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Systematic cell line-based identification of drugs modifying *ACE2* expression

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Abstract

The COVID-19 pandemic caused by SARS-COV-2 has infected over 500,000 people causing over 25,000 deaths in the last 10 weeks. A key host cellular protein required for the virus entry is angiotensin-converting enzyme 2 (*ACE2*). Recent studies have reported that patients with hypertension and diabetes treated with ACE inhibitors or angiotensin receptor blockers might be at a higher risk of COVID-19 infection as these drugs have been reported to increase *ACE2* expression. This has raised the need to systematically investigate the effect of different drugs including antihypertensives on modulating *ACE2* expression. Here, we analyzed a publicly available CMAP dataset of pre/post transcriptomic profiles for drug treatment in cell lines for over 20,000 small molecules. We show that only one subclass of antihypertensives drugs - ACE inhibitors, are significantly enriched for drugs up-regulating *ACE2* expression. Studying the effects of the 672 clinically approved drugs in CMAP, we chart the drug categories that affect *ACE2* expression. Specifically, we find that panobinostat (an HDAC inhibitor) confers the highest

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up-regulation of *ACE2* expression while isotretinoin (a vitamin A derivative) is its strongest down-regulator. Our results provide initial candidates guiding further *in vitro* and *in vivo* studies aimed at assessing drug effects on *ACE2* expression.

Keywords: COVID19, Infectious Diseases

Introduction

The ongoing pandemic of COVID-19, caused by SARS-CoV-2 virus, has plagued over 140 countries and has resulted in over 500,000 cases and 25,000 deaths within the last 10 weeks. A key cellular receptor for SARS-CoV-2 entry in humans is angiotensin-converting enzyme 2 (*ACE2*) [1]. A recent publication by Fang et al. has suggested that patients with hypertension (HT) and diabetes mellitus may be at higher risk of COVID-19 infection [2], as these patients are often treated with ACE inhibitors (ACEIs) or angiotensin II type-I receptor blockers (ARBs), which have been previously suggested to increase *ACE2* expression [3, 4]. This has raised the need to investigate the effect of different drugs including antihypertensives on modulating *ACE2* expression while clinical data is rapidly accumulating.

Addressing this challenge, we set to systematically identify drugs whose treatment can alter *ACE2* expression and, assuming that the latter is an important determinant, possibly increase or decrease the infection risk of COVID-19. To this end, we analyzed the Connectivity Map (CMAP) dataset, which provides transcriptomic data of different cell lines treated by approximately 20,000 small molecules [5]. Utilizing this resource, we mined the *ACE2* expression fold change (logFC) after 24 hours of each drug treatment, to identify clinically approved drugs that result in strong upregulation or downregulation of *ACE2* expression in this data.

Results

First, studying the suggestion of Fang *et al.* that particular antihypertensive drugs may affect *ACE2* expression [2], we focused on 48 clinically approved anti-HT drugs that were tested on the same 4 carcinoma cell lines in the CMAP dataset (we focused on epithelial cancer cell lines,

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which may better resemble epithelial tissues like the lung, Supp. Table 1A). Individually, no major anti-HT drug was found to increase *ACE2* expression significantly in these experiments, but methyldopa (an alpha-2 adrenergic receptor agonist) and molsidomine (an vasodilator) do significantly decrease *ACE2* expression (**Figure 1A**, logFC=-0.605 and -0.290, P=0.002 and 0.005, respectively; adjusted P=0.11 for both). However, when the individual drug results are aggregated to identify the effects of major classes of anti-HT drugs, we find that many ACEIs, but not ARBs, tend to upregulate *ACE2* expression (**Figure 1B**, GSEA method P=0.026, adjusted P<0.1; Supp. Table 1B). Anti-adrenergics other than alpha/beta-blockers tend to downregulate *ACE2* (**Figure 1B**, GSEA P=0.032, adjusted P<0.1; Supp. Table 1C). Notably, we find that calcium channel blockers (CCBs) do not significantly affect *ACE2* expression, providing preliminary *in vitro* support for Fang *et al.* suggestion that this drugs class may be considered as an alternative for ACEIs [2]. This finding concords with results from a large study cohort where hypertensive patients treated with CCBs (amlodipine and nifedipine) had no increase in urinary *ACE2* levels compared with controls with no medication [6]. A similar analysis for the 13 approved anti-diabetic drugs in the CMAP dataset that were tested on the same 4 carcinoma cell lines (Supp. Table 1A) has not identified any individual drug or class of anti-diabetic drugs that significantly altered *ACE2* expression, possibly partly due to the small number of drugs available in this class (Supp. Table 1D,E).

