Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Science Bulletin 66 (2021) 884–888

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Short Communication

A large-scale transcriptional study reveals inhibition of COVID-19 related cytokine storm by traditional Chinese medicines

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ARTICLE INFO

Article history:
Received 10 September 2020
Received in revised form 25 October 2020
Accepted 7 January 2021
Available online 12 January 2021

The application of traditional Chinese medicine (TCM) has made great contributions to the fight against the epidemic of coronavirus disease-2019 (COVID-19). Despite the remarkable therapeutic effects of TCM, the molecular mechanisms of TCM formulae inhibiting COVID-19 are still not fully understood. Here, we combined the automated high throughput sequencing-based high throughput screening (HTS^2) assay with bioinformatics and computer-aided drug design (CADD) to investigate the molecular mechanisms of TCM-mediated therapeutic effects on COVID-19-related cytokine storm (Fig. 1a).

Evidence from a wide range of sources suggests that cytokine storm is an important indicator of the deterioration of COVID-19 [1]. Thereinto, the interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) pathways are likely to be the most important signaling pathways causing cytokine storm [2]. Interestingly, some TCMs might have immunosuppressive features to prevent and treat cytokine storm [3]. Moreover, in view of the characteristics of multiple targets and multiple pathways, TCM is suitable for the treatment of complex signal networks of the immune response.

HTS^2 is a high-throughput screening platform based on the gene expression signature that quantitatively analyzes cell transcriptional profiles at a large scale [4,5]. Therefore, we carried out the HTS^2 assay to detect the regulation of 578 herbal extracts on the IL-6 and TNF-α signaling pathways in immune cells (Table S1 online), which includes most of the herbs reported in the *Pharmacopoeia of the People's Republic of China* (2015 edition). After the correlation analysis for evaluating the reproducibility of HTS^2 assay (Fig. S1 online), Gene Set Enrichment Analysis (GSEA) was conducted.

A total of 16 herbs was identified to have a significant inhibitory effect on the IL-6 pathway (Fig. 1b and Fig. S2a online), while 37 herbs suppress the TNF-α pathway significantly (Fig. 1c and Fig. S2b online). In the heatmap of gene expression induced by these herbs, more than half of the genes are down-regulated, suggesting that these herbs have a significant inhibitory effect on the expression of cytokine storm-related genes (Fig. S2c, e, and Tables S2, S3 online). Notably, some of these herbs have been previously reported to reduce the production of IL-6 in inflammatory cells, such as Mufurongye [6], Xuanfuhua [7], and Guizhi [8]. And some herbs have been reported to decrease the level of TNF-α or down-regulate the gene expression of TNF-α, such as Cheqianzi [9], Dangshen [10], and Guanhuangbo [11]. Interestingly, the correlation

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https://doi.org/10.1016/j.scib.2021.01.005
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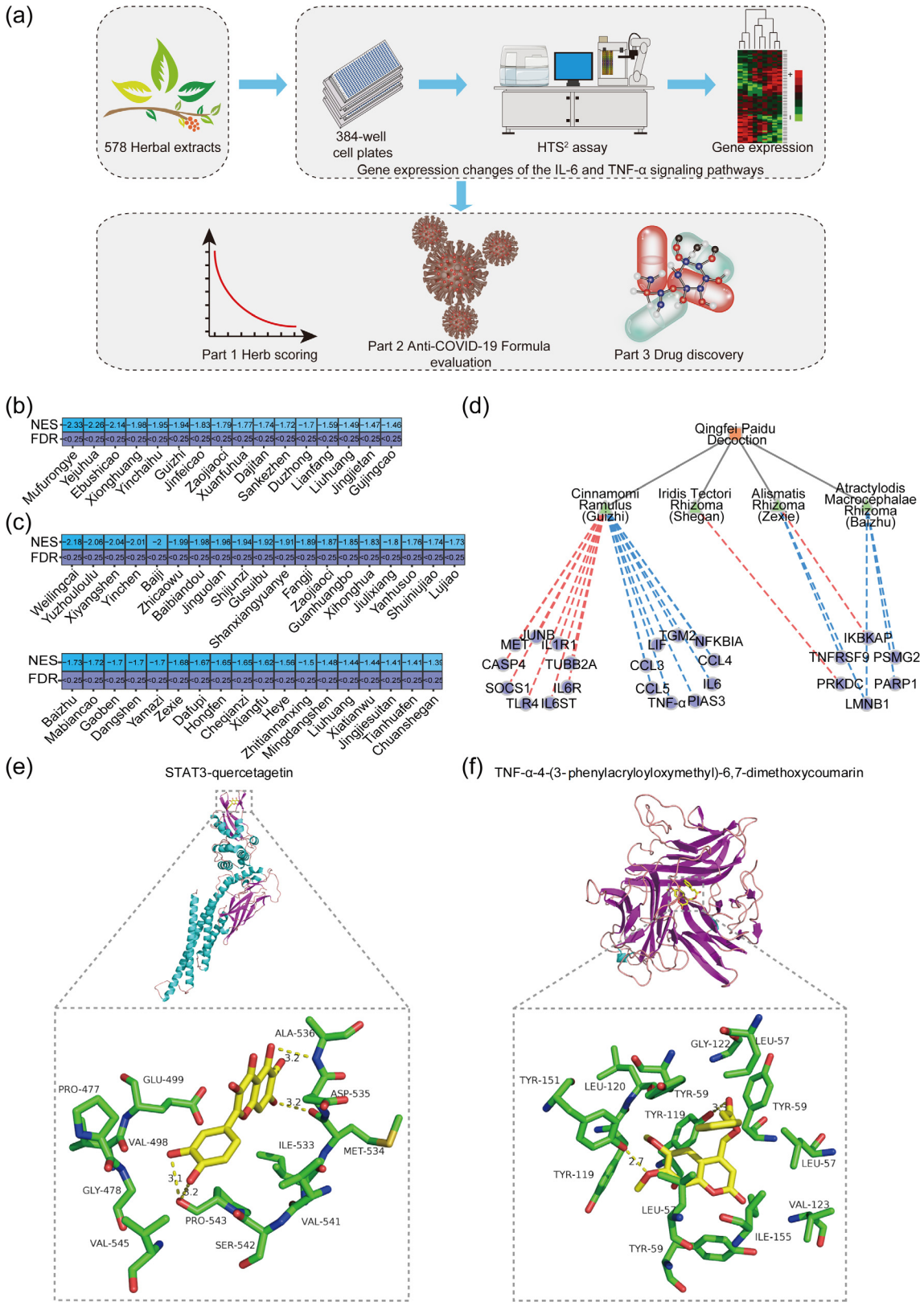

