

Table 1. Results of Differentially Expressed Genes Enrichment Analysis and Drug Repositioning.

Drugs	Class	Score	Pubmed SARS OR MERS OR coronav	Pubmed antiviral OR antivirus	Pubmed Virus OR viral	Pubmed Hsp90 OR Autoph*
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Set 1: top differentially expressed genes in sick vs. convalescent samples (N=100 genes)

KEGG Regulation of actin cytoskeleton

Selected cluster	Up regulated genes					
	Etacrynic acid	Loop diuretic	9.2	2	4	18
	Omeprazole	Proton pump inhibitor	9.2	0	31	79
	Captopril	ACE inhibitor	7.5	0	6	62
	Diphepanil metilsulfate	Anticholenergic	7.5	0	0	0
	Cinchorocaine	Amide local anesthetic	7.5	0	0	0
	Sirolimus	mTOR inhibitor	7.5	6	159	842
	Tolnaftate	Synthetic antifungal	7.5	0	1	1
	ikarugamycin	Natural inhibitor of endocytosis	6.6	0	0	1
	clozapine	Atypical antipsychotic	6.6	1	14	137
	chlorpromazine	Antipsychotic, antiemetic	6.6	8	30	245
	lumicolchicine	Antiinflammatory, anti-tubulin	6.6	0	0	4
	pheniramine	Antihistamine, anticholinergic	6.6	0	2	21
	procaine	Local anesthetic	6.6	0	7	73
	morantel	Antihelmintic	6.6	0	0	1
	sulfadiazine	Antibiotic	6.6	0	21	122
	atropine oxide	Anticholinergic	6.6	1	1	3
	dihydroergotamine	Ergot alkaloid, migraine	6.6	0	2	8
	alpha-estradiol	Weak estrogen	6.6	0	21	222
	iopromide	Contrast media	6.6	0	0	7

Set2: Genes with absolute LFC>1 and FDR<0.05 that were differentially expressed in sick vs. convalescent (N=601)

KEGG Porphyrin and chlorophyll metabolism, Focal adhesion, Ribosome, Complement and coagulation cascades

Selected cluster	Cluster 1					
	Dizocilpine	Anticonvulsant	6.9	1	5	56
	Vincamine	Vinca alkaloid, dementia	5.4	0	0	0
	Nilutamide	Nonsteroidal antiandrogen	5.4	1	0	7
	Exemestane	Aromatase inhibitor	4.8	0	2	4
	Carbamazepine	Anticonvulsant	4.8	1	33	203
	Calcium Pantothenate	Vitamin B5	4.8	1	3	31
	Triflupromazine	Antipsychotic	4.8	0	0	0
	Minaprine	MAO inhibitor, antidepressant	4.8	0	0	0
	Nomifensine	Norepinephrine uptake inh.	4.8	0	0	3
	Sirolimus	mTOR inhibitor	4.8	6	159	843
	Trichostatin A	antifungal, inhibit HDAC	4.8	2	24	362
	Benzamil	Benzyl amiloride, ENac	4.8	0	0	1

Emetine	inhibitor Antiprotozoal	4.8	1	13	69	9
Nabumetone	NSAID	4.8	0	2	2	0
phensuximide	Anticonvulsant	4.8	0	0	0	0
pioglitazone piotamide	Antidiabetic	4.8	1	21	64	17
	Platelet aggregation inhibitor	4.8	0	0	0	0

Set3: top differentially expressed genes in sick vs. healthy samples (N=100)

KEGG Ribosome, Antigen processing and presentation, Prion disease, FC-gamma-R-mediated phagocytosis, MAPK signalling pathway, phosphatidylinositol signalling, cell adhesion molecules CAMS, Huntington's disease, Endocytosis, Lysosome

Selected cluster	Down regulated genes Colchicine Tanespimycin						
		Antiinflammatory, anti-tubulin	6.7	14	62	593	80
	Sulfaphenazole	Antibiotic, anticancer	6.7	0	12	44	669
	Sulfaguanidine	Antibiotic, sulfonamide	6.7	0	0	12	1
	Trimethobenzamide	Antibiotic, sulphanilamide	6.7	0	2	4	0
	Butamben	Antiemetic	6.7	0	0	2	0
	Ethaverine	Local anesthetic	6.7	0	0	0	0
	Rescinnamine	opioid, vasodilator	6.7	0	0	0	0
	15-d prostagl-J2	ACE inhibitor, vinca alkaloid	6.7	0	0	0	0
	Tocainide	PRAP agonist, anti-inflammatory	6.7	0	2	18	10
	Metformin	Antiarrhythmic agent	6.7	0	0	0	0
	Copper sulfate	Antidiabetic	6	0	49	215	329
	Valproic acid	Salt, toxin	6	1	9	64	18
	Novobiocin	Antiepileptic	6	2	444	296	84
	Alvespimycin	Antibiotic	6	1	18	152	147
	LY-294002	Antibiotic, anticancer	6	0	5	15	215
	geldanamycin	Inhibitor of PI3Ks and others	6	3	17	214	101
		Antibiotic, anticancer	6	1	32	101	1373

