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Review

A high-throughput drug screening strategy against coronaviruses

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ABSTRACT

The emergence and re-emergence of coronaviruses (CoV) continually cause circulating epidemics and pandemics worldwide, such as the on-going outbreak of the novel coronavirus SARS-CoV-2. The resultant disease, coronavirus disease 2019 (COVID-19), has rapidly developed into a worldwide pandemic, leading to severe health and economic burdens. Although the recently announced vaccines against COVID-19 has rekindled hope, there is still a major challenge to urgently meet the global need for rapid treatment of the pandemic. Given the urgency of the CoV outbreak, we propose a strategy to screen potential broad-spectrum drugs against CoV in a high-throughput manner, particularly against SARS-CoV-2. Since the essential functional domains of CoV are extensively homologous, the availability of two types of mild CoV, HCoV-OC43 and MHV, should provide a valuable tool for the rapid identification of promising drugs against CoV without the drawbacks of level three biological confinements. The luciferase reporter gene is introduced into HCoV-OC43 and MHV to indicate viral activity, and hence the antiviral efficiency of screened drugs can be quantified by luciferase activity. Compounds with antiviral activity against both HCoV-OC43 and MHV are further evaluated in SARS-CoV-2 after structural optimizations. This system allows large-scale compounds to be screened to search for broad-spectrum drugs against CoV in a high-throughput manner, providing potential alternatives for clinical management of SARS-CoV-2 or other CoV.

Introduction

The emergence and re-emergence of coronavirus (CoV) infections have continually caused serious public health concerns over past decades. Severe acute CoV infections, including severe acute respiratory syndrome-related coronavirus (SARS-CoV) in 2002, Middle East respiratory syndrome-related coronavirus (MERS-CoV) in 2012, and the currently circulating SARS-CoV-2, have become a growing and long-lasting global threat (Gao, 2018). The first case of SARS-CoV-2 was deemed to occur in December 2019 and identified as a new type of coronavirus in early January 2020 (Burki, 2020; Chen et al., 2020a; Gralinski and Menachery, 2020; Wu et al., 2020b; Zhou et al., 2020b). The World Health Organization (WHO) named the resulting pneumonia as Coronavirus Disease-2019 (COVID-19) on Feb 11, 2020 (WHO, 2020). COVID-19 patients suffer acute respiratory distress syndromes (e.g., cough, sore throat, rhinorrhea, fever, and lung damage) as well as other symptoms such as fatigue, myalgia, and diarrhea (Chen et al., 2020b; Guan et al., 2020; Huang et al., 2020). With the rapid global spread of SARS-CoV-2, this disease soon attained the status of the Public Health Emergency of International Concern and currently continues to cause enormous devastation worldwide.

SARS-CoV-2, an enveloped, positive-sense, single-stranded, RNA-type virus, belongs to the orthocoronavirus subfamily of coronaviridae, including four virus genera α, β, γ, and δ (ICTV, 2011; Zhou et al., 2020a). Together with the highly pathogenic

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coronaviruses SARS-CoV and MERS-CoV, SARS-CoV-2 constitutes a β-coronavirus that also contains the mild coronaviruses HCoV-OC43 and mouse hepatitis virus (MHV). SARS-CoV is the most closely related strain to SARS-CoV-2, displaying 79.5% genome sequence identity with SARS-CoV-2 (Wu et al., 2020a; Zhou et al., 2020b, 2020a). The genome of CoV encodes non-structural proteins, including 3C-like protease (3CLpro), helicase, and RNA-dependent RNA polymerase (RdRp), structural proteins like spike glycoprotein, and accessory proteins (Fung and Liu, 2019). The non-structural proteins are highly conserved among CoV and crucial enzymes in the viral life cycle (St-Jean et al., 2004; Zhou et al., 2007; Raj et al., 2013; Lehmann et al., 2015; Smith et al., 2015; Wang et al., 2015; Kindler et al., 2017). The spike glycoprotein contains S1 and S2 subunits associating with virus-cell receptor interactions and membrane fusion during the viral entry, respectively (Fung and Liu, 2019). Therefore, these motifs are potential therapeutic targets for counteracting coronavirus-mediated diseases (Zumla et al., 2016).