We then turned to analyze a broad set of 672 clinically approved drugs that were each tested on the same 4 carcinoma cell lines in the CMAP dataset (Supp. Table 1A), to identify the top drugs that upregulate or downregulate *ACE2* expression. The top up-regulator is panobinostat, an anti-cancer histone deacetylase (HDAC) inhibitor with antifibrotic effects that has been shown to reduced risk of acute respiratory deteriorations [7,8] (**Figure 1C**, logFC=0.457, P=2.29e-5, adjusted P=7.70e-3). The top down-regulator of *ACE2* is isotretinoin, a vitamin A derivative with suggested respiratory side-effects [9] (**Figure 1C**, logFC=-0.478, P=1.59e-4, adjusted P=0.036; Supp. Table 1F). We found 6 drugs in CMAP that are currently being investigated in clinical trials for treating COVID-19 (chloroquine, thalidomide,

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methylprednisolone, losartan, lopinavir and ritonavir, from clinicaltrials.gov), none of which was found to significantly alter *ACE2* expression (P>0.1, Supp. Table 1F).

Analyzing the expression results for these 672 drugs in an aggregated manner, we identified several classes of drugs based on mechanism of action (MOA) that are significantly enriched for up or down-regulation of *ACE2* expression (**Figure 1D**; Supp. Table 1G). Top classes of *ACE2*-upregulating drugs are HDAC inhibitors (GSEA P=0.003, adjusted P=0.02) and dopamine receptor antagonists (GSEA P=0.007, adjusted P=0.02). We further extended this analysis to 989 clinically approved drugs tested on a total of 28 CMAP cell lines and primary cells (16 cancer and 12 normal cells, where each drug may have been tested on a different subset of cell lines, Supp. Table 1A). We examined the enrichment of *ACE2*-modulating drugs in 48 different second-level WHO Anatomical Therapeutic Chemical (ATC) indication categories [12]. We find that the class of drugs targeting the renin-angiotensin system are enriched for up-regulators of *ACE2* (**Figure 1E**, GSEA P=0.044). Additional top enriched ATC classes are shown in **Figure 1E**, the top class being antineoplastic agents and in particular protein kinase inhibitors (GSEA P=0.004, Supp. Table 1H), although these classes do not achieve significance after FDR correction due to limited statistical power (Supp. Table 1H).

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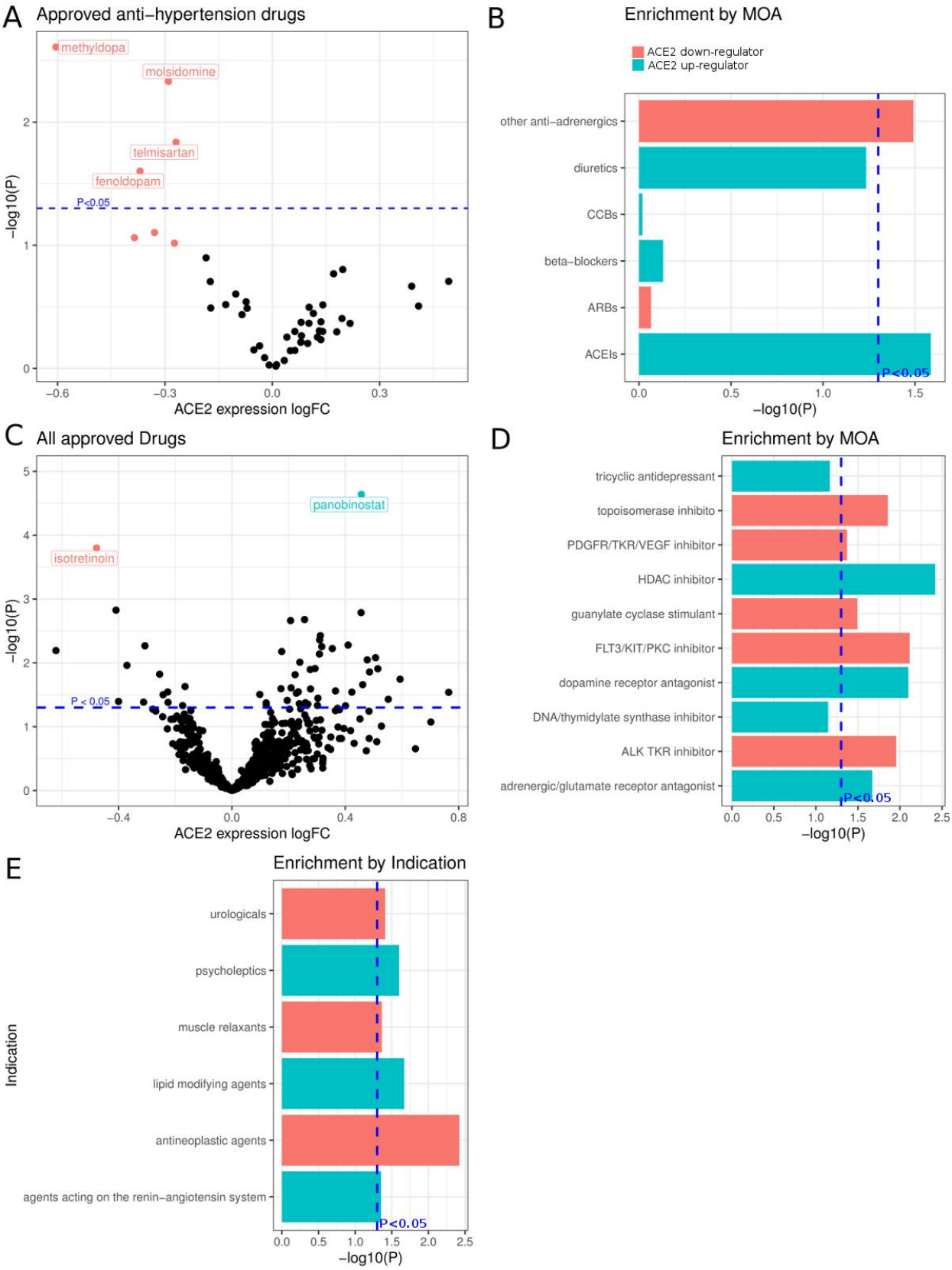

