Heliyon 6 (2020) e05646

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Enhancement of the IFN-β-induced host signature informs repurposed drugs for COVID-19

Chen-Tsung Huang [a], Tai-Ling Chao [b], Han-Chieh Kao [b], Yu-Hao Pang [b], Wen-Hau Lee [b], Chiao-Hui Hsieh [c], Sui-Yuan Chang [b,d,***], Hsuan-Cheng Huang [e,**], Hsueh-Fen Juan [a,c,*]

[a] Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei 10617, Taiwan
[b] Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei 10048, Taiwan
[c] Department of Life Science, National Taiwan University, Taipei 10617, Taiwan
[d] Department of Laboratory Medicine, National Taiwan University Hospital, Taipei 10002, Taiwan
[e] Institute of Biomedical Informatics, National Yang-Ming University, Taipei 11221, Taiwan

ARTICLE INFO

Keywords:
Systems biology
COVID-19
Host-directed therapy
Type I interferon
Drug repurposing
Bioinformatics
Infectious disease
Transcriptomics
Pharmaceutical science

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a causative agent for the outbreak of coronavirus disease 2019 (COVID-19). This global pandemic is now calling for efforts to develop more effective COVID-19 therapies. Here we use a host-directed approach, which focuses on cellular responses to diverse small-molecule treatments, to identify potentially effective drugs for COVID-19. This framework looks at the ability of compounds to elicit a similar transcriptional response to IFN-β, a type I interferon that fails to be induced at notable levels in response to SARS-CoV-2 infection. By correlating the perturbation profiles of ~3,000 small molecules with a high-quality signature of IFN-β-responsive genes in primary normal human bronchial epithelial cells, our analysis revealed four candidate COVID-19 compounds, namely homoharringtonine, narciclasine, anisomycin, and emetine. We experimentally confirmed that the predicted compounds significantly inhibited SARS-CoV-2 replication in Vero E6 cells at nanomolar, relatively non-toxic concentrations, with half-maximal inhibitory concentrations of 165.7 nM, 16.5 nM, and 31.4 nM for homoharringtonine, narciclasine, and anisomycin, respectively. Together, our results corroborate a host-centric strategy to inform protective antiviral therapies for COVID-19.

1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by a new coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and continues to affect people around the world [1]. Paralleling the development of COVID-19 vaccines, the race for effective drug therapies against SARS-CoV-2 infection is also heating up [2, 3]. Although remdesivir, a broad-spectrum inhibitor of viral RNA-dependent RNA polymerase, has revealed some clinical benefits from a recent randomized controlled trial enrolling >1,000 COVID-19 patients [4], high mortality despite its use reflects the need for more efficacious therapies. To date, several drug-discovery approaches have provided a rich set of repurposing opportunities for the treatment of COVID-19 [5, 6, 7].

The interferon (IFN) system is an important first-line defense against viral infections, particularly through activation of type I and III IFNs (IFN-Is and IFN-IIIs, respectively) and subsequent induction of IFN-stimulated genes (ISGs) [8]. In human, IFN-Is comprise IFN-β, various subtypes of IFN-α, as well as IFN-ε and IFN-ω, whereas IFN-IIIs include four subtypes of IFN-λ [8]. IFN-Is help to establish cell-autonomous antiviral states in both infected and neighboring cells, to activate innate immune responses while restraining proinflammatory signals, and to prime adaptive immunity to more efficiently contain the spread of infectious pathogens [9]. Most human cell types can produce IFN-β (which is encoded by a single *IFNB1* gene), whereas hematopoietic cells are the major producers of IFN-α [9]. A full functional characterization of IFN-Is has led to their wide use in clinic, yet IFN-IIIs remain largely unexplored and have not received any approval for clinical indication [10].

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: sychang@ntu.edu.tw (S.-Y. Chang), hsuancheng@ym.edu.tw (H.-C. Huang), yukijuan@ntu.edu.tw (H.-F. Juan).

https://doi.org/10.1016/j.heliyon.2020.e05646
Received 22 September 2020; Received in revised form 6 November 2020; Accepted 30 November 2020

Accumulating evidence has suggested that dysregulation of IFN-I responses may represent an important risk factor for COVID-19 disease severity. However, the clinical benefit of recombinant IFN-Is in the treatment of SARS-CoV-2, and other coronaviruses such as Middle East respiratory syndrome coronavirus and SARS-CoV-1, remains controversial [10, 11]. Recently, Blanco-Melo *et al.* has demonstrated that SARS-CoV-2 infection triggers a modest antiviral response associated with low IFN-Is and IFN-IIIs and high pro-inflammatory cytokines and chemokines across different model systems [12]. Treatment of SARS-CoV-2-infected host cells with recombinant IFN-β appears to effectively reduce viral replication [11, 12, 13], supporting a protective role of early IFN-I in COVID-19 [10, 14]. This recent progress has thus motivated us to develop a drug-discovery approach for COVID-19, with an alternative focus on the host response to treatment.

Specifically, we propose that compounds capable of inducing a similar host response to IFN-I treatment may be effective against COVID-19. To test this idea, we took advantage of a gene-expression-based method that was originally designed to gauge the degree of reversal of a signature by compound treatments via small-molecule-regulated recurring transcripts across multiple cell types [15] and was successfully applied to identify effective drugs targeting non-oncogene dependencies in high-risk neuroblastoma [16]. Here, we used this approach to discover protective compounds against COVID-19 that can largely enhance an IFN-I-induced host signature (Figure 1). Unlike other strategies that target specific viral elements [7] or explore the host dependencies of SARS-CoV-2 [5, 6], our method is quite different and complementary in identifying druggable pathways and proteins for COVID-19. The approach presented here is to explore the ability of thousands of compounds to induce the expression of host antiviral genes and thereby effectively overcome COVID-19 or other potential life-threatening pathogens.