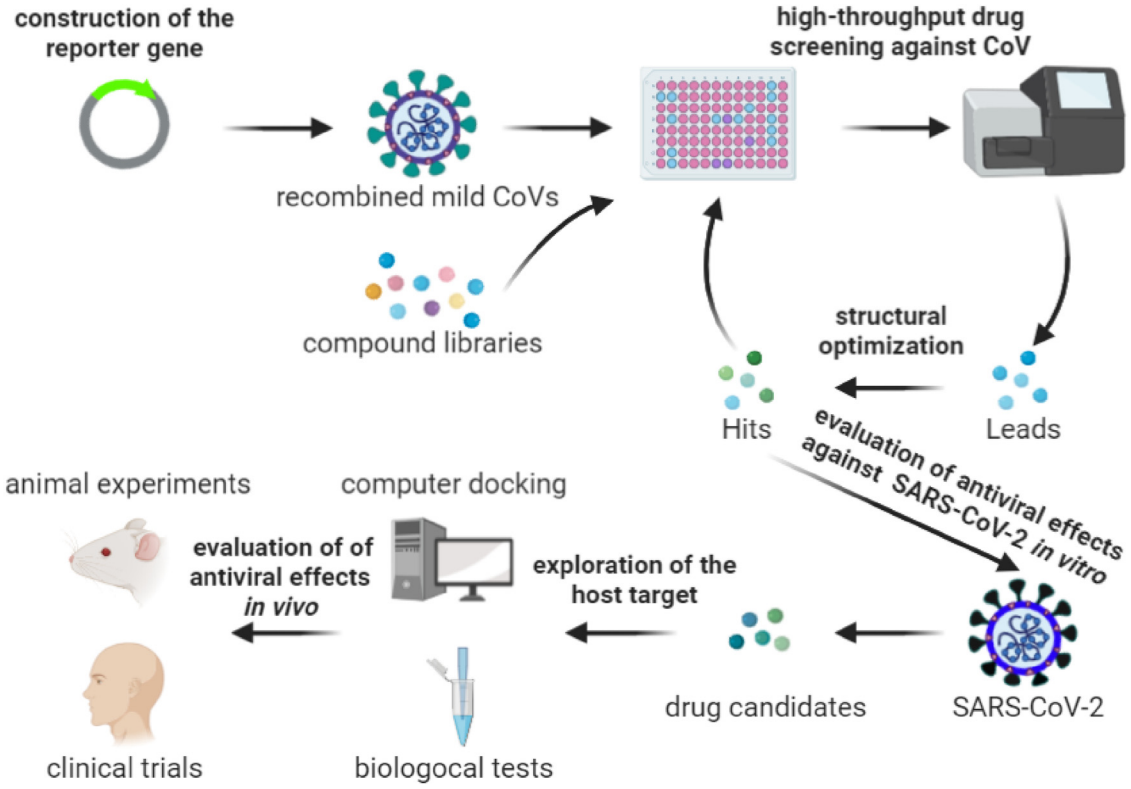
Although researchers have made significant efforts to control or prevent emerging infections of SARS-CoV-2, including the approval of two vaccines, no effective drugs have been approved to cure COVID-19 patients to date. The existing antiviral medications are ineffective for new viruses due to viral specificity, while new interventions are likely to require years to be developed, leading to insufficient strategies to deal with the outbreaks. Therefore, it is imperative to establish a highly effective drug screening system that can quickly and accurately screen large compound libraries against the emerging and re-emerging CoV, particularly SARS-CoV-2. We here describe in brief the conventional paradigm and propose a high-throughput drug screening strategy against SARS-CoV-2, which may provide a new idea for the control of the viral infection.

Challenges in drug screening against SARS-CoV-2

Currently, SARS-CoV-2 drug screening mainly relies on computer-aided drug design against a single viral protein or small-scale drug screening of a few previously approved antiviral drugs using wild-type SARS-CoV-2 (Li and De Clercq, 2020). Both strategies have achieved positive prospects, yet there are also apparent limitations. The computer-aided drug design can only conduct drug screening against a single or several virus targets instead of simulation of the complete virus life cycle, which may result in a loss of a large number of potential antiviral drugs. The resultant medicines from a single-target screening model interact with limited targets, and thus, readily leads to drug resistance. Although this method provides an excellent structural basis for drug design, pharmacological experiments need to be performed to evaluate the actual antiviral activity of drug candidates. Additionally, it is time-consuming to develop drugs against a viral target of which the crystal structure needs to be resolved. Considering the bio-security requirements of SARS-CoV-2, it is commonly applied to identify effective therapeutic agents from a few existing antiviral drugs based on clinical experience, and hence, this method is not compatible for a high-throughput drug screening. Collectively, a high bio-safety CoV infection model with the capability of a high-throughput drug screening against SARS-CoV-2 is urgently required.

