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Therapeutic targets of vitamin C on liver injury and associated biological mechanisms: A study of network pharmacology☆

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ABSTRACT

In our previous studies, vitamin C (VC) exerts potent pharmacological activities against liver injury (LI). Therefore, this report was designed to use network pharmacology-based strategy to predict therapeutic targets of VC against LI, and further to investigate the pharmacological molecular mechanisms. Pathological targets of LI were identified, followed by acquisition of verified targets of VC. After constructing target-functional protein interaction network of VC against LI, the core therapeutic targets of VC against LI were obtained. Further, biological function and pathway enrichment analyses were performed on core therapeutic targets to evaluate the biological processes and key signaling pathways of VC against LI. As revealed in network pharmacology assays, 6 key therapeutic targets for VC against LI were identified, showing tumor necrosis factor (TNF), nuclear factor-kappa-B p65 (RELA), nuclear factor-kappa-B p105 (NFKB1), TNF receptor-associated factor 2 (TRAF2), interleukin 6 (IL-6) and interleukin 1 beta (IL1B). On the basis of data analyses from DAVID database and omicshare cloud platform, bio-functional enrichment assays showed that the therapeutic effects of VC against LI were closely associated with regulating inflammatory reaction and apoptosis. Further, pathway enrichment analysis indicated the anti-LI benefits of VC were principally implicated in regulating the top 20 signaling pathways, such as inflammation-associated TNF signaling pathway, NF-κB signaling pathway. Taken together, the bioinformatics data elucidate that anti-LI pharmacological activities of VC may be predominantly related to inhibition of inflammatory stress, contributing to suppression of LI development. These resultant findings highlight the predicted therapeutic targets may be potential biomarkers for anti-LI.

1. Introduction

Physiologically, liver is a key organ characterized with detoxicated and metabolic functions. If the liver is injured by external aggression, its physiological function will be impaired, gradually developing health risks [1,2]. On the basis of lifestyle and environmental changes around human, various adverse factors can cause liver dysfunctions, such as excessive drinking, unhealthy diet, drug and pollutant exposures [3–5]. Liver injury (LI) may be further developed into diseases of fibrosis, cirrhosis, hepatic carcinoma, and liver failure. In human, LI will seriously affect the quality of life over time [6]. Therefore, it is urgent to develop therapeutic candidate against LI in an attempt to identify molecular mechanisms involved. Vitamin C (VC) is a necessary nutrient associated with the healing of tissue and promotion of key

☆ List of websites used as follows,
HIT: http://lifecenter.sgst.cn/hit/welcome.html,
pharmMapper: http://lilab.ecust.edu.cn/pharmmapper/index.php,
Swiss Target Prediction: http://www.swisstargetprediction.ch/index.php,
Drugbank: https://www.drugbank.ca/,
DisGeNET: http://www.disgenet.org/web/DisGeNET/menu/home,
STRING:https://string-db.org/cgi/input.pl?sessionId=MbW2ndYUZ1B3&input_page_show_search=on,
DAVID: https://david.ncifcrf.gov/home.jsp,
Omicshare cloud platform: http://www.omicshare.com/tools/Home/Soft/gogsea.

