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Review

Drug repurposing for the treatment of COVID-19

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ABSTRACT

Coronavirus disease 2019 (COVID-19) remains prevalent worldwide since its onset was confirmed in Wuhan, China in 2019. Vaccines against the causative virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have shown a preventive effect against the onset and severity of COVID-19, and social and economic activities are gradually recovering. However, the presence of vaccine-resistant variants has been reported, and the development of therapeutic agents for patients with severe COVID-19 and related sequelae remains urgent. Drug repurposing, also called drug repositioning or eco-pharma, is the strategy of using previously approved and safe drugs for a therapeutic indication that is different from their original indication. The risk of severe COVID-19 and mortality increases with advancing age, cardiovascular disease, hypertension, diabetes, and cancer. We have reported three protein–protein interactions that are related to heart failure, and recently identified that one mechanism increases the risk of SARS-CoV-2 infection in mammalian cells. This review outlines the global efforts and outcomes of drug repurposing research for the treatment of severe COVID-19. It also discusses our recent finding of a new protein–protein interaction that is common to COVID-19 aggravation and heart failure.

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1. Introduction

In late 2019, COVID-19 rapidly became prevalent throughout the world.[1,2] Since then, many related studies have been carried out, such as decoding the genome of SARS-CoV-2[3] and elucidating its infection patterns and disease aggravation mechanisms.[4–8] Various COVID-19 sequelae such as vasculitis, pulmonary fibrosis, heart failure, and loss of smell and taste have been reported.[9] Although SARS-CoV-2 vaccination campaigns are underway,[10] several SARS-CoV-2 variants have emerged, and each has caused a wave of infections.[11–15] Worryingly, increasing frequencies of cryptic SARS-CoV-2 lineages not found in GISAID's EpiCoV database have been detected in New York City wastewater. These variants might enable the transfer of SARS-CoV-2 from humans to other animals.[16] Therefore, there is an urgent need to find targets and therapeutic candidates that differ from existing therapeutic drugs.

SARS-CoV-2 invasion of human cells occurs through the following pathway. First, the SARS-CoV-2 spike protein binds to the host receptor, angiotensin-converting enzyme (ACE)2.[4,5] The spike protein is then cleaved by the proteolytic enzyme TMPRSS2 and becomes activated to promote fusion between the outer viral membrane and the host cell membrane, resulting in virus internalization through endocytosis.[5] Severe COVID-19 was initially reported to manifest as acute respiratory distress syndrome (ARDS),

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which is a severe disorder of lung function.[17,18] Several factors have been identified to increase the risk for COVID-19 aggravation; these include advanced age, smoking, and pre-existing diseases such as cancer, diabetes, heart failure, and hypertension.[8,19–22] Because ACE2 is ubiquitously expressed,[23,24] COVID-19 symptoms can occur in organs other than the lungs. As many as 25% of SARS-CoV-2-infected patients have cardiac dysfunction,[25] which is a primary symptom of COVID-19 aggravation. Because ACE2 is expressed in heart tissue, it has been suggested that SARS-CoV-2 can directly infect cardiomyocytes to impair cardiac function.[26–28] In addition, the ACE2 expression level was found to be increased in the lungs of infected patients, and genome-wide analyses have shown that ACE2 expression is low in non-infected people.[29,30] These findings suggest that the ACE2 expression level reflects one's susceptibility to SARS-CoV-2 infection. Viral RNA has been detected in cardiomyocytes taken from heart specimens of COVID-19 patients, revealing that SARS-CoV-2 can infect heart tissue.[31]

Drug repurposing, also known as drug repositioning or ecopharma,[32] is an effective and rapid way to identify new uses of existing drugs that have a well-established safety profile. Drug repurposing can also be cost-effective for treating disease outbreaks and disease progression. At present, there are 7605 clinical trials (including 2030 drug trials and 685 vaccine trials) registered in ClinicalTrials.gov related to COVID-19.[33] Indeed, several existing drugs have been approved or are in clinical trials for the treatment of COVID-19 (Table 1). However, many patients still suffer from COVID-19 aggravation and sequelae; thus, there is a need for the rapid repurposing of drugs with mechanisms of action other than those currently approved for treating COVID-19. In this review, we discuss our efforts related to drug repurposing, focusing on the treatment of heart failure and their relation to finding new treatments for severe COVID-19.