Fig. 1. The inhibitory effect of traditional Chinese medicines on COVID-19 related cytokine storm. (a) Experimental workflow. The GSEA results of 16 herbs inhibiting the IL-6 pathway (b) and 37 herbs inhibiting the TNF-α pathway (c) with the cutoff of FDR < 0.25. (d) The formula-herb-gene-pathway network diagram of Qingfei Paidu Decoction acting on the IL-6 and TNF-α pathways. The red dashed represents the upregulation of genes, and the blue dashed represents the downregulation of genes. (e) Structures and orthogonal views of the pocket of binding between quercetagetin and STAT3 (PDB ID: 6NJS) based on molecular dynamics simulation. (f) Structures and orthogonal views of the pocket of binding between 4-(3-phenylacryloyloxymethyl)-6,7-dimethoxycoumarin and TNF-α (PDB ID: 6OP0) based on molecular dynamics simulation.

heatmap showed a similarity of 16 herbs in suppressing the IL-6 pathway (Fig. S2d online), while 37 herbs may have different modes of action in inhibiting the TNF-α pathway (Fig. S2f online).

We further collected 27 COVID-19 treatment protocols published by the National Health Commission of the People's Republic of China and 26 by different provinces and cities across the country. These protocols include 338 TCM formulae, 196 of which correspond to classical TCM formulae and 142 correspond to newly developed formulae. Through analysis, we found that the most frequently administrated formula is Maxing Shigan Decoction, an effective formula for treating SARS in 2003, followed by some formulae belonging to "three TCM drugs and three formulae" promoted by the National Health Commission of the People's Republic of China (Fig. S3a online). We also counted the frequency of herbs in the 338 prescriptions, revealing that Gancao, Kuxingren, and Guanghuoxiang are used most frequently in the prevention and treatment of COVID-19 (Fig. S3b online). Besides, Baizhu and Shuiniujiao are also in the list, which were identified by our analysis as top suppressors of the TNF-α pathway.

Based on the above analyses, we evaluated the effect of anti-COVID-19 formulae in inhibiting cytokine storm. The number of herbs that we found with suppressing effects on the IL-6 or TNF-α pathways was collected in each COVID-19 prescription, as well as the proportion relative to the total number of these herbs in the prescription was counted. Our results showed that all the top three prescriptions in 196 classical TCM formulae, including Lizhong Decoction, Liujunzi Decoction and Huanglian Jiedu Decoction, contain TNF-α pathway inhibiting herbs (Table S4 online). Consistent with our findings, some previous studies reported that Lizhong Decoction could reduce the yield of inflammatory cytokines [12], Liujunzi Decoction can regulate immunity and release of inflammatory factors [13], and Huanglian Jiedu Decoction exhibits a therapeutic effect on inflammation [14]. Notably, Qingfei Paidu Decoction, one of the most well-known anti-COVID-19 formulae, ranks fourth, and this prescription has been widely used and played a crucial role in the fight against COVID-19 in Wuhan, China. A recent study has shown that the main components of Qingfei Paidu Decoction are polysaccharide, and one of its most remarkable characteristics is an immunomodulatory activity [15]. Nonetheless, the mechanism of action (MOA) of Qingfei Paidu Decoction in inhibiting cytokine storm is still unclear. Thus, we conducted further in-depth research on it.

