

Repurposed drugs for treating lung injury in COVID-19

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Abstract

Lung injury with severe respiratory failure is the leading cause of death in COVID-19. Inhibition of ACE2 caused by spike protein of (SARS)-CoV-2 is the most plausible mechanism of lung injury in COVID-19. We proposed six candidate drugs, including geldanamycin, panobinostat, trichostatin A, narciclasine, COL-3 and CGP-60474, that could best reverse abnormal gene expression caused by (SARS)-CoV-2-induced inhibition of ACE2 in lung cells, for the promise of treating lung injuries in COVID-19.

Main

Coronavirus disease (COVID-19) is an infectious disease discovered in 2019 and currently in outbreak across the world, resulting in more than 0.3 million infections and over fourteen thousand deaths by now. It is causing tens of thousands of new infections and thousands of mortalities every day. Severe viral pneumonia related lung injury with respiratory failure is the main reason of COVID-19 related death¹.

This novel coronavirus (CoV), termed severe acute respiratory syndrome (SARS)-CoV-2, uses the SARS-CoV receptor angiotensin-converting enzyme 2 (ACE2) for entry and the serine protease TMPRSS2 for S protein priming². In SARS-CoV infection, viral spike protein inhibits ACE2 and causes severe lung injury^{3,4}. Since the spike protein of (SARS)-CoV-2 interacts with ACE2 as does the spike protein of SARS-CoV, inhibition of ACE2 may be the pathogenic mechanism in (SARS)-CoV-2 induced lung injury.

Based on this assumption, we performed drug reposition analysis to identify drugs and compounds for treating (SARS)-CoV-2 induced lung injury. We collected differential gene expression profiles in HCC515 and A549 lung cells with the inhibition of ACE2 from LINCS L1000 project⁵. Then we analyzed 12,707 drugs and compounds from LINCS L1000 pharmacogenomics data to find best candidates that could reverse abnormal gene expression. Upon examination of ACE2 expression at different time points (6h and 24h), we opted to focus on HCC515 cells which have reduced ACE2 expression over treatment of moexipril, an ACE2 inhibitor. At 6 h after treatment of moexipril, narciclasine (FDR=0.006) and geldanamycin (FDR=0.006) could significantly (FDR<0.05) recover abnormal gene expressions. At 24h post treatment of moexipril, CGP-60474 (FDR=1.337×10⁻⁷), panobinostat (FDR=2.443×10⁻⁰⁵), trichostatin-a (FDR= 3.546×10⁻⁰³) and COL-3 (FDR= 0.002) could significantly recover abnormal gene expression in HCC515 cell.

In lung, the inhibition of ACE2 promote lung injury via the renin–angiotensin system (RAS)⁶. In pulmonary RAS, ACE2 converts angiotensin II (Ang II), an octapeptide hormone, to Ang-(1-7), an heptapeptide hormone. Ang II is a pulmonary profibrotic mediator and stimulates procollagen production in lung fibroblasts to promote lung injury⁷. Ang-(1-7) protects lung from injury, through reducing both inflammatory response by lowering cytokine release⁸ and pulmonary fibrosis by inhibiting fibrotic signaling pathways⁹. Thus, inhibition of ACE2 will increase Ang II level and decrease Ang-(1-7), promote fibrosis and inflammation and injure lung tissues (Figure 1).

