Network Pharmacology-Based Exploration of the Therapeutic Mechanisms of *Cordyceps cicadae* in Renal Ischemia/Reperfusion

**Background:** *Cordyceps cicadae* is beneficial in treating renal diseases, especially in inhibiting renal ischemia/reperfusion injury (IRI). The aim of this study was to systematically analyze and predict the potential mechanism of *Cordyceps cicadae* in renal IRI therapy using network pharmacology.

**Material/Methods:** Cordycepin, adenosine, and cordycepic acid are the 3 major medicinal ingredients in *Cordyceps cicadae*. Based on network pharmacology, the 3D structure of the 3 compounds were obtained, and then the common targets between these compounds and renal IRI were analyzed and determined. We used the ingredient-target (I-T), protein–protein interaction (PPI) networks, the enrichment analysis of Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) to find the possible pharmacological mechanism of *Cordyceps cicadae* in treating renal IRI.

**Results:** Through target fishing and analysis, the 3 active ingredients of *Cordyceps cicadae* shared 81 target genes with renal IRI. I-T network showed that adenosine had the highest degree, and 5 genes were associated with the 3 active ingredients. PPI network analysis showed that ALB, GAPDH, CASP3, MAPK1, FN1, and IL-10 play a pivotal role. The enrichment analysis of GO and KEGG showed that *Cordyceps cicadae* can treat renal IRI through MAPK, cAMP, PPAR, Rap1, and HIF-1 signaling pathways.

**Conclusions:** *Cordyceps cicadae* exerts its therapeutic effect on renal IRI via multiple targets and pathways. Nevertheless, further experimentation is needed to verify this. The method of network pharmacology provides an effective method of determining the comprehensive action mechanism of Traditional Chinese Medicine (TCM).

**Keywords:** *Cordyceps cicadae* • Forecasting • Molecular Mechanisms of Pharmacological Action • Reperfusion Injury • Systems Biology

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Background

Ischemia reperfusion injury (IRI) refers to a pathological condition in which tissue or organ damage is aggravated instead of alleviated when the tissue or organ regains perfusion or oxygen supply after ischemia [1]. Clinically, renal IRI occurs most commonly following traumatic shock, sepsis, partial nephrectomy, post-partum hemorrhage, and other major surgical procedures [2]. Surprisingly, the probability of IRI in kidney transplant patients is almost 100% [3]. Although the mechanism by which renal IRI occurs is unclear, it is thought to result from apoptosis, necrosis, oxygen free radicals, inflammation, mitochondrial damage, and ferroptosis [4-6]. One of the key factors is the inflammatory cascade induced by immune cells infiltration [7]. Renal ischemia causes significant tissue hypoxia, which triggers the recruitment of immune cells such as dendritic cells (DC cells), neutrophils, macrophages, and natural killer T (NKT) cells to the injured tissues and the release of pro-inflammatory factors [8]. When blood perfusion is restored later, the inflammatory response is further exacerbated, resulting in progressive aggravation of renal damage [9].

Currently, there is still no satisfactory treatment for IRI. On the one hand, new treatments are effective but have limited clinical applications due to insufficient clinical evidence and serious complications [10,11]. On the other hand, the lack of expertise in renal transplantation pathology has affected the evaluation of the IRI caused by transplanted kidneys to some extent [12], although the application of artificial intelligence is expected to improve such events [13,14]. As a traditional form of treatment, pharmacotherapy has always been the focus of research, but despite this, there are no specific drugs available for treating renal IRI [15,16]. Traditional Chinese Medicine (TCM) treatment has the characteristics of holistic management, multi-targets, multi-pathways, and multi-links, and has become the primary source for the exploration of new drugs by researchers. There are a variety of TCMs that can treat kidney failure, such as Cordyceps sinensis or Cordyceps chrysosila, as they are safe, effective, and have few toxic adverse effects [17]. Cordyceps cicadae, named “Chan Hua”, is an insectivorous fungus that parasitizes cicada nymphs in the soil [18]. Cordyceps cicadae has a complex composition, including cordycepin, adenosine, cordycepic acid, nucleotides, and hyaluronic acid [19]. The components extracted from the mycelium of Cordyceps cicadae have been proven to improve renal function, inhibit inflammatory response, and reduce renal fibrosis [20-22]. Furthermore, as compared to Cordyceps, Cordyceps cicadae is relatively inexpensive and easy to obtain [23], making Cordyceps cicadae of great value for clinical research and application. However, the explicit mechanism of its effect is still unclear. It is necessary to further uncover its effective components, molecular targets, and mechanisms for treating renal IRI.

