Anti-novel coronavirus pneumonia biotarget and mechanism of puerarin

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Abstract

As a bioinformatic strategy, the present study was designed to use a network pharmacology analysis to uncover the pharmacological function and mechanism of puerarin to treat novel coronavirus pneumonia (NPC). Following methodological platforms, all theoretical and pivotal targets, anti-NPC mechanism of puerarin were filtrated and disclosed. As results, the pivotal targets of puerarin to treat NPC were collected and identified, comprising of EGFR, TNF, TP53, CASP3, RELA, FOS, CASP8, PTGS2, IL2, PRKCB, BCL2, PRKCA, NOS3, PPARG. Functionally, the anti-NPC action of puerarin was associated with suppression of oxidative stress and inflammatory cascades, cell apoptosis. Mechanically, the signaling pathways of puerarin to treat NPC were uncovered, including modulation of the pathways of Apoptosis, IL-17 signaling, MAPK signaling, TNF signaling. Molecular docking data illustrated the binding capacity of puerarin with NPC, and effective anti-NPC activity of puerarin. Taken together, our current network pharmacology-based findings revealed the pharmacological biotarget and mechanism of puerarin to treat NPC. Further, the bioinformatics findings elucidated that some of these 14 pivotal targets might serve as the potential molecular markers for detecting NPC, a rapidly emerging and evolving disease.

Introduction

Novel coronavirus pneumonia, known as SARS-CoV-2, is a severe and life-threatening epidemic disease, leaving gigantic economic burden and increasing death toll in the word [1]. As an evolving epidemic, NPC is rapidly growing and spreading around the countries, including United States, Brazil, India [2]. Epidemiological evidences suggest that the trend of NPC infection is hard to control due to the insufficient management against NPC, characterized with huge infected cases in different countries [3]. In USA, NPC have already affected millions of population and is characterized with mounting incidence and death-rate [4]. Pathologically, the main clinical characteristics of NPC are related to inflammation, cytokine storms, immunodefficiency, infection [5]. In clinical management against NPC, the common therapy may have supportive care [6], such as breathing assistance, as the developed drug and new vaccine are still ongoing [7]. Traditionally, *Pueraria montana (Gegen)* is found with relieving muscle fever, neutralizing alcoholism hypoglycemic, and hypolipemic actions [8]. Puerarin, rich in *Pueraria montana* , exerts evident pharmacological benefits, such as preventing coronary heart disease, anti-alcoholism, anti-diabetes [9]. In our previous reports, puerarin is found with potent hepatoprotection, neuroprotection, and anti-dysmetabolism effect in preclinical studies [10-13]. There is an interesting report suggest that puerarin may function as a natural amoebicidal agent to manage*Acanthamoeba* -induced pneumonia [14]. In addition, puerarin plays the important role of anti-inflammatory action, including preventing acute lung injury through reducing inflammatory stress [15-16]. However, there is no specific drug for treatment of NPC currently. Although some antiviral drugs and traditional Chinese medicine have been used in clinical treatment of NPC, the therapeutic effectiveness is not clear. Collectively, we aimed to determine and identify the curative effect of puerarin to treat NPC, as well as the therapeutic mechanisms involved would be revealed. It is of great interesting to use the bioinformatics

and computational methods based on network pharmacology for unveiling the potential agent to treat illness [17]. By using the network pharmacology strategy, we have been reported the pharmacological targets and molecular pathways of bioactive agents against complex diseases [18-19]. Thus, in this bioinformatics report, we intended to revealment of a component-target-pathway network and pharmacological mechanism of puerarin to treat NPC.

2. Methods

2.1 Screening of the target of puerarin in the treatment of NCP

Total established genes of puerarin were picked out through the effective tools of traditional Chinese medicine systems pharmacology (TCMSP), Swiss Target Prediction, SuperPred. Meanwhile, other NPC-nosopoietic genes were acquired by use of the DisGeNET, Genecards databases. Additionally, these acknowledged genes of puerarin and NPC were mapped prior to emendation by Uniprot tool. After functional enrichment analysis by Funrich software, all anti-NPC biotargets exerted of puerarin were screened and identified, as reported elsewhere [20-21].

2.2 Screening of core targets of puerarin to treat NCP and construction of PPI network

Total acknowledged targets of puerarin and NCP were used to further determination and construction of functional protein association network via the STRING database following specific algorithm. In addition, these merged targets of puerarin and NCP were to construct a network of protein-protein interaction (PPI) through the Cytoscape software. As revealed in topological parameters using the NetworkAnalyzer tool, the core targets of puerarin to treat NCP were identified and visualized [22-23].