2. Materials and methods

2.1. Generation of IFN-β signature

We used the RNA-sequencing data corresponding to primary normal human bronchial epithelial (NHBE) cells treated with IFN-β for 4, 6, and 12 h, obtained through the Gene Expression Omnibus (GEO) with the accession number GSE147507 [12]. We performed differential expression analysis between IFN-β-treated (4, 6, or 12 h) and untreated conditions, using the DESeq function in the R package DESeq2 (version 1.24.0) with default arguments. Differentially expressed genes (DEGs) for each comparison were obtained with Benjamini–Hochberg (BH)-corrected $P < 0.05$, from which the IFN-β signature was defined as commonly shared DEGs.

2.2. Pathway enrichments of the IFN-β signature

We used the hypergeometric test to determine the enrichments of canonical pathways of the Molecular Signature Database (MSigDB) gene set collection (C2 CP, version 7.1) [17] for the IFN-β signature.

2.3. Predictions of compounds enhancing the IFN-β signature

To predict effective compounds against SARS-CoV-2 infection, we took advantage of a gene-expression-based approach that was originally developed to assess the degree of reversal of a disease signature by single agents or their combinations through their recurrently regulated transcripts across multiple cell types [15]. Here, we used this method to reveal compounds that can significantly enhance a given signature that we hypothesize is protective against SARS-CoV-2. Under this scenario, the algorithm will produce an enhancement score for each considered compound to provide a rough interpretation in a way such that a score of 0.1 corresponds to approximately 10% of enhancement in the signature. Specifically, for each tested compound, we used the IFN-β signature to compute an expression-based enhancement score as the negative of the therapeutic score defined by the original algorithm [15], except that the weight of gene g, $w(g)$, was now replaced by $w(g) = f(g)$, where $f(g) = +1$ or -1 if gene g is upregulated or downregulated in the signature, respectively.

2.4. Prioritization of compounds regulating the IFNB1 transcript

We used recurrent perturbation–transcript regulatory associations inferred between 3,332 compounds and 12,494 transcripts across 10 cell types [15] and prioritized compounds by their tendencies to regulate the expression of *IFNB1* mRNA across cell types.

2.5. Network analysis of IFN-β-responsive genes targetable by the candidate compounds

We investigated drug–target relationships between the four candidate COVID-19 compounds and their significant mRNA regulatory interactions (FDR <0.001) [15] relevant to the IFN-β signature. The results were then visualized as a drug–target network using Cytoscape (version 3.7.1).

2.6. Characterization of commonly targeted IFN-β signature genes by the candidate compounds

We used the significantly regulated mRNA associations to uncover the IFN-β-responsive genes commonly targeted by the four candidate COVID-

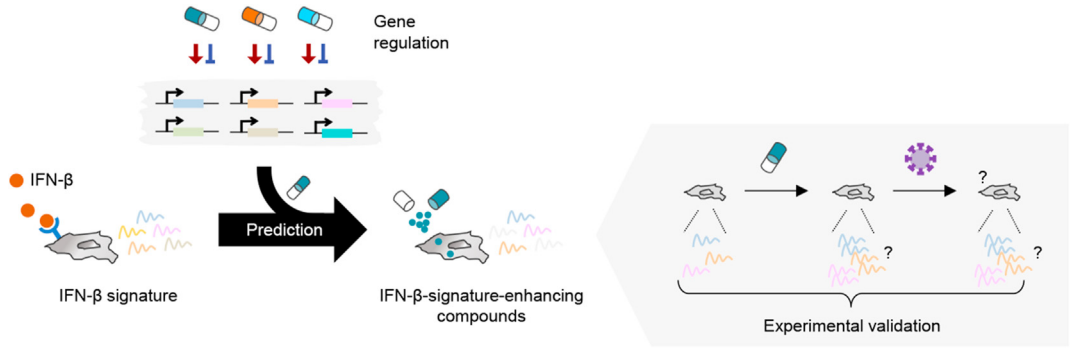

Figure 1. Study overview. We propose that compounds capable of inducing a similar host response to IFN-β may be effective for combating COVID-19. To this end, we used a gene expression-based approach, which was developed to evaluate the degree of reversal of a given signature by small-molecule-regulated recurring transcripts across multiple cell types, to reveal compounds that are able to enhance a large proportion of a IFN-β-induced host signature. The compounds as high-confidence predictions were experimentally confirmed for their anti-SARS-CoV-2 activity.

19 compounds (n = 18). We then used the hypergeometric test to calculate the enrichments of Gene Ontology (GO) Biological Process (BP) (C5 BP) or the entire gene set collection of the MSigDB (version 7.1) [17] for the commonly targeted IFN-β-responsive genes.

2.7. Cell cultures and chemicals

Vero E6 cells (CRL-1586) were obtained from American Type Culture Collection (ATCC). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic (containing 10,000 U/mL penicillin, 10,000 μg/mL streptomycin, and 25 μg/mL amphotericin B) and incubated at 37 °C in humidified atmosphere with 5% CO_2. DMEM (10569-044), FBS (10082-147) and antibiotic/antimycotic (15240-062) were purchased from Gibco. Homoharringtonine (HY-14944) and narciclasine (HY-16563) were obtained from MedChem Express. Anisomycin (S7409) was ordered from Selleckchem. TPCK-trypsin (T1426) was purchased from Sigma-Aldrich.