2. Drug repurposing research for the treatment of COVID-19

Several drugs that have been previously approved to treat other diseases are being repurposed for treating COVID-19 patients; these include hydroxychloroquine, lopinavir/ritonavir, favipiravir, remdesivir, ivermectin, dexamethasone, camostat, and mesylate (Table 1).[34–38] These drugs can be broadly classified into four groups according to their mechanism of action: inhibitors of RNA-dependent RNA polymerase; inhibitors of serine protease (TMPRSS2); inhibitors of the main cysteine protease $3CL^{pro}$; and inhibitors of inflammation and cytokine storm (Fig. 1). Chloroquine and hydroxychloroquine have anti-parasitic activity by increasing the lysosomal pH; these drugs have been used for over 70 years worldwide[39] and also have immunosuppressive effects. Remdesivir was originally developed as a therapeutic agent for Ebola virus infection and exerts an antiviral effect by inhibiting viral RNA-dependent RNA polymerase.[40] To date, four randomized controlled trials that have included a placebo have been conducted to test remdesivir. In Japan, remdesivir was approved as an anti-COVID-19 drug on May 7, 2020. Favipiravir has been approved for treating new or re-emerging influenza virus infection.[41] This drug is converted to its triphosphorylated form *in vivo*, which selectively inhibits virus-derived RNA-dependent RNA polymerase. Camostat and nafamostat inhibit proteolytic enzymes, including TMPRSS2, and can ameliorate the acute symptoms of pancreatitis. They can also prevent coagulation of perfused blood during extracorporeal circulation in patients with hemorrhagic lesions.[42,43] Lopinavir and ritonavir are protease inhibitors and are used as an antiretroviral medication for the treatment and prevention of HIV/AIDS.[44] A fixed-dose combination of lopinavir/ritonavir is thought to block the main cysteine protease of SARS-CoV2. Ritonavir is thought to boost the lopinavir concentration by inhibiting CYP3A4.[45] The anthelmintic drug ivermectin, which is approved for the treatment of intestinal sickness and scabies, is thought to inhibit SARS-CoV-2 proliferation.[46] Dexamethasone is a steroid originally approved for the treatment of severe infections and interstitial pneumonia. Patients with severe COVID-19 develop a systemic inflammatory response that can lead to lung injury and multiple organ failure, suggesting that the anti-inflammatory effects of steroids prevent or suppress the inflammatory response.[47] Other anti-inflammatory drugs target cytokine storm after SARS-CoV-2 infection.

3. Cardiac dysfunction as a major symptom of COVID-19 severity and sequelae

Coronaviruses are known for their impact on the respiratory tract, but SARS-CoV-2 can also infect heart tissue,[25–28] which leads to a spectrum of cardiac manifestations, including inflammation (myocarditis), arrhythmias, heart attack-like symptoms, and heart failure.[48] The tropism of organs has been studied from autopsy specimens. Sequencing data revealed that SARS-CoV-2 genomic RNA was highest in the lungs, but the heart, kidney, and liver also showed substantial amounts.[49] Greater than 1000 copies of SARS-

Table 1
Candidate approved drug for the treatment of COVID-19.