Set4: Gense with absolute LFC>1 and FDR<0.05 that were differentially expressed in sick vs. healthy (N=272)

KEGG Ribosome, Lysosome, Antigen Processing and Presentation, oocyte meiosis, neurotrophin signalling pathway, prion diseases, primary immunodeficiency, regulation of actin cytoskeleton, Fc-gamma-R-mediated phagocytosis, spliceosome, chemokine signalling, focal adhesion

Selected cluster	Cluster 1						
	Tanespimycin	Antibiotic, anticancer	6.4	0	12	44	669
	Alvespimycin	Antibiotic, anticancer	6.4	0	5	15	215
	Propylthiouracil	Hyperthyroidism treatment	4.4	1	3	46	1
	fenbuten	NSAID	3.3	0	0	0	0

MAO monoamine oxidase; HDAC, histone deacetylase; ENaC, epithelial sodium channel; NSAID, nonsteroidal anti-inflammatory drug

Discussion

At the time of writing this manuscript, a rapidly evolving international outbreak of SARS- CoV2 is spreading daily to new communities and the search for effective drugs and vaccines is ongoing, but will require time. Even when a new drug is discovered and manufactured, demand may rapidly outstrip supply if the epidemic continues to grow. Identifying and repositioning existing drugs to treat COVID–19 infections may provide a rapid, inexpensive option with known toxicities. [10, 12, 14, 17, 22]

The purpose of our study was to evaluate the levels of expression of genes in patients while they are sick and compare them to healthy controls and convalescent patients. Genes with different levels of expression reflect pathways that are used by the host in response to viral infection (sick vs. Convalescent) or pathways that are dysregulated by the infection (sick vs. Healthy).[22] Similar to all bioinformatics pipelines, parameter and algorithm selection impact results, and may not be optimal even after tuning, which makes use of a black-box approach for drug repositioning less appealing. [23] However, despite limitations, this approach to identify drugs that interact with differentially expressed genes has proven useful in many cases.[20] The algorithm we used in this paper, cogena, overcomes several limitations of this approach. It clusters genes and enriches the clusters using established ontologic pathways, and thus is more likely to find clinically-relevant drugs. In pursuing this analysis, two questions must be addressed: First, which patient group is more suitable for comparison with sick patients, healthy or convalescent patients? One may argue that healthy controls were not exposed to the virus and identifying genes with different levels of expression in this group does not reflect the physiologic pathways that lead to recovery, which are of great importance in this current problem. The second question concerns the selection of the number of differentially expressed genes for drug repositioning. Obviously, including more genes will yield more pathway enrichment. However, increasing the number of used genes will add more confusion to the model. Many differentially expressed genes reflect pathways that are related to other physiologic processes (e.g. improved tissue nutrition, oxygenation and perfusion). To cover all these possibilities we decided to include 4 analysis sets of gene expression.

It is very impressive that different drug repositioning approaches lead to similar results. For example, our study did not show chloroquine, which was suggested before to be active in treating SARS-FCoVs. [4]

Interestingly, other drugs with antimalarial activity were identified, including sirolimus, chlorpromazine, sulfadiazine, pheniramine, colchicines, metformin, ionomycin and calcium pantothenate. Additionally, when the gene set of sick vs. convalescent (analysis set 1) was altered to include the top 50 DEG (differentially expressed genes) only, chloroquine appeared on the list, possibly as the focus on the very top differentially expressed genes eliminated less important pathways. Again, this shows the robustness of this approach, but the need to refine its work. We claim that our choice for the cogen framework is justified as it provides an unmatched level of transparency.