2.8. Virus preparation

SARS-CoV-2 was isolated from a respiratory specimen in the National Taiwan University Hospital (hCoV-19/Taiwan/NTU13/2020, with the original sequencing data available on GISAID under the Accession ID: EPI_ISL_413592), and propagated in the Vero E6 cell line in DMEM supplemented with 2 μg/mL tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-trypsin.

2.9. Plaque reduction assay

To determine the antiviral activity of the tested compounds against SARS-CoV-2, we used the plaque reduction assay as previously described with minor modifications [18]. In brief, Vero E6 cells were seeded at 2×10^5 per well in 24-well plates in DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic 24 h before infection. About 50–100 plaque-forming units (PFUs) of SARS-CoV-2 were added to the cell monolayer with 2 μg/mL TPCK-trypsin for 1 h at 37 °C. After the adsorption time, viruses were removed, and the cell monolayer was washed once with PBS and covered with the overlay media containing 1% methylcellulose with or without the test compound at indicated concentrations for 120 h. Cells were fixed with 10% formaldehyde overnight. After removal of the overlay media, cells were stained with 0.5% crystal violet and the plaques were counted. The percentage of inhibition was calculated as [1 – (VD/VC)] × 100%, in which VD or VC refer to the virus titer in the presence or absence of the test compound at the indicated concentration, respectively. Data in replicates were fitted with regression analysis to generate a dose–response curve, from which IC_{50} was calculated as the concentration at which a given compound reduces 50% of plaque number.

2.10. Cytotoxicity assay

The acid phosphatase assay was used to determine the cytotoxicity of tested compounds in Vero E6 cells [19]. In brief, Vero E6 cells were seeded at 2×10^4 per well in 96-well plates in DMEM supplemented with 2% FBS. After 24 h, media were removed, and cells were washed once with PBS. One-hundred microliters of fresh media with or without a compound at indicated concentrations was added for 72 h at 37 °C. At the endpoint, media were removed, and cells were washed once with PBS before adding 100 μL of the buffer containing 0.1 M sodium acetate (pH = 5.0), 0.1% Triton X-100, and 5 mM p-nitrophenyl phosphate. After incubation for 30 min at 37 °C, 10 μL of 1 N NaOH was added to stop the reaction. The absorbance at 405 nm (A_{405}) was measured using an ELISA reader (VERSAmax, Molecular Devices, Sunnyvale, CA). Relative growth was represented by A_{405} values with background correction and normalized with the corresponding control group as 100%. Cytotoxicity was calculated as one minus relative growth, and the half-cytotoxic concentration (CC_{50}) was defined as the concentration at which a given compound reduces 50% of cell viability.

3. Results

3.1. Identification of the human IFN-β transcriptional signature

We first performed differential gene expression analysis of primary normal human bronchial epithelial cells treated with IFN-β for 4, 6, or 12 h [12]. This analysis led to identification of a signature of 1,123 genes that were differentially expressed among the three IFN-β-treated conditions (BH-corrected $P < 0.05$; Figure 2A; Supplementary Table S1). We performed the pathway enrichment analysis to validate the IFN-β signature, confirming the IFN-β signature to be most significantly enriched for IFN signaling pathways — for example, REACTOME_INTERFERON_SIGNALING (BH-corrected hypergeometric $P = 1.42 \times 10^{-29}$), REACTOME_INTERFERON_ALPHA_BETA_SIGNALING ($P = 5.29 \times 10^{-29}$), and REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM ($P = 2.61 \times 10^{-28}$) (Figure 2B; for all enrichment results, see Supplementary Table S2). Together, these data establish a qualitative IFN-β host signature that serves to generate predictions of IFN-β-signature-enhancing compounds.

3.2. Predicted compounds capable of enhancing the host responses to IFN-β

We next applied the algorithm [15] to the IFN-β signature, identifying 23 compounds that appear to enhance a significantly large proportion of the signature (enhancement score >0.04, BH-corrected $P < 0.05$)

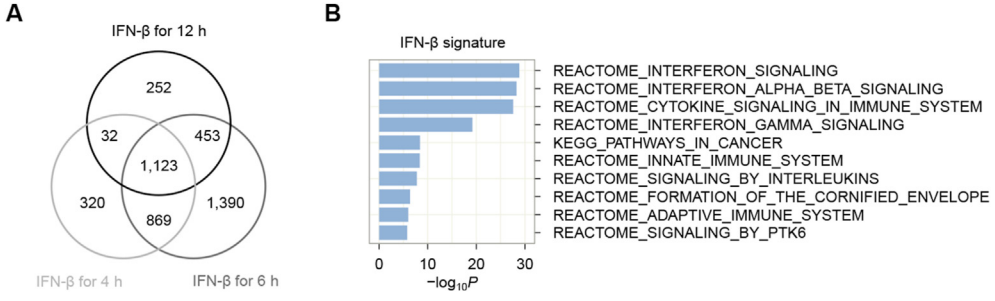

Figure 2. Identification of the human IFN-β signature. (A) Generation of the IFN-β signature. We performed differential gene expression analysis between IFN-β-treated (4, 6, or 12 h) and untreated conditions in normal human bronchial epithelial cells (NHBE) cells. A comparison of differentially expressed genes (DEGs; Benjamini–Hochberg [BH]-corrected $P < 0.05$) led to a commonly shared 1,123 DEGs as the IFN-β signature. For full results, see Supplementary Table S1. (B) Top 10 pathway enrichments of the IFN-β signature. We performed the hypergeometric test to determine the enrichments of canonical pathways of the Molecular Signature Database (MSigDB) gene set collection (C2 CP, version 7.1) (BH-corrected $P < 0.05$). For full results, see Supplementary Table S2.