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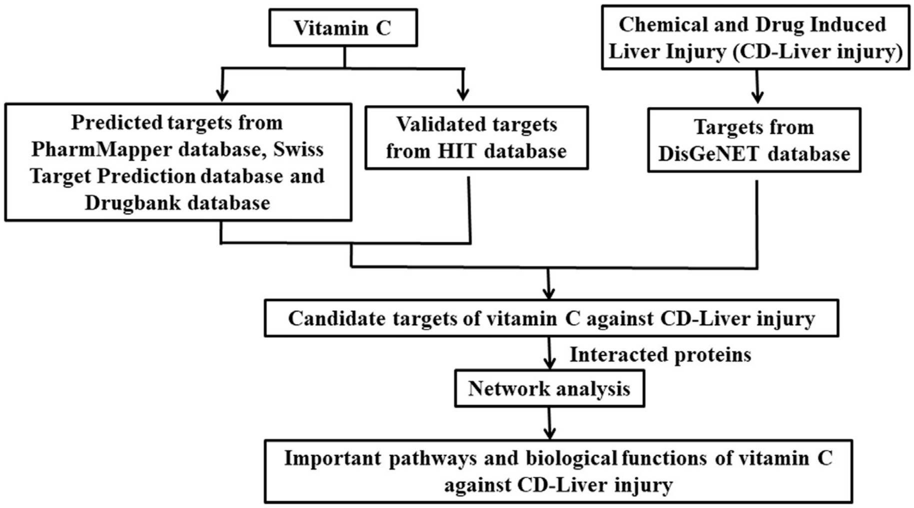

Fig. 1. Flowchart of designed analysis in vitamin C against liver injury.

neurotransmitters. VC functions as a potent antioxidant and helps enhance immunocompetence through regulating functional enzymes [7,8]. Many outstanding studies have shown that VC can protect against LI in clinical and basic studies, because VC is seemed to be cost-effective and low toxicity [9]. Interestingly, our previous evidences showed VC plays potent pharmacological activities against LI in vivo and in vitro [10,11]. However, the detailed therapeutic targets of VC against LI remain unknown. On the basis of the scientific strategy from network pharmacology, the current study aimed to systematically investigate the predicted therapeutic targets and biological signaling pathways of VC against LI, and to further provide bioinformatics data for the follow-up clinical and basic researches on the treatment of LI. The entire design of this study was showed in flowchart proposed (Fig. 1).

2. Methods

2.1. Acquisition of VC anti-LI targets

All verified targets of VC were harvested by use of the HIT database, and all predicted targets of VC were obtained from databases of pharmMapper, Swiss Target Prediction, and Drugbank. In addition, DisGeNET database was employed in detecting pathological targets for LI. Subsequently, VC-associated targets were mapped to pathologic targets of LI, and then therapeutic targets of VC against LI were obtained.

2.2. Construction of PPI network and topological analysis in VC against LI

Further, STRING database was used to collect target and target-functional proteins. Protein interactions with a confidence score $>$ 0.9 were selected in designed setting after eliminating duplicates. Resultant data were introduced into Cytoscape (v3.2.1) to establish protein-protein interaction (PPI) network of VC against LI. Network analyzer in Cytoscape was utilized in analyzing topological parameters of mean and maximum degrees of freedom in PPI network of VC against LI. The core targets were screened according to the setting of the value. The upper limit of the screening range was the maximum degree value in topological data, and the lower limit was twice the average degree of freedom.

2.3. Cluster analysis

In brief, clustering analysis of PPI network of VC against LI was used MCODE algorithm [12].

2.4. Biological function and pathway enrichment analyses of core targets

Available database for annotation, visualization and integrated discovery (DAVID) database was employed to obtain the results of biological function and pathway enrichment in core targets. Visualization of biological processes and signaling pathways associated with therapeutic targets of VC against LI were organized and imported by omicshare cloud platform. And the data were processed by p-value for producing a high-level bubble map of biological processes and signal pathways.

3. Results

3.1. Information of assayed targets

As results, 236 genes were collected by use of DisGeNET database to research disease-related targets. Furthermore, 39 verified targets of VC were detected from the HIT database, and 9 therapeutic targets of VC against LI were obtained after being mapped with pathological targets of LI. A total of 300 predicted targets were screened from the PharmMapper database, and 15 predicted targets were harvested from the Swiss Target Prediction database, as well as other 26 predicted targets were gained from the Drugbank database. Notably, 2 predicted targets were identified with no repetition, characterized with final identification of 11 therapeutic targets of VC against LI.