By combining the HTS^2 assay and herb scoring results, we constructed a formula-herb-gene-pathway network diagram of Qingfei Paidu Decoction (Fig. 1d). In this formula, we identified 4 herbs with an inhibitory effect on the IL-6 or TNF-α pathways, including Guizhi, which significantly inhibits the IL-6 pathway, and Shegan, Baizhu, and Zexie, which significantly inhibit the TNF-α pathway. In particular, Guizhi down-regulates the expression of *IL-6* and *TNF-α* genes. Shegan up-regulates the expression of protein kinase, DNA-activated, catalytic subunit (*PRKDC*) gene, while Baizhu down-regulates the expression of proteasome assembly chaperone 2 (*PSMG2*), poly (ADP-ribose) polymerase 1 (*PARP1*), and lamin B1 (*LMNB1*) genes. Moreover, Zexie down-regulates the expression of TNF receptor superfamily member 9 (*TNFRSF9*) and *LMNB1* genes, and up-regulates the expression of elongator acetyltransferase complex subunit 1 (*IKBKAP*) gene. In addition, we also conducted network pharmacology analysis on Qingfei Paidu Decoction affecting IL-6 and TNF-α pathways (Fig. S4 online), which further strengthens our conclusion that Qingfei Paidu Decoction might significantly inhibit IL-6 and TNF-α signaling pathways.

To explore the key targets of herbs suppressing the IL-6 and TNF-α pathway, we built the protein–protein interaction network (Fig. S5a, c online). It was established based on the genes that represent components of these two pathways. Subsequently, three centrality algorithms were used to calculate the whole network, and the top 10 targets were identified according to the results of topological analysis (Fig. S5b, d online). Thereinto, signal transducer and activator of transcription 3 (STAT3) and TNF-α were selected as the key target of the IL-6 and TNF-α pathway, respectively. Studies have shown that STAT3 is a potential molecular target for clinical syndromes characterized by systemic inflammation [16], and the TNF-α inhibitors could show high therapeutic efficacy in treating chronic inflammatory diseases via the promotion of a rapidly decreased number of cells [17].

In order to predict active ingredients with the binding potential to STAT3 and TNF-α, we collected all chemical components contained in 48 out of the 53 herbs showing inhibitory activity on the expression of the IL-6 and TNF-α-related gene signatures. We could not find chemical information for the remaining five herbs (Dajitan, Jinjietan, Shanxiangyuanye, Zhitiannanxing, and Jingjiesuitan). Afterwards, all compounds with oral bioavailability $\geq$30% and drug-likeness $\geq$0.18 were identified as the active ingredients. A total of 375 and 1402 active ingredients was identified for the IL-6-related gene signature and TNF-α-related gene signature, respectively (Table S5 online). Further, molecular docking was applied to predict the active ingredients that could bind to STAT3 or TNF-α, the two key proteins in the IL-6 and TNF-α signaling pathways. With the lowest Glide gscore, the compounds with the highest binding stability towards STAT3 and TNF-α are quercetagetin and 4-(3-phenylacryloyloxymethyl)-6,7-dimethoxycoumarin, respectively (Table S6 online).

To interrogate the binding potential of the optimal active ingredient towards STAT3 and TNF-α, we next conducted molecular dynamics (MD) analysis (Fig. S6 online, Table S7 online). Through MD simulation, we identified the optimal conformation of the active ingredient binding to the target. The results showed that quercetagetin could bind to the C-terminal domain of STAT3 by forming hydrogen bonds with the key residues Ala-536, Met-534, and Pro-543 (Fig. 1e). A number of neighboring hydrophilic residues (Glu-499 and Asp-535) and hydrophobic residues (Ile-533 and Val-545) may also contribute to the interaction's stability. Meanwhile, MD simulation infers 4-(3-phenylacryloyloxymethyl)-6,7-dimethoxy coumarin binding to the hydrophobic core of the β domain of TNF-α (Fig. 1f). As the residues at their binding sites are mainly hydrophobic residues, they could form strong hydrophobic interactions and Van der Waals forces. An orthogonal view also suggests the formation of hydrogen bonds with the residues Tyr-119 and Tyr-151. In combination, these simulations further suggest the stable binding of quercetagetin to STAT3 and 4-(3-phenylacryloyloxymethyl)-6,7-dimethoxycoumarin to TNF-α. The overall results are summarized (Fig. 2), which shows the docking results of active ingredients binding with key targets, and the top three herbs with inhibitory effects on key nodes in the IL-6 and TNF-α pathways, and the main targets of Qingfei Paidu Decoction.