2.3 Enrichment analyses in biological process and KEGG pathway of intersection targets

Using the R-language packages, such as "ClusterProfiler", "ReactomePA", "org.Hs.eg.Db" and "GOplot" in the R language (3.6.1), the enrichment analysis and visualization of the biological process and KEGG pathways of the intersection targets were performed and obtained accordingly. Moreover, information of gene annotation resulted from "org.Hs.eg.Db", p-value Cutoff = 0.05, q-value Cutoff = 0.05 for enrichment prior to plotting the corresponding bubble chart, histogram and Circos circle chart [24-25].

2.4 Molecular docking analysis

Using chemical-protein binding method, the core targets were screened out and identified for puerarin-based molecular docking analysis. After searching specific proteins through the PDB database, 5UGC, 6OOY ligands were selected to dock with the puerarin compound. The ChemBio3D Draw in Chem Bio Office 2010 software was used to conduct three-dimensional structure of puerarin before docking the molecular structures following MGLTools in Autodock Vina software. The rationality of the docking parameter setting was assessed according to the root mean square deviation (RMSD) of the ligand molecule. And the RMSD [?] 4 Å was the threshold for the conformation of the ligand molecule [26-27].

3. Results

3.1 Findings of putative genes of puerarin and NCP

As revealed in assays, a group of 393 NCP-morbid genes were selected and detected. Meanwhile, a total of 138 putative genes of puerarin were specified following the databases. As shown in Venn diagram, 34 shared genes of puerarin to treat NPC were screened and identified. Further, these mapped genes were re-used to plot a interaction network (Figure 1).

3.2 Identification of all core targets

All the mapped intersection proteins were input into Cytoscape 3.7.1 software, to calculate the topological parameters of the interaction network of targets and functionally related proteins of puerarin in the treatment of NCP. The algorithm from the network freedom median degree of 6.129 and freedom maximum degree of 15 was used to identify the core targets of puerarin to treat NCP. Therefore, the core target screening condition

range was set to 7-15, and finally 14 core targets were obtained. These genes included EGFR, TNF, TP53, CASP3, RELA, FOS, CASP8, PTGS2, IL2, PRKCB, BCL2, PRKCA, NOS3, and PPARG, as shown in Figure 2 and Supplemental Table 1.

3.3 Enrichment findings of core targets

Following the assays using R language-related packages, the anti-NPC molecular pathways from puerarin-achieved core targets were determined and revealed accordingly. As results, puerarin in treatment of NCP's targets related biological processes mainly involved in response to oxidative stress, response to lipopolysaccharide, response to molecule of bacterial origin, response to steroid hormone, extrinsic apoptotic signaling pathway, response to reactive oxygen species, response to UV, response to glucocorticoid, cellular response to oxidative stress, response to corticosteroid, response to light stimulus, cellular response to external stimulus, response to osmotic stress, response to mechanical stimulus, positive regulation of neuron death, regulation of apoptotic signaling pathway, myeloid cell differentiation, negative regulation of apoptotic signaling pathway, negative regulation of extrinsic apoptotic signaling pathway, reproductive structure development (Figure 3A, Supplemental Table 2). Meanwhile, related cell components of puerarin in treatment of NCP target were mainly related to membrane raft, membrane microdomain, membrane region, transcription factor complex, RNA polymerase II transcription factor complex, nuclear transcription factor complex, caveola, plasma membrane raft, mitochondrial outer membrane. In addition, the molecular functions of puerarin in the treatment of NCP targets mainly had ubiquitin protein ligase binding, protease binding, ubiquitin-like protein ligase binding, protein phosphatase binding, tumor necrosis factor receptor superfamily binding, phosphatase binding, activating transcription factor binding, cytokine receptor binding, cysteine-type endopeptidase activity involved in apoptotic process, protein kinase C activity, death receptor binding, histone kinase activity, RNA polymerase II transcription factor binding, tumor necrosis factor receptor binding, protein phosphatase 2A binding, core promoter sequence-specific DNA binding, actinin binding, core promoter binding, protein self-association, scaffold protein binding (Figure 3B). Other 138 KEGG pathways (P -adjust <0.05) of core targets were identified and involved mainly in Hepatitis B, Human cytomegalovirus infection, AGE-RAGE signaling pathway in diabetic complications, Sphingolipid signaling pathway, Human immunodeficiency virus 1 infection, Apoptosis, Measles, IL-17 signaling pathway, MAPK signaling pathway, HIF-1 signaling pathway, TNF signaling pathway, Fluid shear stress and atherosclerosis, Oxytocin signaling pathway, Hepatitis C, Leishmaniasis, Influenza A, Colorectal cancer, MicroRNAs in cancer, Small cell lung cancer, Kaposi sarcoma-associated herpesvirus infection (Figure 4A-B, Supplemental Table 3).