Name	Mechanism of action	Target diseases
Hydroxychloroquine	Increasing lysosomal pH	Parasitic infection
Lopinavir/Ritonavir	Inhibition of the HIV protease	AIDS
Favipiravir	Inhibition of viral RNA-dependent RNA polymerase	Pandemic influenza
Remdesivir	Inhibition of viral RNA-dependent RNA polymerase	Ebola virus disease
Ivermectin	Activating glutamate-gated chloride channels	Parasitic infection
Dexamethasone	Corticosteroid	Severe allergies etc.
Camostat mesylate	Serine protease inhibition	Chronic pancreatitis
Baricitinib	JAK inhibition	Rheumatoid arthritis
Tocilizumab	IL-6 inhibition	Rheumatoid arthritis
Mavrilimumab	Human monoclonal antibody against GM-CSF	Rheumatoid arthritis
Azithromycin	Inhibition of bacterial protein synthesis	Bacterial infection
Thalidomide	IL-6 inhibition	Cancers
Methylprednisolone	Corticosteroid	Inflammation
Pirfenidone	Suppression of fibroblast proliferation and collagen production	Idiopathic pulmonary fibrosis
Bromhexine hydrochloride	Increasing of serous production in the respiratory tract and pulmonary surfactant secretion	Bronchitis
Bevacizumab	Human monoclonal antibody against VEGF	Cancers
Fluvoxamine	Inhibition of selective serotonin reuptake	Depression
Ibudilast	PDE3A, PDE4, PDE10 and PDE11 inhibition	Bronchitis

HIV: human immunodeficiency virus, AIDS: acquired immunedeficiency syndrome.
JAK: Janus kinase, IL: interleukin, GM-CSF: granulocyte macrophage colony-stimulating factor.
VEGF: vascular endothelial growth factor, PDE: phosphodiesterase.

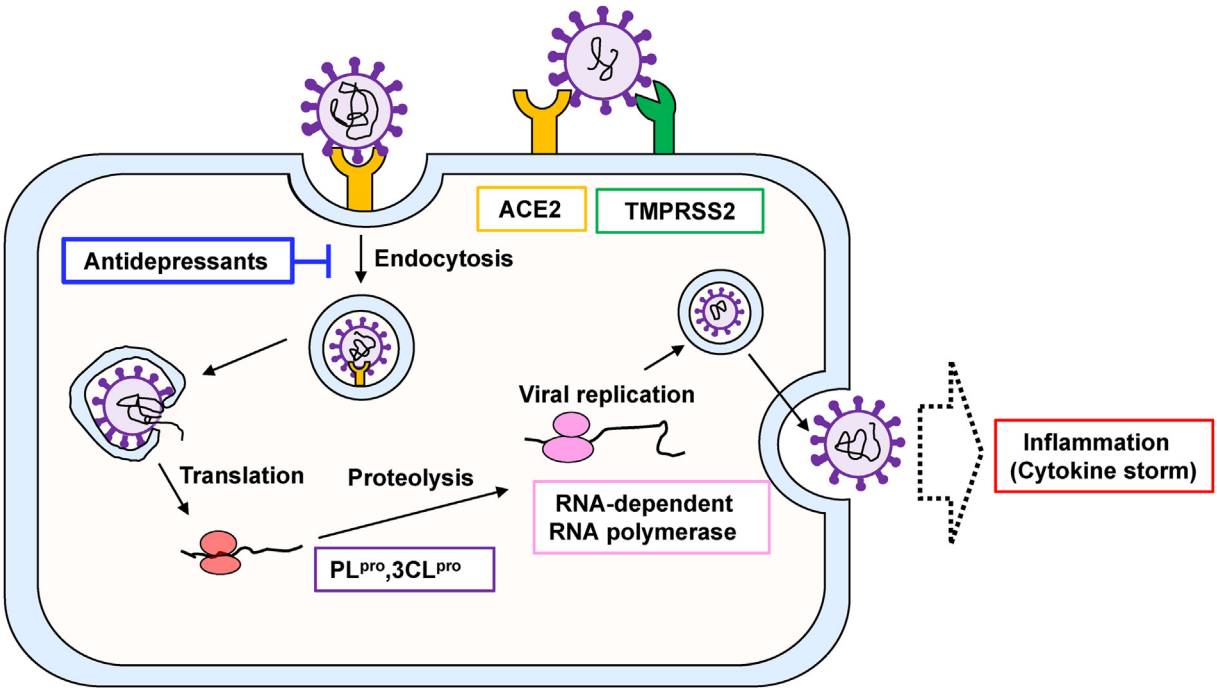

Fig. 1. Possible drug discovery targets for developing COVID-19 treatments. PL^{pro}: papain-like protease, CL^{Pro}: 3-chymotrypsin-like protease (main protease), ACE2: angiotensin-converting enzyme 2, TMPRSS2: transmembrane protease, serine 2.