SARS-FCoVs obtain entry into the cells by attaching to ACEII surface protein. Moving the virus into the cell depends on clathrin-mediated endocytosis.[9] Identified drugs that interfere with endocytosis in our study are ikarugamycin, triflupromazine, clozapine and chlorpromazine; the last three drugs are typically used as antipsychotics. Acidification of vacuoles to eliminate the spike protein is an essential step in activating the virus.[9] This can be repressed by inhibiting v-ATPase activity to block the vacuolar protein pump. Omeprazole, an H⁺K⁺ proton pump inhibitor inhibits v-ATPase at higher concentration.[24] Additionally, chloroquine is known to increase the pH of endosomes.[25] Colchicine and vinca alkaloids interact with cellular microtubules, which cause inhibition of lysosomal formation.[26] Non-steroidal anti-inflammatory drugs (e.g. Nabumetone) exhibit their antiviral/antitumor effect by via inhibition of endoplasmic-reticulum-resident protein kinase (PERK) mediated phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2A (eIF2a). This leads to protein synthesis inhibition and cellular death. [27] Etacrynic acid, a drug identified in analysis set 1, was shown before to be a suitable inhibitor of the main coronavirus protease (M pro) due to its electrophilic binding of its active domain. This binding inhibits the function of this vital protease which cleaves viral proteins at 9 specific sites in preparation for viral assembly. [28] Amiloride derivatives interacts with protein ion channel (protein E of coronavirus) which prevents viral formation and stops replication, at least in “in-vitro” models.[29] Figure 1 shows a simplified representation of some of the drugs we identified and their mode of action.

One drug of great interest that was identified in our study was captopril. This commonly used antihypertensive medication from the Angiotensin-converting enzyme (ACE) inhibitor class works by blocking the active domain of this enzyme, leading to decreased conversion of angiotensin I to angiotensin II. Another rarely used ACE inhibitor appeared in analysis set 3, rescinamine; which is a vinca alkaloid derivative. While the SARS-FCoVs get access to cells via the ACE-II protein, which carries similarity to ACE-I protein as more than 60% of their sequences are identical. We wonder why captopril was never studied in-vitro or in-vivo to treat coronaviruses. Using this drug in particular, despite proposed benefit, should be only attempted in non-human models first. Prolonged use of captopril is associated with increasing levels of ACE-II. Whether this could possibly lead to enhanced entry of the virus is a question that can only be answered through careful preclinical tests.

Geldanamycin and its derivatives (tanespimycin and alvespimycin) were identified in analysis sets 3 and 4. Geldanamycin is an antibiotic that is believed to be targeting the ADP/ATP binding site of the Heat-shock protein (HSP90). It was shown to be targeting many viruses, including SARS coronavirus, where it inhibits SARS replication with a selectivity index of >300 and an EC₅₀ of 0.91 μM; for comparison the

EC50 for chloroquine is 8.8uM. The drug was submitted for a Chinese patent (application 03146591, July 2003).[30] Interestingly, geldanamycin is produced by soil bacteria, *streptomyces hygroscopicus*, the same source of sirolimus.

In a recent study that evaluated 87 drugs effective in MCF7 breast cancer epithelial cells, 17-allyloamino-geldanamycin, LY294002, valproic acid, trichostatin A and sirolimus (in addition to wortmannin), were evaluated using pathway gene enrichment. Two pathways (ERBB_signaling and Insulin_signaling) were the 2 most combined pathways.[31] Wortmannin, another PI3K inhibitor, similar to LY294002, is known to inhibit RSV replication by inhibiting autophagy, a process that favors viral replication by inhibiting apoptosis.[32] This finding is also supported by wortmannin inhibition of coxsackievirus replication in infected mice treated with a histone-deacetylase which induced replication by inhibiting autophagy and inducing apoptosis.[33]

Geldanamycin is a benzoquinone with known antimalarial activity. It belongs to a group of drugs called ansamycins. One of the potent antiviral ansamycins is rifampin. This commonly used antibiotic has a known antiviral function due to its inhibition of DNA- dependent RNA polymerase.[34] This selective action makes rifampin of no value in treating RNA viruses, like coronavirus. To the contrary, geldanamycin is a potent HSP90 inhibitor and works on a broad spectrum of viruses through modifying host response.[30] While effective in treating resistant multiple cancer types, hepatotoxicity of geldanamycin limited its use and lead to the development of derivatives with less toxicity and improved oral bioavailability, namely tanespimycin and alvespimycin.[35]

Autophagy, a process where the internal cellular components degenerate, with or without integration with lysosomes, resulting in autophagosomes, which are hijacked by some viruses and used as safe sanctuary. This is opposed by programmed cell death, or apoptosis, which programmatically destroys the cell and calls for its clearance. Several studies showed the ability of SARS-CoV to induce autophagy.[36, 37] Notably, autophagy plays a role in cancer resistance to chemotherapy and modulation of its components may restore drug sensitivity.[38] Chloroquine and hydroxychloroquine show promising results in restoring chemotherapy sensitivity by modulating autophagy.[39] It is suggested that chloroquine function as antimalarial and antitumor drug can be linked to Hsp90 inhibition with secondary modulation of autophagy, among other pathways. [40]