Network pharmacology is a method for network analysis of biological systems based on the theory of systems biology. At present, network pharmacology, as a new and advanced analytical technique, is widely used in pharmacological research, with its superior reliability and efficiency, and is the current research frontier of TCM [24]. Network pharmacology integrates the ideas of systems biology and multidirectional pharmacology to analyze the mechanism of action of drugs by constructing complex drug-ingredient-target-disease networks, enabling pharmacological research to shift from the traditional search for a single target to comprehensive network analysis [25]. The complex chemical composition and multi-target and multi-level pharmacological effects of Chinese herbs are naturally compatible with network pharmacology. In this study, we comprehensively analyzed the mechanism of action of Cordyceps cicadae using network pharmacology.

The purpose of this study was to comprehensively uncover the active ingredients, distinct targets, and accurate molecular mechanism of Cordyceps cicadae in the treatment of renal IRI by using network pharmacology. We integrated the predicted molecular targets related to the components of Cordyceps cicadae. By comparing these targets with the therapeutic targets associated with renal IRI, the repeated targets were identified as potential targets and mapped to the ingredient-target (I-T) network. The biological effects of these repeated targets were analyzed by Gene Ontology (GO), and the corresponding pathways were obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. This approach provides a fresh strategy for further understanding the renal-protective activity of this TCM therapy.

Material and Methods

Identifying the Molecular Targets of Cordyceps cicadae

With reference to published articles, cordycepin, adenosine, and cordycepic acid were considered likely to be the 3 key active ingredients of Cordyceps cicadae that exert therapeutic effects by targeting diverse proteins. Subsequently, a crucial step was to identify the molecular targets that could disclose the pharmacological mechanisms of Cordyceps cicadae. In this study, using the 3D structural data of the 3 ingredients (Figure 1) obtained in PubChem (PubChem: https://pubchem.ncbi.nlm.nih.gov/), the target prediction was completed based on the PhammerMap database (PhammerMap: http://lilab-ecust.cn/phammapper/) and the SwissTargetPrediction database (SwissTargetPrediction: http://www.swistargetprediction.ch/). Due to the irregular description of the identified candidate targets, the UniProtKB database (UniProtKB: www.uniprot.org) [26] was used to standardize the candidate targets under the category of “Homo sapiens”. Finally, the unified
the proteins mostly constitute macromolecular complexes by interacting with each other in intracellular biochemical processes to complete biological functions. Exploring protein–protein interactions is essential to reveal pharmacodynamic mechanisms, optimize drug efficacy, and reduce adverse effects. To systematically clarify the protein–protein interactions, the relevant targets were retrieved using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; Version 11.0; https://string-db.org/) to construct the PPI network. The STRING database is generally applied to retrieve and construct PPI networks, and its results are derived from experimental research data, literature mining, bioinformatics predictive analysis, and other relevant databases [30]. With STRING’s inherent scoring criteria, the higher the score setting, the higher the reliability of the predicted PPI results. Hence, the lowest score was set at a confidence of 0.4 to guarantee the reliability of the prediction results. The proteins isolated were removed from the network. Finally, the PPI network was the output, and statistical analyses of the PPIs were conducted. The proteins in the network are represented by nodes, while the edges indicate the association among proteins.

Identifying the Targets of Renal IR

The target genes related to renal IR were screened from GeneCards (http://www.genecards.org/). This human gene database is user-friendly, integrative, and searchable. Integrative information in relation to all annotated human diseases, proteins, and genes is available from this database [27]. The database encompasses diverse resources from 125 databases, including NCBI, HGNC, UniProtKB, and ENSEMBL, as well as multitudinous other relevant databases [28]. It is highly reliable in terms of data reliability. Using GeneCards, the information about related targets can be easily retrieved by keyword search, and we identified 1497 renal IR-related targets using the keywords “kidney ischemia reperfusion” and “renal ischemia reperfusion”.