3.2. Anti-LI targets of VC and function-related protein interaction network

Further, STRING database was used to produce data of 11 target-related PPI. And data with confidence score $>$ 0.9 were selected and then introduced into Cytoscape to construct functional-related protein interaction network of VC against LI. As revealed in Fig. 2, loop network had 101 nodes, in which were interconnected and associated by 584 edges.

3.3. Clustering analysis in PPI network

In addition, clustering subnetworks were produced by using the MCODE algorithm in Cytoscape software. As shown in Fig. 3, the predicted targets of SOD1, SOD3 and CAT were grouped together.

3.4. Topology parameter analysis and identification of core targets

Network analyzer was used to assay the topological parameters of VC against LI and function-related protein interaction network. Because

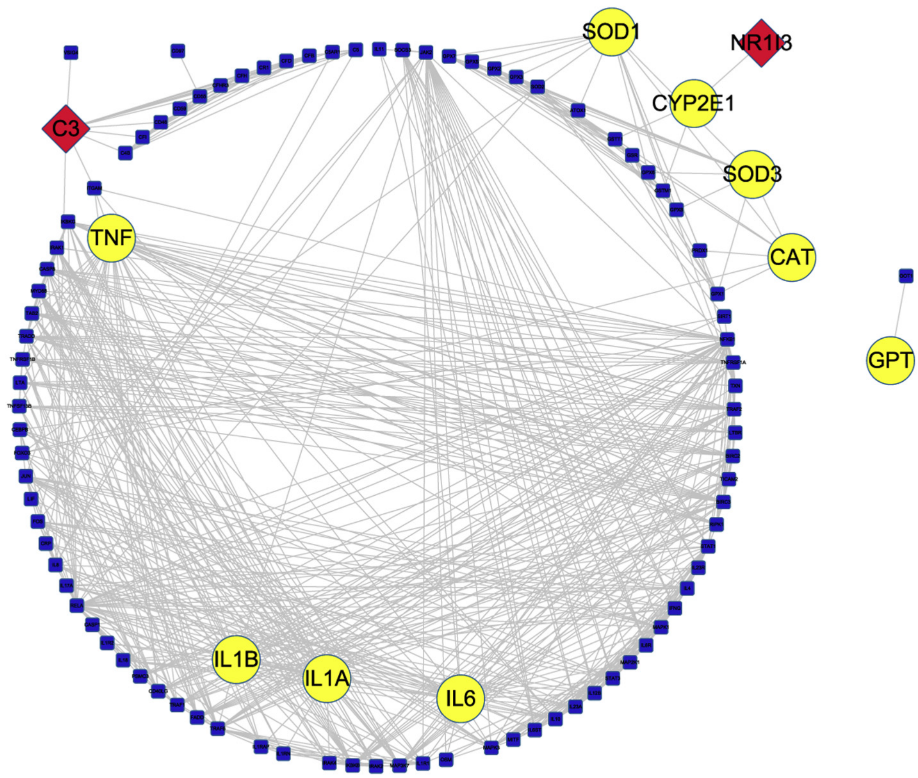

Fig. 2. PPI network of vitamin C against liver injury. As results, 11 therapeutic targets of VC against LI were identified, in which loop network had 101 nodes that were interconnected and associated by 584 edges.

the smaller and shortest path length was showed, and proposed proteins were more important. The calculated median degree of freedom was 11.564, and the maximum degree of freedom was 38. Therefore, the screening conditions of core targets were ranged from 23.128 to 38. Correspondingly, the resultant 6 core target proteins were obtained, showing TNF, RELA, NFKB1, TRAF2, IL6 and IL1B (Fig. 4).