An estrogen (alpha-estradiol), an anti-androgenic drug (nilutamide) and an aromatase inhibitor (exemestane) appeared on the analysis set 1 and set 2. This is noteworthy, particularly as the SARS-FCoVs are known to cause more severe infections in males and are associated with higher mortality; a phenomenon that proved true in male mice and female mice treated with estrogen receptor antagonist. [41] Complex interaction between estrogens, Hsp90 and lysine methyltransferase (SMYD2) point to the interaction of sex hormones with the function of this molecular chaperone in modulating autophagy.[42] Antidiabetic drugs (metformin, pioglitazone and possibly acetohexamide) restrict viral replication by activating AMP-activated protein kinase which modulates autophagy.[43, 44]

Multiple sulfonamides were identified, including antibiotics (sulfaphenazole, sulfaguanidine, sulfadiazine). In addition, diphemanil metisulfate and copper sulfate are sulfur containing drugs. Of note, the family of sulfonamides includes multiple drugs that are potent antivirals.[45] Many sulfonamides are known to inhibit the ATP-binding pocket of Hsp90.[46] Sirlomus, also known as rapamycin, is a macrolide mTOR inhibitor and was reported in analysis sets 1 and 2. Sirolimus is known to have antiviral effect on multiple viruses, including CMV, HIV, EBV and others.[47–49] Interestingly, the mTOR pathway functions in parallel with Hsp90 in modulating autophagy.[50] Inhibition of both pathways is synergistic in infected cells. The suggested link between these pathways involves viral-induced activation of the Hsp90 client, Akt, which in turn phosphorylates mTOR to facilitate translation of viral mRNA.[51] All of these identified drugs highlight Hsp90 as a druggable target that deserves more attention (Fig 2).

Based on our findings, we suggest adding more drugs to the experimental arsenal deployed against SARS-FCoVs. We suggest using nontoxic drugs that are readily available for wide scale prophylaxis or the treatment of mild cases of COVID–19. These drugs would include omeprazole, nonsteroidal anti-inflammatory drugs, colchicine, sulfoanamide antibiotics, antimalarial drugs and clozapine/chlorpromazine. For patients with serious COVID–19 illnesses, the above drugs can be combined with immune modulators like sirolimus, and direct Hsp90 inhibitors under well-designed clinical trials. Given the high pathogenicity of the current ongoing epidemic and its potential to become a pandemic,[52] it seems prudent to attempt to use multiple drugs in combination if low risk for toxicity and drug-drug interaction is established. Choosing a combination of drugs identified in this study or other drug-repositioning studies and careful reporting of the efficacy of these experiences can result in rapid accumulation of knowledge.

In conclusion, we showed that a drug repositioning framework using simple and unambiguous methodology can yield a plethora of drugs that are active for SARS-FCoVs. We believe that a combination of some of these relatively nontoxic drugs can be worth trying (Fig 3). Serious efforts should be shifted to Hsp90 and autophagy pathways. Multiple candidate drugs can be of great value; most notably, geldanamycin and its derivatives. Hsp90 inhibitors are being studied for cancer patients. It seems there is an urgent need to focus on this important pathway to overcome the ongoing outbreak.

Methods

We searched for a well annotated dataset and identified an efficient algorithm to generate a list of drugs that are potentially useful to treat this disease. [23] Our methodology is based on classifying patients into “sick”, “convalescent” and “healthy controls” categories; followed by identifying gene signatures that are amenable for drug-repositioning analysis.

After searching the Gene Expression Omnibus (GEO) for the transcriptomic data related to SARS-FCoVs, GSE5972 was identified as a suitable candidate. Gene expression of 40 individuals infected with SARS and 10 healthy controls were included in our analysis. Studied RNA was extracted from peripheral blood mononuclear cells. Expression levels were analyzed using the noncommercial UHNMAC Homo sapiens

19K Hu19Kv8 microarray platform. Applied microarray procedures are mentioned in the study paper.[21] Phenotypic data provided with the dataset included a unique patient identifier, gender, age, days-since-onset of symptoms, and status. This latter variable indicated if the sample was collected at time of active infection (pre-O2 nadir and post-O2 nadir), or convalescence. Data was submitted to NCBI on October 5th, 2006 and was made available on July 31st, 2007. We downloaded this file on February 20th, 2020.