Model for drug screening against SARS-CoV-2

A feasible high-throughput drug screening method against SARS-CoV-2 is to employ a highly homologous virus in genomics as a drug screening model that presents superior bio-safety and can be easily cultured. The HCoV-OC43 infection mainly causes mild



Scheme 1. Conceptual roadmap of the high-throughput drug screening strategy against CoV.

upper respiratory symptoms that can be cured by the patient's autoimmunity and requires no special treatments. Except for the discrepancy in the spike S1 protein, the genomic sequence of OC43 can be readily aligned to SARS-CoV-2 (Bill Gallaher, 2020). Several critical studies have shown that HCoV-OC43 is closely related to SARS-CoV (St-Jean et al., 2004; Bill Gallaher, 2020). The comparison of nucleotide and amino acid sequences revealed that the two viruses have extensive identities in important motifs involving viral replication and pathogenesis, such as RdRp, helicase motifs, and 3CLpro. MHV, which does not infect humans, is a standard model for studying coronaviruses' molecular biology, including virus classification, replication, and infection of SARS-CoV (Bárcena et al., 2009; Almazán et al., 2014). Indeed, all coronaviruses show a high level of conservation in essential functional domains, especially within RdRp, RNA helicase, and 3CLpro hydrolase and capping enzyme (Hegyi and Ziebuhr, 2002; Novella, 2003; St-Jean et al., 2004). All these motifs represent potential drug targets for the therapy of coronavirus-mediated diseases given that they are necessary for the virus to complete replication in the host (Hung et al., 2002; Dragovich et al., 2003). Inhibiting any of these targets enables the blockade of the viral life cycle and reduction of the viral activity. One advantage of the live virus drug screening model is that it is a kind of *in vivo* phenotypic screening, which allows drugs to be evaluated against various targets. Therefore, this mild CoV model facilitates the discovery of drugs targeting different proteins, which can be applied to combination therapy to more effectively treat COVID-19 and reduce drug resistance. Accordingly, HCoV-OC43 and MHV are excellent models for the preliminary drug screening against CoV as well as SARS-CoV-2 without the drawbacks of level III biological confinement.

The high-throughput drug screening strategy

To achieve a high-throughput drug screening system, a luciferase reporter gene is designed and inserted into HCoV-OC43 and MHV to indicate the viral activity. The antiviral efficiency of drugs can be monitored and quantified by luciferase activity, and thus, this system allows for screening large-scale compounds in a fast, economical, and effective manner. To reduce false-positives, compounds showing antiviral efficiency in both CoVs are selected as lead compounds. After the comprehensive structural modification and optimization of leads, the antiviral activity of newly synthesized compounds will be screened using HCoV-OC43 and MHV models, and the resultant hits will be further evaluated using SARS-CoV-2 in the BSL-3 laboratory. Subsequently, the specific targets and corresponding action mechanisms of antiviral activity of drugs will be thoroughly explored. Simultaneously, drug-likeness properties, *in vivo* experiments, and clinical trials of promising drug candidates will be evaluated (Scheme 1).

Potential compounds against SARS-CoV-2

An efficient approach for drug discovery is to repurpose existing approved drugs in treating new diseases. Since FDA-approved medications have been used in people without serious safety issues, they are suggested to be tested first to combat COVID-19. In addition, many clinical practices have demonstrated that traditional Chinese medicine (TCM) plays a useful role in the prevention and treatment of COVID-19, which opens a new avenue for developing novel drugs against the new virus (Li et al., 2020; Ren et al., 2020; Yang et al., 2020; Zhang, 2020). Although TCM has been proven effective in treating COVID-19, its mechanism of action remains elusive. TCM could suppress and alleviate excessive immune responses, thereby eliminating complications of COVID-19.

Furthermore, evidence has shown that TCM compounds have the potential to function as antiviral drugs by directly interacting with critical proteins of the virus (Wu et al., 2020c). Consequently, the abundance of the TCM library provides a wealth of exquisite candidates for discovering novel antiviral drugs. As well, home-made compounds are a potential source for the search of new chemical entities against SARS-CoV-2. Overall, the libraries of marketed drugs, TCM medications, and home-made compounds provide sufficient candidates for screening potential drugs against SARS-CoV-2.