3.5. Biological function and pathway enrichment analyses of core targets

As highlighted in Fig. 5, results exhibited that biological processes of predicted core targets were mainly involved in positive regulation of NF-κB transcription factor activity, inflammatory response, cellular response to nicotine, positive regulation of sequence-specific DNA binding transcription factor activity, cellular response to lipopolysaccharide, positive regulation of transcription, DNA-templated,

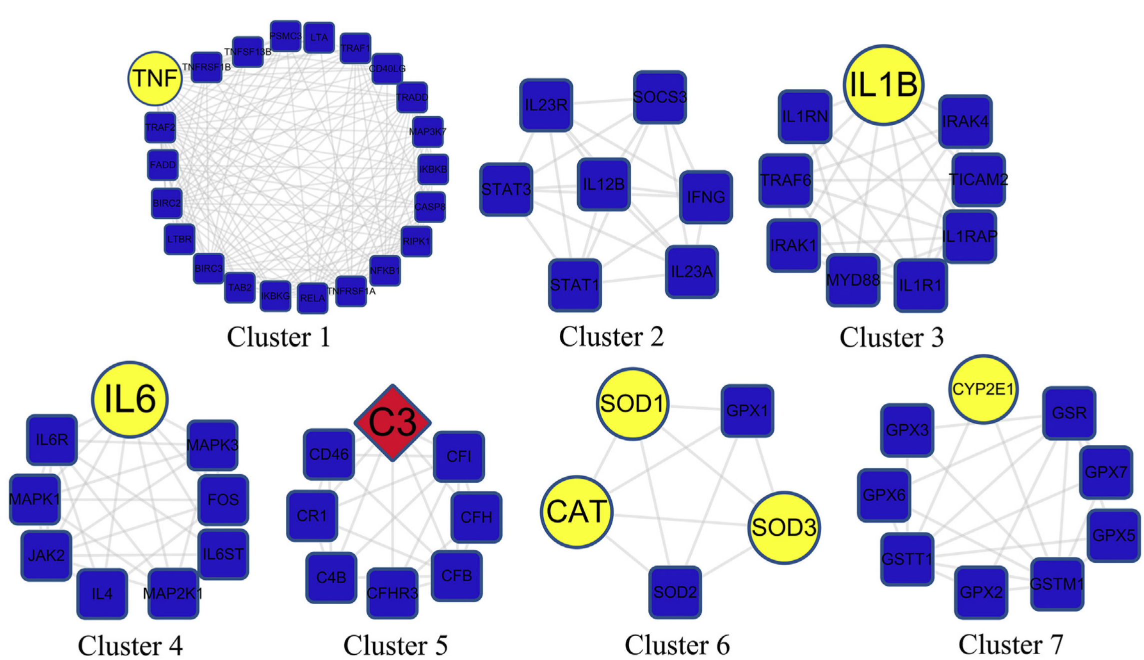

Fig. 3. Clusters of interacted proteins in vitamin C against liver injury by use of MCODE algorithm. The predicted targets of SOD1, SOD3 and CAT were clustered in connected with other biological targets.

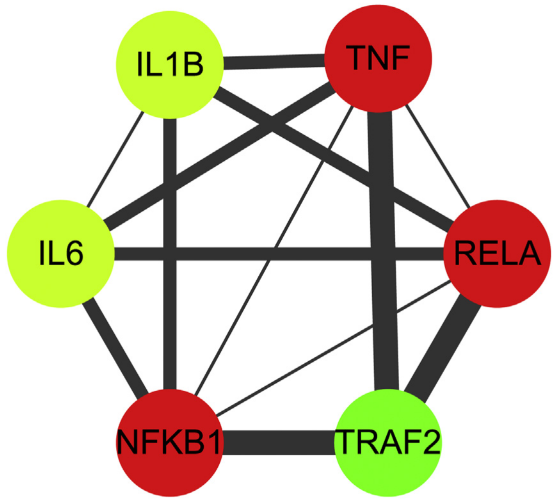

Fig. 4. Hub targets of vitamin C. As results, the 6 key regulator targets were finally identified, showing TNF, RELA, NFKB1, TRAF2, IL6 and IL1B.

positive regulation of transcription from RNA polymerase II promoter, positive regulation of nitric oxide biosynthetic process, positive regulation of interleukin-6 production, IκB kinase/NF-κB signaling, cellular response to interleukin-1, cytokine-mediated signaling pathway, positive regulation of miRNA metabolic process, positive regulation of calcidiol 1-monooxygenase activity, sequestering of triglyceride, positive regulation of fever generation, regulation of immunoglobulin secretion, epithelial cell proliferation involved in salivary gland morphogenesis, regulation of establishment of endothelial barrier, positive regulation of gene expression. Overall, these biological processes were mostly related to the development of inflammatory stress, DNA damage repair, oxidative stress.