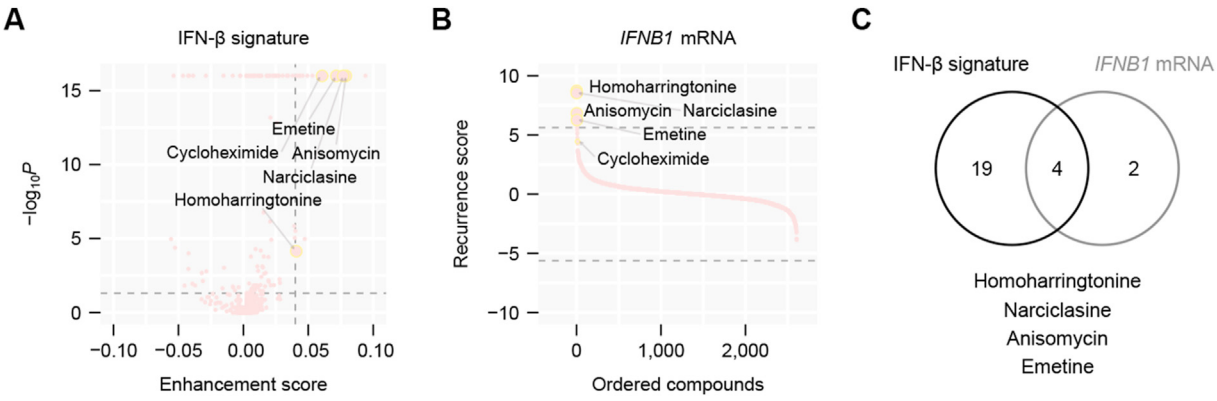

Figure 3. Predicted compounds capable of enhancing the host responses to IFN-β. (A) Predicted compounds enhancing the IFN-β signature. We used a gene-expression-based approach, which was developed to evaluate the degree of reversal of a given signature by small-molecule-regulated recurring transcripts across many cell types, to discover compounds that are able to enhance the human IFN-β-induced host signature. The algorithm generated an enhancement score for each compound to provide a rough interpretation such that a score of 0.1 corresponds to ~10% of enhancement in the signature. The horizontal dash line indicates a BH-corrected *P*-value of 0.05. The vertical dash line indicates an enhancement score of 0.04. For full results, see Supplementary Table S3. (B) Compounds associated with *IFNB1* regulatory recurrences. We used recurrent perturbation–transcript regulatory associations inferred between 3,332 compounds and 12,494 genes across 10 cell types and prioritized compounds according to their propensity to regulate the expression of *IFNB1* mRNA. The horizontal dash lines indicate mRNA recurrence scores for which FDR is 0.001. For full results, see Supplementary Table S4. (C) Overlap of significant predictions. Shown below the panel are compounds that significantly enhance the IFN-β signature and increase the expression of *IFNB1* mRNA.

(Figure 3A; Table 1; for full results, see Supplementary Table S3). These compounds were the protein kinase C (PKC) activators (ingenol, phorbol-12-myristate-13-acetate, and prostratin), the cardiac glycosides (digoxin, digitoxin, ouabain, and proscillaridin), the protein synthesis inhibitors (anisomycin, narciclasine, emetine, cycloheximide, puromycin, and homoharringtonine), the IκB kinase (IKK) inhibitors (IKK-2-inhibitor-V and BX-795), the anthelmintics (niclosamide and pyrvinium pamoate), the proteasome inhibitor bortezomib, the NF-κB pathway inhibitor parthenolide, the JAK–STAT signaling inhibitor cucurbitacin I, and other experimental compounds (F-1566-0341 and CT-200783).

To increase the confidence of the predictions, we further prioritized six compounds (homoharringtonine, narciclasine, anisomycin, BNTX [an opioid receptor antagonist], emetine, and QL-XII-47 [a Bruton tyrosine kinase inhibitor]) that show a tendency to recurrently upregulate *IFNB1* mRNA after treatment across cell types [15] (FDR <0.001) (Figure 3B; Table 2; for full results, see Supplementary Table S4). A final comparison of IFN-β-signature-enhancing and *IFNB1*-expression-increasing compounds led to four drug candidates for COVID-19 treatment (Figure 3C).

The four candidate compounds, namely homoharringtonine, narciclasine, anisomycin, and emetine, have a mechanism of action that involves inhibition of eukaryotic

Table 1. The 23 significant IFN-β-signature-enhancing compounds.