In conclusion, facilitated by HTS^2 technology, we evaluated the effects of 513 herbs and all 338 reported anti-COVID-19 TCM formulae on cytokine storm-related signaling pathways, and identified the key targets of the relevant pathways and potential active ingredients in these herbs. This study uncovered molecular mechanisms of TCM-mediated therapeutic effects on COVID-19-related cytokine storm, and provided scientific evidence for the understanding of MOA of TCMs.

Conflict of interest

The authors declare that they have no conflict of interest.

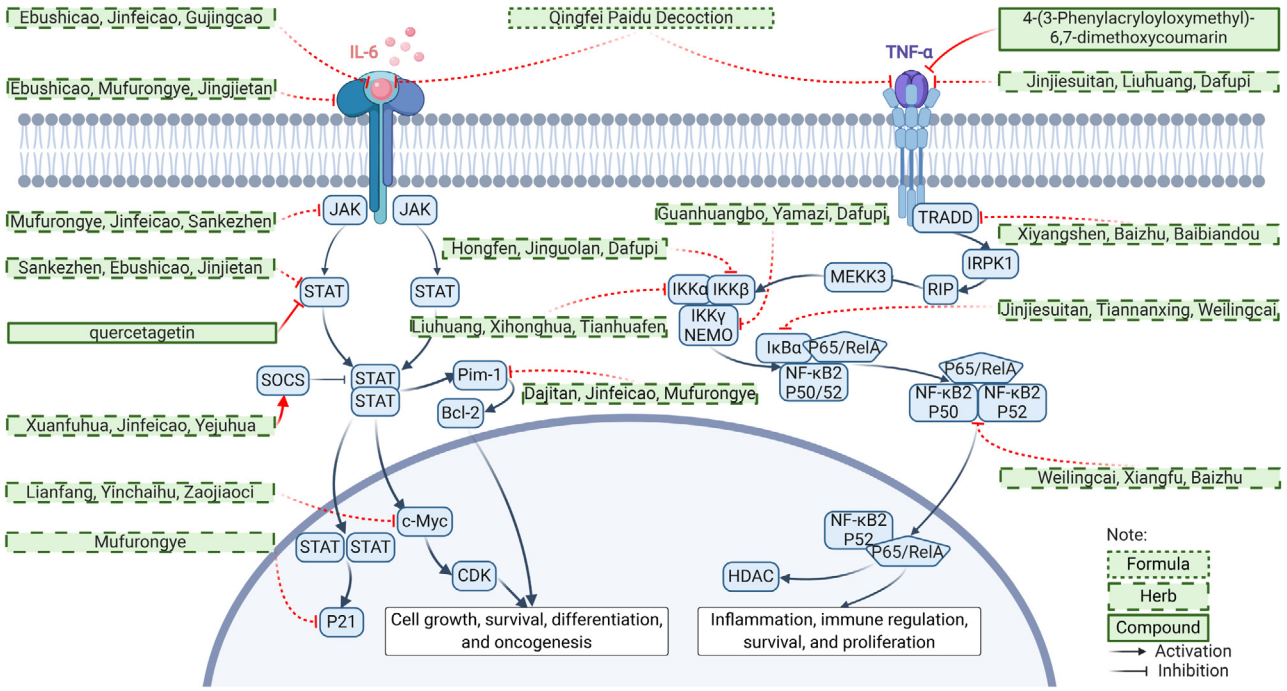

Fig. 2. Summary diagram concerning the molecular mechanisms of formula, herb, and compound that inhibit cytokine storm-related pathways.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81673460) and Sichuan Youth Science and Technology Innovation Research Team of Experimental Formulology (2020JDTD0022).

Author contributions

Yifei Dai, Lan Xie, Xun Lan, and Dong Wang conceived the study and designed the experiments. Yifei Dai and Weijie Qiang performed the experiments with all the other authors help. Yifei Dai, Weijie Qiang, Yu Gui, Xue Tan, Tianli Pei, and Lan Xie collected the data. Yifei Dai and Weijie Qiang wrote the manuscript. Kequan Lin, Siwei Cai, Liang Sun, Guochen Ning, Jianxun Wang, Hongyan Guo, Yimin Sun, Jing Cheng, Lan Xie, Xun Lan, and Dong Wang discussed and edited the manuscript. All authors read and gave final approval to submit the manuscript.

Appendix A. Supplementary materials

Supplementary materials to this article can be found online at https://doi.org/10.1016/j.scib.2021.01.005.

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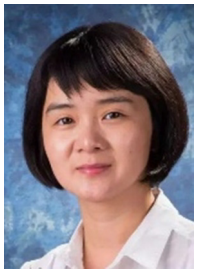

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