CoV-2 virus were detected in the heart of 31% of patients who died from COVID-19.[31] In addition, a cohort study of 153,760 individuals with COVID-19 included in a national health care databases from the US Department of Veterans Affairs found that beyond the first 30 days after SARS-CoV-2 infection, individuals who has symptomatic COVID-19 were at increased risk of many types of incident cardiovascular disease, including arrhythmias, ischemic heart disease, myocarditis, and heart failure, indicating the long-term cardiovascular outcomes of COVID-19.[48]

SARS-CoV-2 gains entry into human cells by binding to ACE2, the metallocarboxyl peptidase angiotensin receptor.[50] ACE2 is a key enzyme that converts angiotensin (Ang) II to Ang 1–7, which in turn binds to the Mas receptor to negatively regulate Ang II-dependent signaling in the cardiovascular system.[50] There are two isoforms of ACE2.[51] The full-length ACE2 is located on the plasma membrane and contains the receptor binding site for the SARS-CoV-2 spike protein. The internalization of membrane ACE2 as a result of SARS-CoV-2 binding is thought to enhance Ang II-dependent signaling and to reduce Ang 1–7/Mas-mediated signaling, resulting in the increased risk of cardiovascular events. ACE2 can also occur as a soluble form that is shed into the circulation. Soluble ACE circulates at low concentrations but also contains the receptor site for the spike protein.[51] Therefore, it is expected that an increase in soluble ACE2 relative to membrane-bound ACE2 may reduce the risk of developing cardiovascular events.

4. Drug repurposing research targeting protein–protein interactions in heart failure

We have reported three pathology-dependent protein–protein interactions (PPIs) that participate in the progression of cardiovascular remodeling in mice (Fig. 2). The first is related to the canonical transient receptor potential (TRPC) proteins, which are thought to be molecular entities of receptor-activated cation channels. We reported that one of the diacylglycerol-activated TRPC isoforms, TRPC3, participates in oxidative stress-dependent cardiac fibrosis and left ventricular dysfunction in pressure-overloaded mouse hearts by forming a protein complex with NADPH oxidase (Nox) 2.[52–55] The basal expression level of Nox2 protein is negatively regulated by the endoplasmic reticulum-associated degradation (ERAD) system. However, the interaction between TRPC3 and Nox2 prevents ERAD-dependent Nox2 degradation, leading to amplification of the Nox2-dependent production of reactive oxygen species (ROS) and ROS-mediated fibrotic signaling in cardiac cells.[53] By screening nearly 1200 approved drugs for their ability to inhibit TRPC3–Nox2 PPI, we found that ibudilast (an anti-asthma drug) potently inhibits the formation of the TRPC3–Nox2 protein complex.[56] Ibudilast is now in a clinical trial for the treatment of ARDS in patients hospitalized with severe COVID-19. Second, we found that G protein-coupled purinergic $P2Y_6$ receptor ($P2Y_6R$) mRNA expression increases with age and can interact with Ang II type 1 receptor (AT1R), which leads to the progression of Ang II-stimulated hypertension.[57] The heterodimerization of AT1R with $P2Y_6R$ preferentially activated Ang II-stimulated G_q protein-dependent signaling, which is required for vascular smooth muscle hypertrophy.[57] We also found that AT1R–$P2Y_6R$ heterodimerization was suppressed by treatment with the $P2Y_6R$ antagonist MRS2578, which has two isothiocyanate (ITC) residues.[57] ITC is electrophilic, allowing it to react with the thiol residue of Cys^{220} located in the 3rd intracellular loop, leading to internalization and ubiquitylation-dependent proteasomal degradation of $P2Y_6R$.[58] This redox-dependent alternative internalization (REDAI) of $P2Y_6R$ is suggested to contribute to the anti-inflammatory effects of ITC-containing compounds in brightly colored vegetables, such as sulforaphane and iberin. As several GPCRs contain the redox-sensitive Cys in the 3rd intracellular loop, the REDAI system of GPCRs is a promising new target for covalent drug development. Third, we revealed that mitochondrial hyperfission occurs in mouse chronic heart failure after myocardial infarction through interaction between dynamin-related protein (Drp) 1 and filamin, an actin-binding protein.[59,60] We also found that cilnidipine, a dihydropyridine-derivative voltage-dependent L/N-type Ca^{2+} channel blocker used as an anti-hypertensive drug,