Compound	Enhancement score	Adjusted *P*-value	Primary mechanism of action
Ingenol	0.0940	0	PKC activator
Digoxin	0.0809	0	Cardiac glycoside
Anisomycin	0.0790	0	Protein synthesis inhibitor
Narciclasine	0.0769	0	Protein synthesis inhibitor
Phorbol-12-myristate-13-acetate	0.0759	0	PKC activator
Emetine	0.0716	0	Protein synthesis inhibitor
F-1566-0341	0.0642	0	Others
IKK-2-inhibitor-V	0.0636	0	IKK inhibitor
Prostratin	0.0622	0	PKC activator
Cycloheximide	0.0607	0	Protein synthesis inhibitor
Ouabain	0.0601	0	Cardiac glycoside
NSC-632839	0.0574	0	Ubiquitin-specific protease inhibitor
Digitoxin	0.0532	0	Cardiac glycoside
Niclosamide	0.0493	0	Anthelmintic
CT-200783	0.0473	1.07E-05	Others
Proscillaridin	0.0457	0	Cardiac glycoside
Puromycin	0.0434	0	Protein synthesis inhibitor
Bortezomib	0.0433	0	Proteasome inhibitor
Parthenolide	0.0432	0	NF-κB pathway inhibitor
BX-795	0.0422	0	IKK inhibitor
Cucurbitacin-i	0.0409	0	JAK–STAT signaling inhibitor
Homoharringtonine	0.0407	7.1E-05	Protein synthesis inhibitor
Pyrvinium pamoate	0.0401	3.03E-06	Anthelmintic

Table 2. The six significant *IFNB1*-expression-increasing compounds.

Compound	*IFNB1* mRNA regulatory recurrence score	FDR	Primary mechanism of action
Homoharringtonine	8.738	3.97E-06	Protein synthesis inhibitor
Narciclasine	8.550	5.60E-06	Protein synthesis inhibitor
Anisomycin	6.842	1.19E-04	Protein synthesis inhibitor
BNTX	6.412	2.54E-04	Opioid receptor antagonist
Emetine	6.281	3.20E-04	Protein synthesis inhibitor
QL-XII-47	5.632	9.83E-04	Bruton tyrosine kinase inhibitor

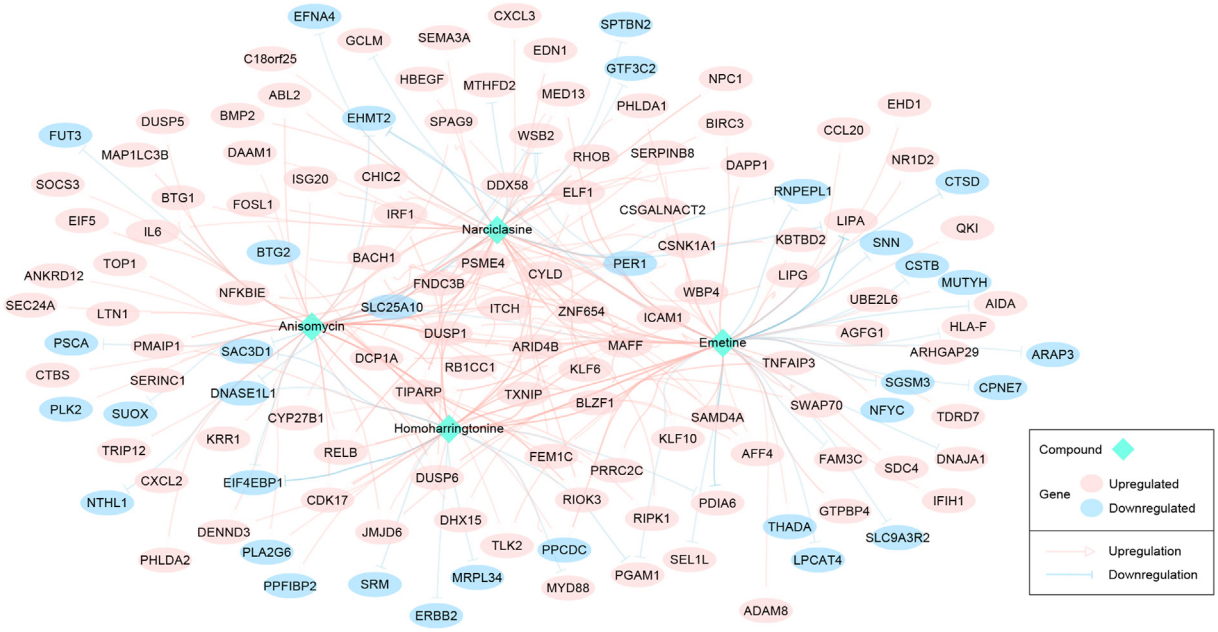

Figure 4. Drug–target network involving IFN-β-responsive genes and the candidate compounds. Shown are significant compound–transcript regulatory relationships (FDR <0.001, for which an edge of light red indicates an upregulation whereas light blue indicates a downregulation) between the four candidate compounds (cyan diamond) and IFN-β-responsive genes (ellipse for which a light red color indicates an upregulated gene whereas light blue indicates a downregulated gene).