In addition, the molecular signaling pathways of core targets in VC against LI were closely associated with TNF signaling pathway, Non-alcoholic fatty liver disease (NAFLD), Herpes simplex infection, Legionellosis, NOD-like receptor signaling pathway, Inflammatory bowel disease (IBD), Pertussis, NF-κB signaling pathway, Toll-like receptor signaling pathway, Tuberculosis-, Apoptosis-, Cytosolic DNA-sensing pathway, MAPK signaling pathway, et al. (Fig. 5).

4. Discussion

LI is induced by a variety of causes of cyto-architectural damage and inflammatory necrosis, resulting in impaired activities of hepatic functional enzymes. The occurrence and development of LI may eventually lead to liver failure in time-dependent manner, characterized with serious health problems to humankind [13,14]. The pathological onsets of LI are found to be involved in activation of interlaced signaling mechanisms, including oxidative stress, lipid peroxidation, and mitochondrial damage [15,16]. Our previous experiments have shown that VC can fight against drug-induced liver injury [17], however, the detailed molecular mechanisms of beneficial effect of CV against LI remains totally clear. Therefore, it is important to screen and identify the therapeutic targets of VC against LI before VC-associated clinical application.

In order to screen the key therapeutic targets of VC against LI, this study was used network pharmacology to identify 380 VC-verified targets and 236 LI-pathogenetic targets. As results, 11 therapeutic targets VC against LI were obtained, and resultant 6 core targets were screened, showing TNF, RELA, NFKB1, TRAF2, IL6 and IL1B. Based on the analyses of gene ontology (GO) annotation and Kyoto encyclopedia of genes and genomes (KEGG) pathway in target proteins, the data uncovered that a majority of the enrichment pathways was associated with hepatocellular apoptosis and inflammation. Therefore, anti-LI of pharmacological activities in VC may be benefited through regulating apoptosis and inflammation-related pathways in liver cells. In current bioinformatics findings, the proposed pharmacological mechanism is chiefly linked to suppression of hepatocellular TNF signaling pathway.

Apoptosis refers to is a type of programmed cell death in multicellular tissue, and this biological process may cause cell morphological changes and death. Currently, it is believed that three critical avenues