3.4 Construction of network diagram

Further, the network visualization of puerarin-target-BP-KEGG-NPC was created through the Cytoscape 3.7.1 software**,** as shown in Figure. 5.

3.5 Molecular docking findings

From the interaction diagram of the original ligand and the protein-ligand complex, the data showed the amino acid residues where the original ligand GWS binding to the 5R84 protein. In the three-dimensional picture of puerarin, the binding force of puerarin small molecules with the protein included the residues of the original ligand, indicating that puerarin had a good binding activity with the 5R84 protein of NPC, as revealed in Figure 6.

4. Discussion

In our current study, the these in silico findings from network pharmacology approach and molecular docking analysis revealed the pharmacological targets, functions, and signaling pathways of puerarin-achieved anti-NPC. In details, all core biotargets of puerarin to treat NPC were identified via bioinformatics determination, including EGFR, TNF, TP53, CASP3, RELA, FOS, CASP8, PTGS2, IL2, PRKCB, BCL2, PRKCA, NOS3, and PPARG. Molecular docking data indicated that the binding capacity of puerarin with NPC was significant, indicating the potential and pharmacological activities of puerarin to treat NPC. FOS, a proto-oncogene, is considered to be a regulator of cell proliferation, differentiation and transformation. And the

over-expression of FOS gene is also related to apoptotic cell death [28]. Prostaglandin-endoperoxide synthase (PTGS) is responsible for the prostanoid biosynthesis involved in inflammation and mitogenesis when it is induced by specific stimulatory genes [29]. Some interesting data indicate that targeting suppression of PGE2 synthesis is likely to reduce bacterial infection in mice [30]. Protein kinase C (PKC), including PRKCB, PRKCA, are found to be involved in diverse cellular signaling pathways, including cancers [31]. The proteins encoded by PRKCB, PRKCA genes have been reported to be associated with numerous cellular functions, such as B cell activation, apoptosis induction, endothelial cell proliferation [32]. NOS3 (nitric oxide synthase 3), may induce nitric oxide release for regulation of angiogenesis and cell proliferation [33]. Interestingly, a NOS3 may functionally suppress the sepsis-caused systemic inflammation and myocardial dysfunction in mice [34]. Collectively, there is no associated investigation for the associations between FOS, PTGS, PRKCB, PRKCA, NOS3 and NPC. Therefore, the current *in silico* findings using network pharmacology uncovered all pharmacological targets, functions and signaling pathways of puerarin to treat NPC, marked by inhibition of cytonecrosis- and inflammation-associated signaling pathways, such as IL-17 signaling pathway, TNF signaling pathway, MAPK signaling pathway, HIF-1 signaling pathway. Potentially, puerarin is likely a promising active compound against NPC prior to future experimental report.

5. Conclusion

Briefly, the *in silico* approach based on network pharmacology can be used to harvest the mapped and core biotargets, pharmacological functions, therapeutic mechanisms of puerarin to treat NPC. Additionally, total core biotargets of puerarin to treat NPC are identified and determined, and some new-found anti-NPC targets may comprise FOS, PTGS, PRKCB, PRKCA, NOS3 and NPC.

Declaration of interest

None.

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Figure captions

Figure 1. All common genes of puerarin and NPC were identified prior to further identification of the shared targets of puerarin to treat NPC. All common targets of puerarin to treat NPC were harvested and plotted for construction of a PPI network.

Figure 2. All core targets of puerarin to treat NPC was screened and identified.

Figure 3. All top biological processes of puerarin to treat NPC from enrichment analyses were revealed and visualized.

Figure 4. All top molecular pathways of puerarin to treat NPC from enrichment analyses were uncoverd and visualized.

Figure 5. An integrated network from vital targets the was plotted and revealed the intersection association of target-disease-function-pathway in puerarin to treat NPC.

Figure 6. Molecular docking findings suggested that the binding capacity of puerarin against NPC was significant in the core targets.