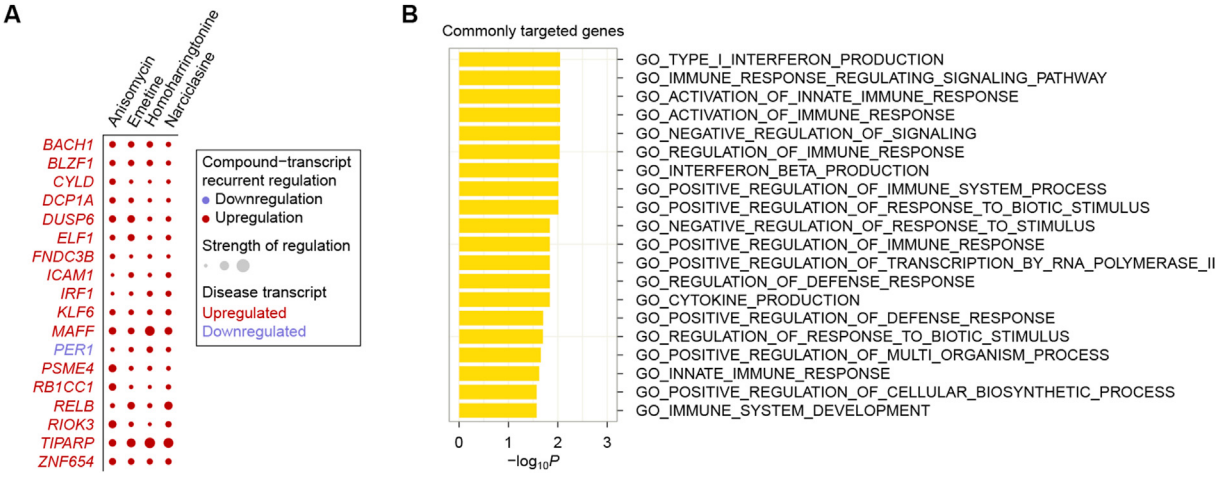

Figure 5. Commonly targeted IFN-β signature genes by the candidate compounds. (A) Target relationships between the candidate compounds and their commonly shared IFN-β signature genes. (B) Gene ontology (GO) enrichments of the commonly targeted IFN-β-responsive genes by the candidate compounds. Shown are the top 20 significant enrichments of GO biological processes (BH-corrected $P < 0.05$) defined in the MSigDB database (C5 BP, version 7.1). For full results, see Supplementary Table S5.

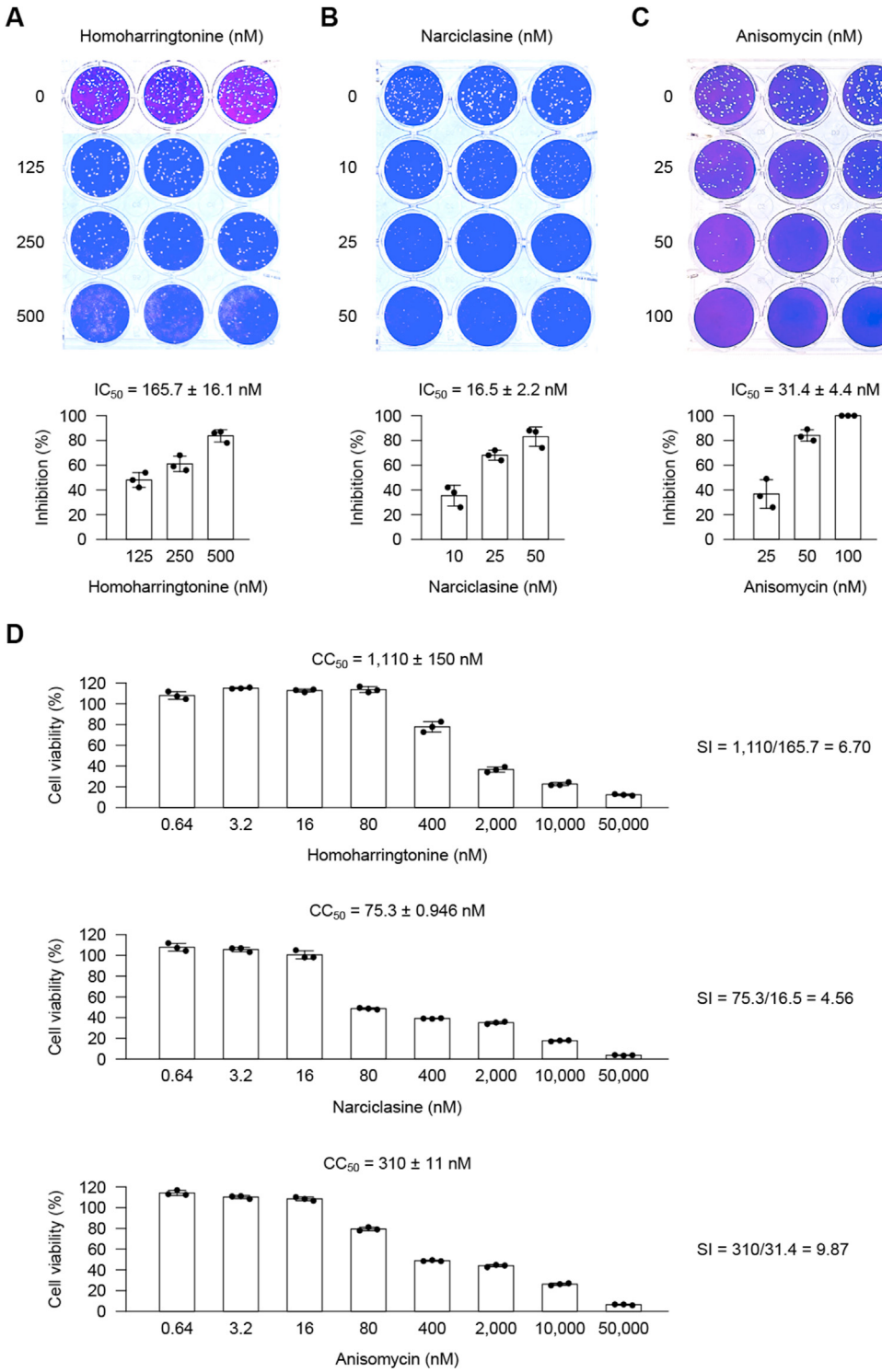

Figure 6. Validation of anti-SARS-CoV-2 activity of select compounds. (A–C) Inhibition of SARS-CoV-2 infection in Vero E6 cells after treatment with homoharringtonine (A), narciclasine (B), or anisomycin (C) for 120 h. IC_{50}, half-maximal inhibitory concentration. (D) Cytotoxicity of selected compounds in Vero E6 cells. CC_{50}, half-cytotoxic concentration. SI, selectivity index. Data were presented as mean ± SD.