For the purpose of differential gene expression, samples in the dataset were classified into sick (pre-O2 status, N = 26), convalescent (N = 6 samples) and healthy (N = 10). The Limma package and R code provided by the GEO analyzer were used to perform differential gene expression (DGE) of sick vs. convalescent patients and sick vs. healthy. The choice of these 2 settings was simply to compare gene expression of sick patients to those recovering, and to healthy noninfected controls. While the first setting is more interesting as it reflects pathways used by the host to overcome this serious infection, the influence of different medications received by patients and their impact on gene expression was considered a drawback.

The resulting top tables with differentially expressed features were filtered for duplicates; only rows with lowest false discovery rate (FDR), equivalent to adjusted p-values, were counted when a gene had multiple records. Rows of features with no gene name were excluded. The resulting top tables for the 2 settings (sick vs. health, sick vs. convalescent) were then filtered using 2 different parameters: 1- Selection of the top 100 genes of each analysis set, based on lowest-to-highest FDR; and, 2- Selection of all genes that had an absolute log-fold-change more than 1 -indicating doubling in one direction, and $FDR < 0.05$ indicating significant DGE. The resulting model yielded 4 analysis sets of differentially expressed genes. As a first step, principal component analysis (PCA) was used to confirm proper clustering of samples using the selected gene sets.

Drug repositioning and Kyoto-Encyclopedia-for-Genes-and-Genomes (KEGG) pathways enrichment was conducted using cogena (co-expressed gene-set enrichment analysis) package, available via Biocoductor platform. The methods used to deploy this package are illustrated in the package vignette, its manual, and its publication. [23] In summary, after feeding cogena with each set of differentially expressed genes, hierarchical and partitioning around medoids (PAM) clustering of genes resulted in defining clusters of genes that had similar differential expression. After multiple attempts, the number of clusters was set to 5 in order to keep consistency and enhance downstream enrichment analysis, i.e. identify the best number of enriched pathways. We attempted to use 3 to 10 clusters for each analysis set, enriched clinically relevant pathways using the reactome gene set (based on best practices when using cogena), then selected 5 clusters for each analysis set as shown in extended Fig1,2,3,4. Enrichment of these clusters using KEGG pathways was conducted and visually inspected. This enrichment used correlation distance metric to set up the pathway analysis. The resulting genes were grouped into 2 “Up” or “Down” expressed genes with reference to the sick group. If a cluster was found to be significantly enriched in KEGG pathways that are related to viral infection, it was selected for drug repositioning. Otherwise, the group of “Up” or “Down” regulated genes, as a whole, was selected based on the predominant pattern of expression. The package has pre-built drug sets that are imported from the Molecular Signatures

Database (Msigdb) [53] and Connectivity Map.[54] Together, the set includes 6100 instances of 1309 drugs and chemicals, with many drugs having more than one instance.

Resulting drugs were then queried using easy PubMed package.[55] Three queries were iterated for all drugs: 1- drug AND (coronavirus OR SARS OR MERS), 2- drug AND (antiviral OR antivirus), 3- drug AND (virus OR viral). A fourth additional search was added based on further analysis: drug AND (Hsp90 OR autophagy). For queries with resulting hits, further review of hits with brief synopsis was conducted. This was not intended to be a comprehensive literature review of these drugs but rather an exploratory search. Results of this search were used to write the discussion.

In order to find recent interventional papers that address recently evaluated drugs, the following search was used: (Drug OR Therapy OR Medication) AND (SARS OR MERS OR Coronavirus OR COVID-19 OR SARS-CoV2) with publication data filter set from 01/01/2020 to 02/28/2020. Data was analyzed using R software v3.6.1. A Markdown file with code that was used to download the dataset, conduct Limma analysis, PCA, perform cogen analysis (including pathway enrichment, heatmap clustering and drug repositioning), and search Pubmed is available online.

Declarations

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Authors contributions:

I.S., concept, design, analysis, writing first draft, approving final draft S.H., concept, approving final draft

A.T., concept, design, approving final draft

Competing interests:

No competing interests for any of the authors.

Data availability:

Data used for this study is already available through the NCBI-GEO website.

Supplementary material:

All code used in this study is available as a Markdown file.

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Figures

Figure 1

A simplified cartoon showing suggested pathways that some of the drugs indentified in our study exploit. Some drugs work on more than one pathway.

Figure 2

Interactions between the Heat Shock Protein (Hsp90) and different drugs, placing Hsp90 and its action by modulating autophagy vs. apoptosis at center of host response. Example references retrieved by our Pubmed search are mentioned by their Pubmed unique identification number (PMID).

Figure 3

Categories of drugs used to treat SARS-FCoVs according to our analysis.

Supplementary Files

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