Constructing the Ingredient–Target (I-T) Network

To better understand and analyze the molecular mechanism, the visualization software Cytoscape 3.7.2 was used in this study to construct an I-T network. The candidate ingredients targets and renal IR targets were retrieved to obtain the related targets shared with them. The ingredients and the shared targets were then used to map the I-T interaction network using the software. An attribute circle layout algorithm was used when building the network. To arrange each node reasonably and achieve clearer and understandable visualization effect, users can set each node and symbol with appropriate geometric position and set the network topology with personalized graphics and colors through Cytoscape [29]. The importance of every target and ingredient can be estimated by the 2 most critical parameters, degree and betweenness centrality, of the topology. Consequently, the ingredients and targets of Cordyceps cicadae exerting the core effect against renal IR were analyzed and identified.

Constructing the Protein–Protein Interaction (PPI) Network

It is difficult to individually identify specific proteins functions; the proteins mostly constitute macromolecular complexes by constructing the Protein–Protein Interaction (PPI) Network. For each protein, a comprehensive analysis of the association among proteins was conducted to complete biological functions. Exploring protein–protein interactions is essential to reveal pharmacodynamic mechanisms, optimize drug efficacy, and reduce adverse effects. To systematically clarify the protein–protein interactions, the relevant targets were retrieved using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; Version 11.0; https://string-db.org/) to construct the PPI network. The STRING database is generally applied to retrieve and construct PPI networks, and its results are derived from experimental research data, literature mining, bioinformatics predictive analysis, and other relevant databases [30]. With STRING’s inherent scoring criteria, the higher the score setting, the higher the reliability of the predicted PPI results. Hence, the lowest score was set at a confidence of 0.4 to guarantee the reliability of the prediction results. The proteins isolated were removed from the network. Finally, the PPI network was the output, and statistical analyses of the PPIs were conducted. The proteins in the network are represented by nodes, while the edges indicate the association among proteins.

GO and KEGG Enrichment Analysis

GO analysis is a common approach to describe and annotate the biological processes, cellular components, and molecular functions of genes and gene products [31]. The KEGG is a useful resource that helps to systematically analyze gene functions and related advanced genome functions information [32,33]. In the study, we mainly explored the biological effects of Cordyceps cicadae and realized enrichment analysis by using 3 packages of R software (version “3.6”): (1) “DOSE”, an R package that explores the similarities in diseases and gene functions from a disease perspective by computing semantic similarity involving genes and disease ontology terms [34]; (2) “clusterProfiler”, an R package that mainly functions to compare biological themes and the enrichment analyses of gene clusters [35]; and (3) “pathview”, the R package that can visualize as well as integrate data according to the known pathways [36].
Results

Target Identification and Analysis

After previous target fishing, it was forecast that 288 targets interacted with the 3 active compounds identified in Cordyceps cicadae. Using keywords retrieval, 1497 genes with relevant to the pathogenesis and progression of renal IR were screened by GeneCards database. By combining the active compounds targets of Cordyceps cicadae with renal IR-related targets, 81 targets shared among them were identified to be potential targets for treating renal IR (Figure 2A), and the details are presented in Supplementary Table 1. From the perspective of network topology, the key targets of the network are regarded as the cores, so the 81 targets determined in this study could be considered as the effective targets for Cordyceps cicadae in the treatment of renal IR.

I-T Network Construction and Analysis

To comprehend the relationships between the active components in Cordyceps cicadae and their potential targets common to renal IR, we constructed an I-T network of interactions between ingredients and targets, where every one of the ingredients was connected to its potential targets. The targets, interactions, and active ingredients presented in Figure 2B refer to the 3 active ingredients of Cordyceps cicadae respectively mapped to 81 potential targets. The ingredients and targets are indicated by yellow nodes and green nodes, respectively. And the interactions among the nodes are represented by the edges. The degree of an ingredient node is usually considered to reflect its potential value in drugs. The I-T network analysis showed that adenosine had the highest degree (degree=50), followed by cordycepic acid (degree=36) and cordycepin (degree=13), suggesting the priority of their potential value. Moreover, out of the 81 potential target genes, a total of 8 genes (IL-10, TGM3, MAOA, SMURF2, DPP4, CA2, CA1, and CA9) were linked to 2 ingredients, and 5 genes (TGM2, ERBB4, PTGIS, IGFR1, and SIRT3) were linked to 3 ingredients, indicating that these genes might be crucial in repairing renal IR.