protein synthesis [20]. Our analysis revealed that a significant proportion of IFN-β-responsive genes are intensively regulated by the four candidate COVID-19 drugs (Figure 4). A detailed investigation of their commonly shared genes further verified the ability of these compounds to regulate the production of type I IFNs and the process of immune responses (Figure 5A) — for example, GO_TYPE_I_INTERFERON_PRODUCTION (BH-corrected hypergeometric $P = 8.95 \times 10^{-3}$), GO_IMMUNE_RESPONSE_REGULATING_SIGNALING_PATHWAY ($P = 8.95 \times 10^{-3}$), and GO_ACTIVATION_OF_INNATE_IMMUNE_RESPONSE ($P = 8.95 \times 10^{-3}$) (Figure 5B; for all enrichment results, see Supplementary Table S5). Notably, these common genes were also significantly enriched for DNA-binding transcription factor activity (GO_DNA_BINDING_TRANSCRIPTION_FACTOR_ACTIVITY, $P = 0.0131$), including *BACH1* (BTB domain and CNC homolog 1), *BLZF1* (basic leucine zipper

nuclear factor 1), *ELF1* (E74 like ETS transcription factor 1), *IRF1* (interferon regulatory factor 1), *KLF6* (Kruppel like factor 6), *MAFF* (MAF bZIP transcription factor F), and *RELB* (RELB proto-oncogene, NF-kB subunit) (Supplementary Table S6).

3.3. Validation of anti-SARS-CoV-2 activity of select compounds

Given that the anti-SARS-CoV-2 activity of emetine has been reported [5], we performed a plaque reduction assay [18] to assess the antiviral activity of three selected compounds against SARS-CoV-2 in Vero E6 cells, and an ACP assay [19] to determine their cytotoxicity. From these assays, we were able to determine a half-maximal inhibitory concentration (IC_{50}), half-cytotoxic concentration (CC_{50}), and selective index (SI) value ($SI = CC_{50}/IC_{50}$) for each compound. We found that homoharringtonine (IC_{50} = 165.7 nM, CC_{50} = 1,110 nM, SI = 6.70), narciclasine (IC_{50} = 16.5 nM, CC_{50} = 75.3 nM, SI = 4.56), and anisomycin (IC_{50} = 31.4 nM, CC_{50} = 310 nM, SI = 9.87) profoundly reduced viral replication at low concentrations at which they had modest cytotoxicity (Figure 6). Collectively, these data demonstrate that the three compounds strongly inhibited SARS-CoV-2 replication at nanomolar, relatively non-toxic concentrations.

4. Discussion

Beyond targeting SARS-CoV-2 proteins, our host-centric exploration of the compounds' ability to induce protective antiviral responses has successfully revealed some promising therapies for COVID-19. This was achieved by comparing thousands of small-molecule-perturbed transcriptional responses with a high-quality IFN-β-induced host signature. Of 23 significant IFN-β-signature-enhancing compounds, some have been hypothesized to possess the likely antiviral mechanisms against coronavirus infections, such as cardiac glycosides [21] and niclosamide [22]. We then proceeded to identify four compounds, namely homoharringtonine, narciclasine, anisomycin and emetine, that were found to be able to enhance a significant fraction of the core IFN-β response genes, with some of them known for their roles in IFN-I gene induction [23]. Interestingly, it has been suggested that, in mouse cells transfected with the human *IFNB1* gene, treatment with cycloheximide, emetine, or puromycin alone could stimulate *IFNB1* gene expression and augment IFN production in response to poly (I:C), a synthetic analog of double-stranded RNA that binds to toll-like receptor 3 and activates downstream signaling [24, 25]. Together, our data substantiate the capability of the four candidate compounds to induce a similar host response to IFN-β, providing a molecular mechanism for their potential efficacy against COVID-19.

In this study, we performed *in vitro* experiments to confirm the antiviral effects of select compounds against SARS-CoV-2 infection. In support of our findings, the anti-SARS-CoV-2 activity of emetine, as well as another commonly used translation inhibitor cycloheximide, has been reported recently [5]. However, the extent to which the observed anti-SARS-CoV-2 effects of select compounds correlate with IFN-I signaling and ISGs warrants further investigation. Alternatively, the clear efficacy of select compounds has validated our approach of comparing host transcriptional responses to IFN-I treatment, while suggesting intriguing opportunities to repurpose these compounds for COVID-19. Our analysis was further supported by the most recent evidence revealing that SARS-CoV-2 proteins can antagonize IFN-I responses [26, 27] and early IFN-Is may be protective against COVID-19 [27, 28, 29, 30].

We note that while the four candidate compounds have been shown to exert antiviral effects against certain virus infections [31, 32, 33], they may also display broad antitumor activity [34, 35, 36, 37]. Homoharringtonine is the only drug currently approved by the US Food and Drug Administration for the treatment of chronic myeloid leukemia [38]. Given their promising anticancer activity, together with a recent finding demonstrating that patients with cancer are more vulnerable to SARS-CoV-2 outbreak [39], the potential benefits of these compounds in the treatment of COVID-19 patients with cancer deserve further clinical studies.