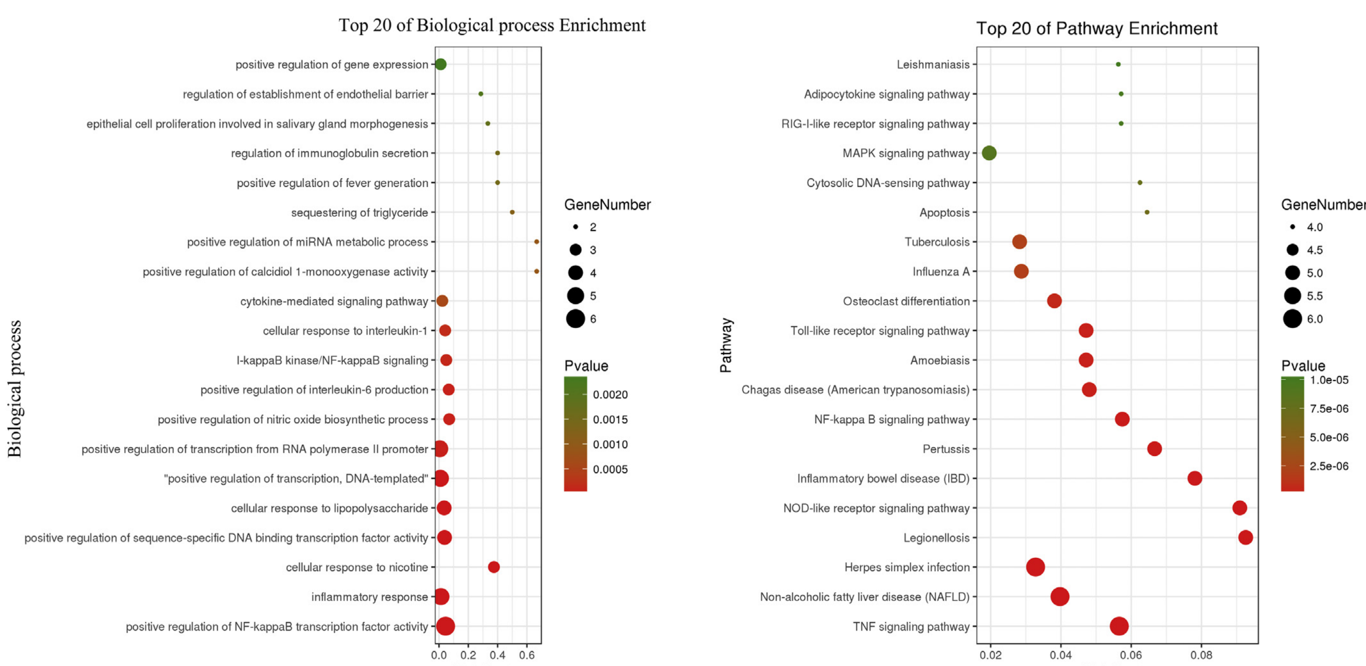

Fig. 5. Biological process and pathway analyses from bioinformatics data. Results showed that main biological processes of vitamin C against liver injury were highlighted, such as regulation of NF-κB transcription factor activity, inflammatory response and apoptosis. In addition, molecular signaling pathways of vitamin C against liver injury were closely related to inflammation-based pathways, such as TNF signaling pathway, NF-κB signaling pathway, toll-like receptor signaling pathway, apoptosis signaling pathway.

are involved in apoptosis, such as the endoplasmic reticulum pathway, the mitochondrial pathway, and the death receptor pathway [18,19]. In addition, apoptotic event is found to be related to the development of LI [20]. Acute and chronic hepatic inflammation results in LI, and inflammatory cells are responsible for induction of liver inflammation, including neutrophils, lymphocytes, and infiltrating macrophages [21]. TNF is a class of most important inflammatory molecules that can indirectly activate NF-κB, and then up-regulate the expression of TRAR-related factor family members TRAF1 and TRAF2 to inhibit apoptosis [22,23]. Activation of RELA (NF-κBp65) are significant post-translational modification necessary for initiation of NF-κB activity. Further, RELA has been related to regulate inflammatory responses in LI [24]. In addition, inappropriate activation of NF-κB can lead to excessive release of inflammatory cytokines, such as IL-1β, IL-6 [25]. Increasing evidences show interleukin factors are closely linked to the progression of chronic liver injury [26]. Therefore, these data highlighted that suppression of TNF signaling pathway in the liver and inactivation of hepatocellular TNF, RELA, NFKB1, TRAF2, IL6 and IL1B expressions may be key pharmacological mechanism of VC against LI. In further clinical perspective, some clinical drugs, such as TNF-inhibitor medicine, may play dual efficacy in combination of VC.

5. Conclusion

In summary, the therapeutic mechanism of VC against LI may be closely related to the inhibition of apoptosis and inflammation-related pathways in liver cells. Network pharmacology method may predict the key therapeutic targets. In addition, these target-based inhibitors (medicines) may enhance the efficacy in combination of VC for treating LI.

Declaration of interest

The authors declare that they have no conflict of interest.

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