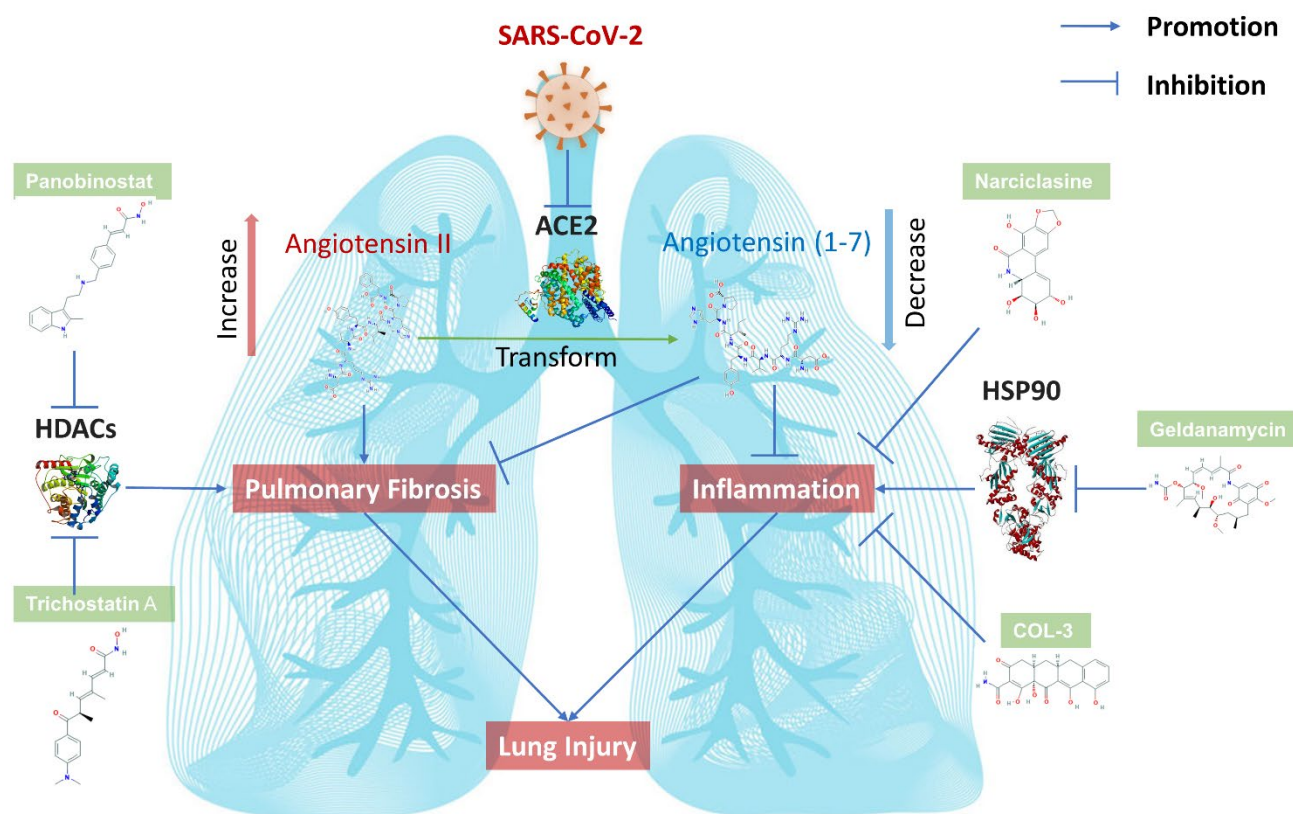


Figure 1. Mechanisms of candidate drugs for treating lung injury in COVID-19.

Among proposed candidate drugs, panobinostat and trichostatin-a were shown to inhibit pulmonary fibrosis^{10,11}, while geldanamycin, COL-3 and narciclasine inhibit inflammation¹²⁻¹⁴, to attenuate lung injury in animal model (Figure 1). Panobinostat is a hydroxamic acid and

can act as a non-selective histone deacetylase (HDAC) inhibitor. It was approved for the treatment of multiple myeloma. Panobinostat was reported to reduce pulmonary fibrosis by inducing cell cycle arrest and apoptosis in idiopathic pulmonary fibrosis fibroblasts¹⁰. Trichostatin A is an organic compound that serves as an antifungal antibiotic and selectively inhibits the class I and II mammalian HDACs, but not class III HDACs. It attenuated lung injury in various diseases^{15,16}. Geldanamycin is an inhibitor of heat shock protein 90. It reduced H5N1 infection induced lung injury¹². Narciclasine is an isocarbostryril alkaloid found in the Amaryllidaceae (amaryllis) family of flowering plants. It can alleviate inflammatory responses and reduce lung injury in sepsis¹⁴. COL-3 is a chemically modified tetracycline that prevented lung injury and reduced morbidity associated with sepsis¹³. COL-3 was used in clinical trials for the treatment of HIV Infection (Clinical trial: NCT00020683). CGP-60474, an inhibitor of cyclin-dependent kinase, is the only molecule that has no known relationship with lung injury.

In summary, we propose the six candidate drugs above, which could be used to inhibit pulmonary fibrosis or inflammation and thus attenuate lung injury induced by (SARS)-CoV-2. It is our hope to solicit the interest of the biomedical community, to save more lives from severe respiratory failure in COVID-19.

Methods and Materials

Data Preparation

We downloaded level 5 LINCS L1000 data, a collection of gene expression profiles for thousands of perturbagens at a variety of time points, doses, and cell lines, from Gene Expression Omnibus (GEO) database (GEO id: GSE70138 and GSE92742). Gene expression profiles in lung cells were extracted from downloaded dataset. The extracted data

includes 37,366 treatments of 12,707 drugs in 13 lung cell lines at different time points and doses. There were two lung cell lines, A549 and HCC515, that were treated with 10 μ M moexipril, an inhibitor of ACE2 and ACE, a homologue of ACE2. Gene expression profiles were collected from A549 and HCC515 cells at 6 and 24 h after treatment. Upon moexipril treatment, ACE2 level decreased with time in HCC515 but increased in A549, prompting us to focus the analysis using HCC515 line, which showed the inhibition effect of moexipril. The gene expression profiles used to simulate gene expressions in lung with the inhibition of ACE2 by spike protein of (SARS)-CoV-2. Differential expression of gene was obtained by comparing the sample cell to all other cells in the same plate after all cells' gene expression being normalized by a z-scoring procedure.

Drug Repurposing Analysis

The differential gene expression of 12,328 genes, simulating (SARS)-CoV-2 related inhibition of ACE2 in lung, were transformed to a gene rank list. The reference dataset contains gene rank lists of treatments of 12,707 drugs from LINCS L1000 data as mentioned above. In our hypothesis, an effective drug treatment is one that reverts the aberrant gene expression back to the normal levels. Therefore, an outlier-sum (OS) based statistic was used to model the overall disease-drug connectivity by aggregating disease transcriptome changes with drug perturbation using DrInsight Package¹⁷. The Kolmogorov–Smirnov (K-S) test was used to determine whether the OS from one drug is larger than those from the rest drugs and compounds in the reference dataset.

Statistical Analysis

The false discovery rate (FDR) method was used to adjust P-values from the K-S test to avoid false discovery in multiple comparisons. A drug with FDR<0.05 was selected as the candidate drug for the disease.

Code Availability

All the codes and data are available at:

<https://github.com/lanagarmire/COVID19-Drugs-LungInjury>

Competing interests

The authors declare no competing financial interests.

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