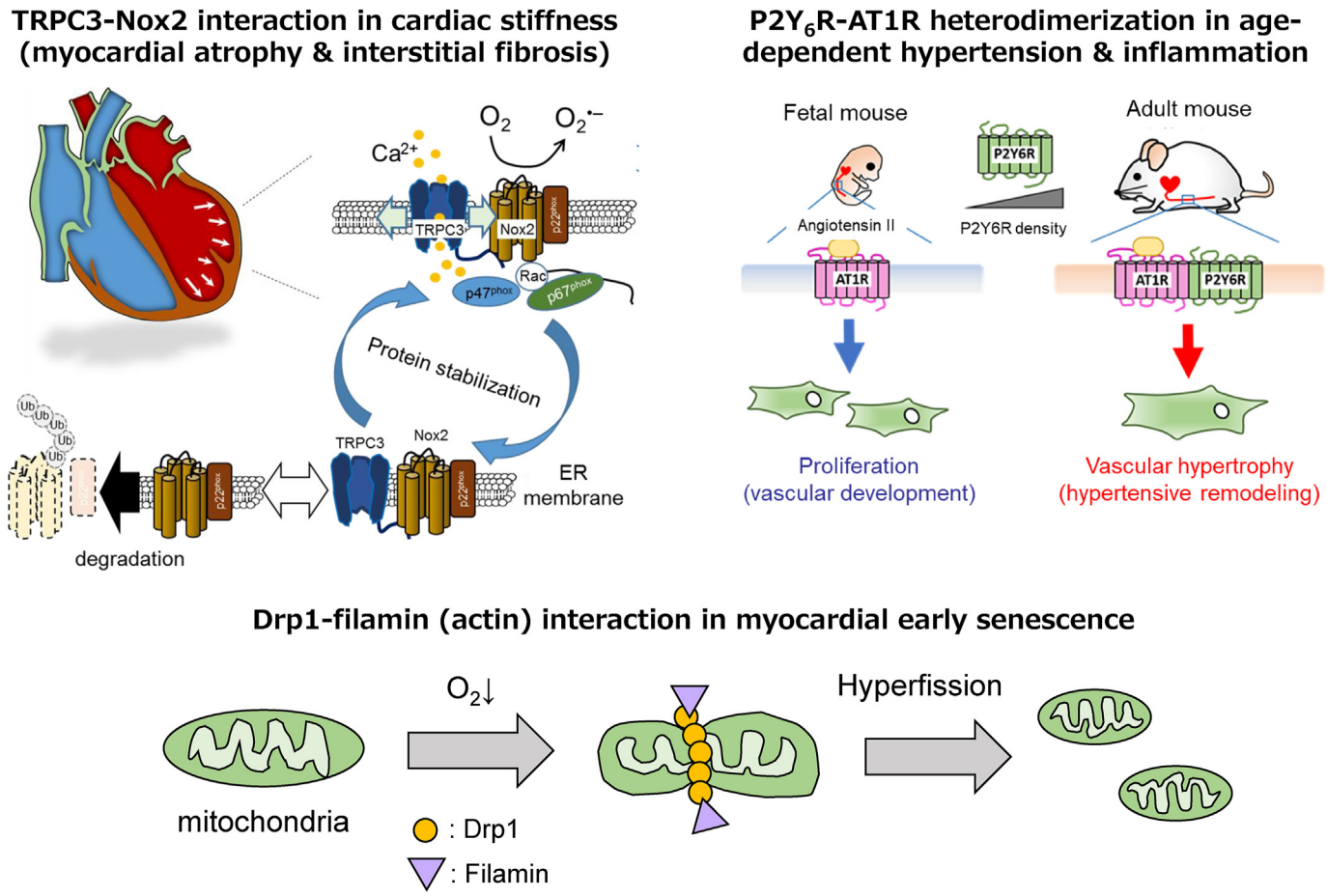

Fig. 2. Protein–protein interactions in pathological cardiovascular tissue that could be targets of drug repurposing efforts. TRPC3: transient receptor potential canonical subfamily member 3, Nox2: NADPH oxidase, $P2Y_6R$: purinergic $P2Y_6$ receptor, AT1R: angiotensin II type 1 receptor, Drp1: dynamin-related protein 1.