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Figure 1: The landscape of *ACE2* expression levels alterations in response to drug treatments across cell lines in CMAP [5]. Differential expression analysis for *ACE2* expression changes in treated vs control samples was performed with the level 3 CMAP data of 24hrs response values using limma [10]. Volcano plots showing the log fold-change (X-axes) and uncorrected negative $\log_{10}$P value (Y-axes) of *ACE2* expression changes are given for **(A)** 48 antihypertensive drugs and **(C)** 672 clinically approved drugs that are each tested on 4 carcinoma cell lines from CMAP [5]. The enrichment of positive/negative *ACE2* expression regulators in different drug classes based on their mechanism of action (MOA) was tested with the GSEA method as implemented in the R package fgsea [11], and the enrichment significance (negative log10P values) is shown in bar plots in the corresponding right hand side panels (B, D). We extend the analysis in (D) to a larger set of 989 clinically approved drugs tested on a total of 28 cell lines and performed enrichment analysis using the second level WHO ATC drug classification data [12], and top enriched drug classes are shown in (E). The horizontal and the vertical dashed lines denote a P value of 0.05. Drug indication and ATC classification annotations are obtained from the drugbank database [13]. Abbreviations: ERAs, endothelin receptor antagonists; CCBs, calcium channel blockers; ARBs, angiotensin II type-I receptor blockers; ACEIs, angiotensin-converting enzyme inhibitors; TKR, tyrosine kinase receptor.

Discussion

To date, solid evidence concerning whether *ACE2* expression can alter the risk of COVID-19 infection is lacking. The role of ACEIs/ARBs in modulating the clinical course of viral pneumonia and COVID-19 infection is also under debate [14-16], both being beyond the scope of this study. If *ACE2* expression does have an effect, though, it is important to chart the landscape of approved drugs effects on its expression. Addressing this challenge, here we performed a systematic *in vitro* analysis of the CMAP cell line data that shows that ACEIs, although not most other antihypertensives, are enriched for up-regulators of *ACE2* expression (**Figure 1B**). At a broader scope, we identified additional clinically approved drugs and drug categories that affect *ACE2* expression in vitro (**Figure 1C-E**). Caution is obviously warranted, as these are in vitro results, limited to measurements of expression levels after 24 hours in a variety of cancer and

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normal cell lines. However, keeping these reservations in mind, we believe these results provide a baseline of candidate leads guiding further *in vitro* and *in vivo* studies aimed at carefully assessing specific drug effects on *ACE2* expression.

Methods

The level 3 data of the Connectivity Map (CMAP) dataset [5] was downloaded from the GEO database (GSE92742 and GSE70138). For consistency, we focused on expression profile measures after 24 hours of treatment with various small molecules at a concentration of 10 uM (this represents the most frequent treatment condition present in the dataset). Using drug information from the drugbank database [13] and matching by drug common name, we obtain the subset of CMAP data for: 1. 48 clinically approved antihypertensive drugs that were each tested on the same 4 carcinoma cell lines; 2. 13 approved anti-diabetic drugs that were each tested on the same 4 carcinoma cell lines; 3. 672 clinically approved drugs that were each tested on the same 4 carcinoma cell lines; 4. 989 clinically approved drugs tested on a total of 28 cell lines, including cancer and primary cell lines where each drug may have been tested on a different subset of cells. For each of the drug treatment differential expression analysis for ACE2 expression changes in treated samples vs population controls (as described in the CMAP publication [5]) was performed using limma [10]. The enrichment of positive/negative ACE2 expression regulators in different drug classes based on their mechanism of action (MOA) or indication was tested with the GSEA method as implemented in the R package fgsea [11]. For drug indication we used the second level WHO ATC classification [12], obtained also from the drugbank database [13]. P values were adjusted with the Benjamini-Hochberg method.

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Conflict of interest statement

The authors declare no competing interests.

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