PPI Network Construction and Analysis

To better analyze and understand the therapeutic mechanism of Cordyceps cicadae, the PPI network of the targets for the treatment of renal IR was constructed. The confidence level was set above 0.4, and the proteins independent of the network were removed. Then, 75 proteins with 377 relationships were obtained by PPI network analysis (Figure 3A). The priority of key proteins was analyzed by the degree of nodes output by the STRING database. Among them, the ALB value (degree=43) was the highest, followed by GAPDH (degree=39), CASP3 (degree=28), MAPK1 (degree=28), FN1 (degree=27), and IL-10 (degree=27), indicating that these proteins might act as bridges to other nodes in the PPI network (Figure 3B).

Gene Ontology Functional Enrichment Analysis

To verify whether the 81 shared targets are associated with renal IR, GO functional enrichment analysis was carried out with R software 3.6.0, and the associated biological processes were clarified using the “DOSE” and “clusterProfiler” packages. Figure 4A displays the first 30 most significantly enriched GO terms, including biological processes (BP), molecular function (MF), and cellular component (CC) (P value ≤0.01). Supplementary Table 2 provides the P values, q-values, and counts of the remarkable enrichment items in BP, CC, and MF. The results indicate that the development of renal IR is regulated by many specific biological activities, including those involved in adenosine receptor signaling pathway (GO: 0001973), regulation of inflammatory response (GO: 0050727), regulation of body fluid levels (GO: 0050878), and response to hypoxia (GO: 0001666). Figure 4B presents the first 8 most significantly enriched GO terms in a circle-plot style. Among them, combined with the previous research results of our team [37], we focused on the adenosine receptor signaling pathway (GO: 0001973) and present the possible regulatory mechanism in Figure 5.

KEGG Enrichment Analysis of Pathways

The pathways correlated to Cordyceps cicadae were obtained using the KEGG pathway enrichment analysis via R software 3.6.0. The significant pathways were enriched, and their corresponding P values were computed (calibrated by the Bonferroni method, the pathways with P values ≤0.01 were regarded as remarkable enrichment items). After sorting the P values, the first 30 pathways were shown in Figure 6A. Supplementary Table 3 provides the P values, q-values, and counts of the prominent enrichment pathways. The results indicated that multiple pathways participated in the process of renal IR, such as the cAMP signaling pathway (hsa04024), MAPK signaling pathway (hsa04010), PPAR signaling pathway (hsa03320), HIF-1 signaling pathway (hsa04066), and Rap1 signaling pathway (hsa04015). Figure 6B presents the first 8 most significantly enriched KEGG pathways in a circle-plot style.

Discussion

Renal IRI often occurs after recovery from renal ischemia, usually in septicemia, hypovolemic shock, and kidney transplantation [2]. In kidney transplant patients, IRI can lead to delayed graft function and cause a variety of long-term and short-term complications, thereby shortening the long-term survival rate of patients and bringing a heavy financial and medical burden to families and society [6,38]. Clinically, anti-oxidation,
Figure 2. (A) The blue circle represents the related targets of renal ischemia/reperfusion injury (IRI), the red circle represents the related targets of Cordyceps cicadae, and the intersecting part of the 2 circles is the potential targets of Cordyceps cicadae acting on renal IRI. Figure created by R 3.6.0 with Rstudio, R: The R Project for Statistical Computing (r-project.org). (B) The ingredient–target (I-T) network in this study. The green nodes represent potential targets associated with renal IRI, the blue nodes represent Cordyceps cicadae, and the yellow nodes represent active ingredients of Cordyceps cicadae; the edges indicate the interactions among the nodes. Figure created by Cytoscape 3.7.2 software. IR – ischemia/reperfusion.
anti-inflammatory, inhibiting apoptosis, and improving renal perfusion are usually used for treatment. However, there is currently no treatment plan to completely avoid renal reperfusion injury. In China, Cordyceps cicadae has been widely used in the treatment of renal ischemia and chronic kidney disease. Numerous studies have also shown that Cordyceps cicadae has value in treating kidney-related diseases [21,22,39-41]. To further elucidate the possible mechanism of Cordyceps cicadae against renal IRI, this study analyzed it through network pharmacology.