potently inhibits hypoxia-induced Drp1–filamin interaction, followed by the induction of myocardial senescence after myocardial infarction.[59] Because mitochondrial fission is also observed in other pathological organs and tissues, cilnidipine might be repurposed for the treatment of mitochondrial fission-associated intractable diseases, such as amyotrophic lateral sclerosis and muscular dystrophy. Although cilnidipine is a quite safe drug and has few side effects, its major Ca^{2+} channel blocking action will be the limitation to the repurposing of this drug for the treatment of mitochondrial fission-associated diseases. Therefore, we now synthesize cilnidipine-based derivatives to create a new compound with the same (or higher) efficacy but with lower Ca^{2+} channel blocking action.

5. ACE2 expression regulation in cardiomyocytes

Studies have verified that SARS-CoV-2 infection affects cardiac function.[25–28] When human iPS cell-derived cardiomyocytes (hiPS-CMs) were infected with SARS-CoV-2, the spontaneous beating of hiPS-CMs was temporarily increased but was subsequently arrested after a few days.[28] The initial spike protein-mediated viral entry process might be the cause of the observed cardiac dysfunction. Therefore, in this review, we focus on how the ACE2 expression level is regulated in cardiomyocytes. Several diseases and lifestyle factors are reportedly risk factors for severe COVID-19; these include hypertension, diabetes, obesity, cardiac disease, older age, smoking, and anti-cancer drug treatment.[8,19–22] We found that the myocardial ACE2 mRNA expression level increased when neonatal rat cardiac myocytes (NRCMs) were exposed to high glucose, doxorubicin (an anthracycline anticancer drug), cigarette side-stream smoke (CSS), and methylmercury. The treatment of NRCMs with ibudilast, but not cilnidipine or ITC-containing compounds, significantly suppressed the CSS-induced increase of ACE2 mRNA, suggesting the involvement of PPIs between TRPC3 and Nox2. Indeed, the increase of ACE2 protein expression in doxorubicin-treated mouse hearts was canceled by *trpc3* gene deletion, and the CSS-induced increase in ACE2 mRNA expression was abolished by silencing *trpc3* and *nox2* genes in NRCMs. These results strongly suggest that the formation of a TRPC3–Nox2 protein complex induced by anticancer drug treatment or CSS exposure would mediate COVID-19 aggravation through ACE2 upregulation. The formation of the TRPC3–Nox2 protein complex amplifies Nox2-dependent ROS production by stabilizing the Nox2 protein. We previously reported that Nox2-dependent ROS production mediates cardiomyocyte atrophy accompanied by E3 ubiquitin ligase, atrogin-1, and muscle RING finger 1 upregulation.[53,55,56] Thus, Nox2-dependent ROS protein may be involved in ACE2 upregulation in rodent cardiomyocytes induced by risk factors for severe COVID-19 (Fig. 3).

6. Antidepressants inhibit spike protein-induced ACE2 internalization

ACE2 receptor-dependent endocytosis is thought to be the main SARS-CoV-2 entry pathway.[4,5] Several antidepressant drugs have