Importantly, despite the demonstrated ability of the four candidate compounds to elicit similar transcriptional responses to IFN-β, we note that Vero E6 cells (an African green monkey kidney cell line used for validation in this study) are devoid of IFN-I production [40], owing to homozygous deletion on chromosome 12 containing the IFN-I gene loci [41]. Although it is unlikely that these compounds induce such transcriptional responses by directly acting on the IFN-I receptor complex (composed of IFNAR1 and IFNAR2 to which IFN-β binds and relays the signal), we reasoned that it is either compounds' primary mechanisms of action (MoAs) or other possible secondary mechanisms that initiate a series of signaling events culminating in transactivation of IFN-β-responsive genes. Therefore, the observed anti-SARS-CoV-2 effects of the tested compounds in IFN-I-deficient Vero E6 cells may be explained by the ability of the compounds to transactivate IFN-β-responsive genes through their primary MoAs (or other unknown mechanisms).

Recent studies suggest that IFN-IIIs might be an important area of coronavirus research given the fact that the IFN-III receptor complex (composed of INFLR1 and IL-10Rβ) is preferentially expressed on epithelial cells of respiratory and mucosal barriers, on which SARS-CoV-2 infection occurs and IFN-IIIs are the predominant antiviral cytokines [42, 43]. Compared with IFN-Is, the effects of IFN-IIIs are focused, long-lasting, and non-inflammatory, thus making IFN-λ as an attractive therapeutic strategy for COVID-19. To date, IFN-IIIs have not yet been approved for any indications, but several clinical trials are under way to evaluate their potential use in COVID-19 (for example, NCT04331899, NCT04343976, NCT04354259, NCT04388709, and NCT04344600). Although the antiviral activity of IFN-IIIs is less explored, it has been shown that IFN-Is and IFN-IIIs drive a similar transcriptional signature and ISG response during influenza virus infection [44], suggesting that the IFN-I and IFN-III system may compensate for each other. Given also the fact that several signaling molecules downstream of IFN-Is and IFN-IIIs are shared (such as JAK–STAT signaling effectors or IRF family members) [8, 45], it is reasonable to expect that the transcriptional responses to IFN-λ in primary normal human bronchial epithelial cells (used for identifying IFN-β-responsive signature in this study) might have significant overlap with those of IFN-β. Also, although the IFN-III receptor is mainly restricted to epithelial cells, the IFN-I receptor is ubiquitously expressed in almost all human cell types (including epithelial cells), providing broad antiviral protection [45]. Together, these current understandings support our rationale of using IFN-β instead of IFN-λ to predict effective COVID-19 therapies.

However, it should still be noted that delayed and prolonged IFN responses are not helpful for viral clearance but may instead exacerbate inflammation, as evidenced by two recent studies demonstrating that chronic IFN-Is and IFN-IIIs may disrupt lung epithelial repair during recovery from viral infection [46, 47]. This suggests that early administration of IFN-based therapies may be critical for effective treatment of COVID-19 [10].

Our approach for predicting effective COVID-19 treatment is different and complementary to other recent large-scale screening efforts, such as proteomics-based strategies that explore the host dependencies of SARS-CoV-2 to identify druggable pathways and proteins [5, 6] or structure-based virtual and high-throughput screening against a specific viral element [7]. Instead, our approach is based on two assumptions: that low IFN-Is correlate with severe COVID-19 (which is informed by our increasing knowledge of the disease), and that compounds capable of inducing IFN-I responses are therefore protective against COVID-19 (which can be realized through our previous gene expression approach). Importantly, compared with other existing computational approaches, such as network-biology-based drug repurposing [48, 49, 50], our analysis was supported by experimental validation in cell-based assays. This constitutes an advantage of our approach over other

computational predictions that often fail to translate into success even in the cell models [51].

As our understanding of COVID-19 grows, combined targeting of different aspects of SARS-CoV-2 biology may inform better treatments. For example, it might be beneficial for COVID-19 patients when combining one drug targeting the viral element (such as remdesivir) with another drug targeting the host factor (such as those identified in [5, 6]). In this study, we demonstrated the IFN-I-enhancing capability of the four candidate COVID-19 drugs, providing a rationale for their potential use in combination therapy.

The strategy of repurposing existing drugs for the treatment of COVID-19 remains to be a powerful solution and could be facilitated with the use of artificial intelligence [51]. In this work, we realize a computational drug repurposing solution to COVID-19 by building a prediction model based on our current understanding of the disease biology. With this success, we expect more artificial intelligence-informed effective treatments could be identified to stop the COVID-19 pandemic in the near future.

Declarations

Author contribution statement

H.F. Juan: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

C.T. Huang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

H.C. Kao, Y.H. Pang, W.H. Lee, C.H. Hsieh and T.L. Chao: Performed the experiments; Analyzed and interpreted the data.

S.Y. Chang: Contributed reagents, materials, analysis tools or data; Wrote the paper.

H.C. Huang: Conceived and designed the experiments; Wrote the paper.

Funding statement

This work was supported by the Higher Education Sprout Project (NTU-109L8837A), the Ministry of Science and Technology (MOST 109-2221-E-002-161-MY3, MOST 109-2320-B-002-017-MY3 and MOST 109-2327-B-002-009) and the National Health Research Institutes (NHRI-EX109-10709BI) in Taiwan.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e05646.

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