Cordycepin, adenosine, and cordycepic acid are the most important potential components of Cordyceps cicadae used to relieve renal IRI [42,43]. Aiming to further explore the specific pharmacological mechanism of these 3 compounds, the network pharmacology technique was used to analyze and obtain their target genes. It was found that there were 81 shared targets between the 3 compounds and renal IR. Adenosine had the highest degree of 50, followed by cordycepic acid and cordycepin with 36 and 13 degrees, respectively. Adenosine exists widely in and out of cells and exerts different physiological effects, mainly by binding to different receptors. Activation or inhibition of corresponding receptors can reduce the inflammatory response caused by renal reperfusion injury [44,45]. Studies have found that an increase in intracellular adenosine concentration is beneficial for alleviating renal IRI [46]. Cordycepic acid (mannitol) exhibits various biological effects, including antioxidant, anti-lipid peroxidation, anti-hepatic fibrosis, and antibacterial activities [47]. Studies have suggested that mannitol can reduce IRI after renal transplantation and prevent acute renal failure (ARF), but its use has been controversial [48]. Cordycepin has been reported to reduce the
expression of inflammatory cytokines such as IL-1β, IL-6, and TNF-α, as well as reduce the early inflammatory reaction, reduce the expression of apoptotic genes, reduce the production of reactive oxygen species (ROS), and increase the concentration of antioxidant factors, thereby alleviating injury of renal tissue and deterioration of renal function [49-51].

With the results of I-T network analysis, 5 genes (TGM2, ERBB4, PTGIS, IGF1R, and SIRT3) were noted to be linked to the 3 active compounds identified, which indicated that these 3 components of Cordyceps cicadae had potential synergistic effects. TGM2 is a calcium-dependent transaminase that catalyzes extracellular matrix proteins to form stable e-(γ-glutamyl)-lysine isopeptide cross-links to resist proteolysis [52]. Activation and expression of TGM2 can cause extracellular matrix protein remodeling and tissue fibrosis, which is associated with fibrosis in the liver, lungs, heart, and kidneys [53-56]. During the peak of acute rejection of renal transplantation, TGM2 is highly expressed in leukocytes, indicating that it may be involved in the immune response of renal transplantation [57]. ERBB4 is a member of the tyrosine protein kinase receptor family, which can bind to many different forms of epidermal growth factor, thus affecting cell proliferation, differentiation, and death [58]. Previous studies have found that deletion of ERBB4 can accelerate renal IR-induced renal fibrosis, while the expression of ERBB4 is significantly reduced in the model of moderate and severe renal fibrosis caused by renal IRI [59]. PTGIS, a prostacyclin synthase that can synthesize prostaglandin H2 (PGH2) into prostacyclin (PGI2), has an atypical cytochrome P450 enzyme mainly expressed in renal mesenchymal

![Figure 3. (A) The protein–protein interaction (PPI) network of potential targets correlated to Cordyceps cicadae against renal IRI. The nodes refer to proteins, and the edges indicate the interactions among them. The more edges on the same node, the greater the degree of the node. Figure created by STRING 11.0, https://string-db.org/. (B) Bar graph of the top 30 target proteins sorted by degree value. The longer the bar, the more significant the connection of the protein. Figure created by R 3.6.0 with Rstudio, R: The R Project for Statistical Computing (r-project.org).](image-url)
Adenosine receptor signaling pathway
G protein-coupled purinergic receptor signaling pathway
Purinergic receptor signaling pathway
Rhythmic process
Response to extracellular stimulus
Response to nutrient levels
Regulation of inflammatory response
Regulation of body fluid levels
Muscle cell proliferation
Vascular process in circulatory system

Membrane raft
Membrane microdomain
Membrane region
Plasma membrane raft
Caveola
Basolateral plasma membrane
Side of membrane
Cell projection membrane
Blood microparticle
Leading edge membrane

Purinergic receptor activity
G protein-coupled receptor binding
Fatty acid binding
Protease binding
Oxidoreductase activity, acting on the CH-NH2 group of donors, oxygen as receptor
Cofactor binding
Long-chain fatty acid binding
Purinergic nucleotide receptor activity
Carbonate dehydratase activity
Nucleotide receptor activity