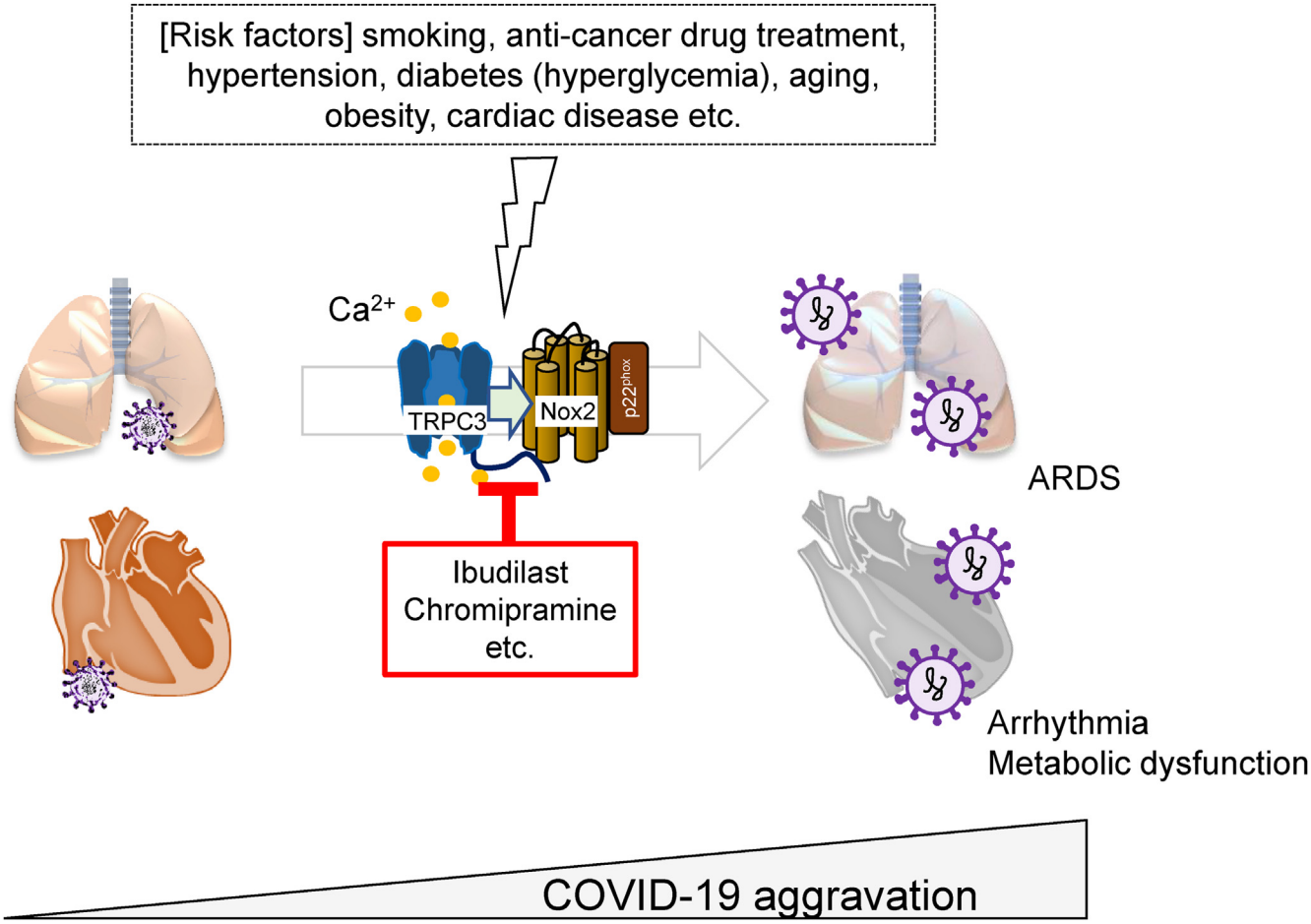

Fig. 3. Risk factor-induced TRPC3–Nox2 complex formation mediates COVID-19 aggravation. ARDS: acute respiratory distress syndrome.