The main research contents of the proposed project

Construction of the recombined MHV and HCoV-OC43 expressing luciferase

A coronavirus reversed genetics system will be used to construct recombined MHV and HCoV-OC43 expressing firefly luciferase (Fluc), i.e., MHV-Fluc and HCoV-OC43-Fluc. First, the Fluc gene will be integrated into a plasmid containing 3'-end of the viral genome. The edited gene is then inserted into the viral genome as a separate transcription module initiated by the viral transcription regulating sequences (TRS). During virus replication, it will be recognized as a non-structural gene of the virus. After the plasmid has been transcribed into RNA, the RNA transcripts are then transfected into cells infected with helper coronaviruses using electro-transducers. The recombined virus will be purified by plaque purification and limiting dilution for MHV-Fluc and HCoV-OC43-Fluc, respectively. The chimera of the Fluc gene in the virus genome will be identified by RT-PCR. Viruses with correct genetic information will be further confirmed by the luciferase activity assay after cell infection. Subsequently, the recombined virus will be extensively amplified for the follow-up studies.

Discovery of the leads

Standard assays will be performed to test the antiviral activity of these compounds. To this end, relative cells are infected with the corresponding virus in the presence of test compounds. Efficacies are evaluated by quantification of the luciferase activity. To exclude false-positives caused by cytotoxicity, the cytotoxicity of screened drugs from TCM and home-made compound libraries will be determined in advance by CCK8 assay. Also, the hERG cardiotoxicity of these compounds will be determined following standard practices. The compounds with high antiviral activity and low toxicities will be chosen as lead compounds.

Structural optimization of leads

Subsequently, the systematic structural modification and optimization of leads will be performed according to classic medicinal chemistry theories and experience. Meanwhile, the physicochemical, pharmacokinetic, and toxicological properties of newly designed compounds will be considered. The purity and chemical structures of newly synthesized compounds will be identified by high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and high-resolution mass spectrometry (HRMS). The antiviral activity of optimized compounds will be measured, and their chemical structures will be further optimized based on structure-activity relationships to search for ideal, safe, and reliable antiviral hits.

Evaluation of the anti-SARS-CoV-2 efficiency of hits in vitro

The antiviral effects of hits against wild type SARS-CoV-2 will be further evaluated according to the previous study. Briefly, Vero E6

cells are infected with SARS-CoV-2 in the treatment of different hit compounds. The viral yields in the cell supernatant can be quantified by quantitative real-time RT-PCR (qRT-PCR). Immunofluorescence microscopy can be used to visualize virus nucleoprotein (NP) upon drug treatment. The standard time-of-addition assay is employed to determine whether antiviral hits function at the entry or post-entry stages of the virus infection in cells.

Exploration of the host target of drug candidates

The specific targets and corresponding action mechanisms of antiviral drugs will be first analyzed through computer docking. The plausible drug targets will then be verified using a series of cellular and biochemical experiments by constructing recombinant pseudo-viruses with missing or mutated fragments.

Animal experiments and clinical trials

For the FDA-approved drugs with high antiviral efficiency against SARS-CoV-2 infection in cell culture, pharmacodynamic tests in animals and further clinical trials will be urgently launched to evaluate their efficacy in combating the disease. Meanwhile, the newly identified drug candidates with excellent antiviral activity *in vitro* are proposed to be actively assessed in animal tests and then in clinical trials. The translational studies of these drugs in the treatment of COVID-19 will be enthusiastically promoted.

Conclusions

In summary, the outbreak of SARS-CoV-2 underscores the urgent need for renewed efforts to develop broad-spectrum drugs to combat emerging and re-emerging CoV. Herein, we proposed a high-throughput drug screen system against CoV. Given the high conservation in crucial domains among CoV and avoidance of level three, aerosol-aware biological confinement, two types of mild CoV, HCoV-OC43 and MHV, are employed as ideal models for the rapid preliminary identification of potential drugs against CoV. Since the luciferase gene is introduced in HCoV-OC43 and MHV to indicate the degree of viral activity, the antiviral efficiency of screened drugs can be reported by a light signal, which can be readily quantified by a microplate reader. The microplate reader, handling thousands of samples in minutes, or even seconds, helps minimize the operational time, reduce reagent costs, and screen a large number of compounds in a high-throughput manner. We speculate that the antiviral drugs screened by this strategy will exhibit potential therapeutic value to quickly conquer the challenges of emerging and re-emerging pathogenic CoV.

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Ethical approval

Ethical approval was not required for the preparation of this manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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