been reported to inhibit SARS-CoV viral entry by inhibiting clathrin-dependent endocytosis.[61–63] We found that clomipramine, a tricycle anti-depressant drug, and trifluoperazine, a phenothiazine-derived antipsychotic drug, can inhibit TRPC3–Nox2 protein complex formation.[56] Among the 12 most commonly prescribed drugs that are able to inhibit TRPC3–Nox2 complex formation, clomipramine showed the strongest inhibitory effect on ACE2 receptor internalization in HEK293T cells exposed to artificial trimeric spike protein.[64,65] Clomipramine was also found to suppress the increase in SARS-CoV-2 RNA copy number in hiPS-CMs and TMPRSS2-expressing VeroE6 cells after exposure to SARS-CoV-2.[65] ACE2-dependent SARS-CoV-2 viral entry is reportedly achieved through endocytosis regulated by phosphatidylinositol 3-phosphate 5-kinase[66] (the main enzyme that synthesizes phosphatidylinositol-3,5-bisphosphate ($PI(3,5)P_2$) in the early endosome[67]), two-pore channel subtype 2[66] (a major downstream effector of $PI(3,5)P_2$[68]), and cathepsin L[66,69] (a cysteine protease that cleaves S protein to facilitate viral entry into the lysosome). Although the molecular target of clomipramine has not yet been identified, determining the pleiotropic effects of clomipramine will promote the repurposing of this drug for the prevention and treatment of severe COVID-19. Importantly, the mechanism of action by which clomipramine inhibits SARS-CoV-2 spike protein-induced ACE2 internalization is completely different from that of remdesivir and dexamethasone, suggesting the potential for the concomitant use of clomipramine and these approved drugs for patients with severe COVID-19.

7. Future perspectives

Using knowledge of the chemicals and bioresources gained through our previous drug repurposing research, we suggest that pathology-specific PPIs (i.e., the TRPC3–Nox2 interaction) contribute to the risk of severe COVID-19 outcomes related to human heart tissue. We also believe that a pleiotropic drug that can inhibit both ACE2-dependent viral entry and ACE2 upregulation through TRPC3–Nox2 interaction would be a promising strategy for the treatment of severe COVID-19 patients with cardiac sequelae. However, there are several challenges to be overcome. Demonstrating the *in vivo* efficacy of clomipramine will be required before it can be trialed in humans. Additionally, *in vivo* COVID-19 studies are limited by the need for laboratories with a high biosafety level. Another problem is that because the price of repurposed drugs is low, expected profits will not be commensurate with the costs of conducting clinical trials. Clomipramine reportedly causes several adverse effects, such as cardiac arrhythimia (QT prolongation) and anti-cholinergic actions, limiting the dosage for this repurposing. However, derivatives could be synthesized based on the structure of approved drugs to create new compounds with the same (or higher) efficacy but with lower adverse effects.

More than two years have passed since the onset of the global COVID-19 pandemic. In addition to therapeutic agents, vaccines and neutralizing antibodies such as casirivimab/imdevimab, bamlanivimab/etesevimab, and sotrovimab have been developed to prevent

SARS-CoV-2 viral entry through ACE2-dependent endocytosis.[70] These antivirals and neutralizing antibodies are administrated intravenously, but there is a demand for oral drugs that are easier to administer. The oral antivirals molnupiravir (an RNA-dependent RNA polymerase inhibitor) and nirmatrelvir (a $3CL^{pro}$ inhibitor); ritonavir (a CYP3A inhibitor) combination have recently been developed and are now used to treat COVID-19.[71,72] Vaccination has helped to decrease the number of COVID-19 patients,[73,74] while new SARS-CoV-2 variants (e.g., Delta and Omicron) have emerged consecutively, with the number of COVID-19 patients rapidly increasing with the rise of each new variant.[75] The wild-type virus and the known SARS-CoV-2 variants (including Omicron) require ACE2-mediated cell entry.[76] The pharmacological advantage of clomipramine lies in its potential to inhibit ACE2-mediated viral entry needed by the ancestral SARS-CoV-2 virus and its variants, as well as SARS-CoV and MERS. This advantage will promote the development of antivirals that can efficiently inhibit viral entry while reducing adverse side effects.

It is hoped that researchers will quickly identify new drugs that can be used to treat COVID-19 and deliver them to patients suffering from severe COVID-19 as soon as possible. Moreover, the number of patients suffering from COVID-19 sequelae is increasing. Various symptoms of COVID-19 sequelae have been reported, such as fatigue and depression, taste and smell disorders, and myocarditis and arrhythmia.[48,77] In the future, it will be necessary to develop animal models that mimic COVID-19 sequelae to elucidate the related underlying mechanisms and to identify new treatment strategies.

Declaration of competing interest

The authors declare no conflicts of interest.

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