A

BP

EC

SE

p. adjust
5e-04
1e-03
Figure 4. Gene Ontology (GO) analysis and significantly enriched GO terms of target genes related to *Cordyceps cicadae* in renal IR. Figures created by R 3.6.0 with Rstudio, R: The R Project for Statistical Computing (r-project.org). (A) The top 30 GO terms in biological processes (BP), molecular function (MF) and cell component (CC). The x-axis represents significant enrichment counts of these terms, and the y-axis represents the categories of GO terms of the target genes (P value ≤0.01). (B) The first 8 significantly enriched GO terms in a circle-plot style. FC - Fold Change.
A study confirmed that the loss of PTGIS in renal IR aggravates renal injury, increases BUN level, and aggravates renal tubular injury [61]. IGF1R is a tyrosine kinase receptor, which can regulate cell proliferation, differentiation, and survival by binding to insulin growth factor 1 [62]. IGF1R is highly expressed in renal microvascular endothelial cells and maintains vascular homeostasis and regulating endothelial function [63]. The expression of IGF1R gene has been reported to inhibit inflammatory cell infiltration and renal fibrosis during renal injury, and to modulate vascular homeostasis and endothelial function by stabilizing vascular endothelial protein tyrosine phosphatase/vascular endothelial-cadherin complex to alleviate renal injury [64]. SIRT3, a kind of NAD⁺-dependent histone deacetylase located in the mitochondrial matrix, works with SIRT1 to regulate tissue inflammation, apoptosis, and fibrosis in the kidneys [65,66]. In a study of cisplatin nephrotoxicity, renal tubular injury and inflammatory cell infiltration were worse in SIRT3 knockout mice, indicating that SIRT3 has anti-inflammatory and anti-apoptotic effects in renal injury [67]. SIRT3 can protect against IRI by reducing the production of ROS, increasing the content of antioxidants in cells, promoting apoptosis of inactivated mitochondria, and enhancing...
opticatrophy1 (OPA-1) triggering mitochondrial fusion, while IRI leads to a decrease in SIRT3 expression [68]. In addition, the increase of acetylation of manganese-dependent superoxide dismutase (SOD2) and p53 proteins in the mitochondria of renal tubular epithelial cells is one of the mechanisms of IRI, and the inactivation of SIRT3 strengthens this mechanism [69].

PPI network analysis showed that the protein node ALB had the highest value (degree=43), followed by GAPDH (degree=39), CASP3 (degree=28), MAPK1 (degree=28), FN1 (degree=27), and IL-10 (degree=27), indicating that these proteins might act as bridges connecting to other nodes in the PPI network (Figure 3B). In a related study, NO produced by IRI induced GAPDHs-nitrosylation at cysteine 150, which binds GAPDH to Siah1 (an E3 ubiquitinase), binds, promoting nuclear transformation of GAPDH, and then causing apoptosis [70]. Another study demonstrated that pre-silencing of GAPDH in cardiomyocyte IR by siRNA increased the autophagy in injured cells and the level of oxidative protective factors such as superoxide dismutase (SOD) and glutathione, and decreased the production of ROS, thereby alleviating the damage of cells and tissue caused by IRI [71]. CASP3 is a key protease involved in the caspase-dependent apoptosis pathway. It has been found that the expression of caspase-3 in renal IR increases significantly [72]. Deletion of the CASP3 gene can increase the levels of urinary cystatin C and serum creatinine and the increase the score of renal tubular injury in the early stage of AKI, and promote the death of renal tubular epithelial cells, while in long-term studies, it decreased the expression of α-smooth muscle actin and collagen deposition in peri-tubular capillaries, thus attenuating the degree of renal fibrosis [73]. Animal studies of renal fibrosis found that the expression of fibronectin 1 (FN1) generally increases with the increase of renal fibrosis [74]. Mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinases 1 and 2 (ERK1/2), the c-Jun N-terminal kinases (JNK), and p38 mitogen-activated protein kinases (p38-MAPK), participate in proliferation and differentiation in the process of renal growth and development, and also exert an important role in a variety of renal injuries such as inflammation, apoptosis, and fibrosis [75]. During IRI,
Figure 6. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the target genes related to renal IR that are modulated by *Cordyceps cicadae*. Figures created by R 3.6.0 with Rstudio, R: The R Project for Statistical Computing (r-project.org). (A) The top 30 pathways sorted by $P$ values. The x-axis represents significant enrichment counts of these pathways, and the y-axis represents the categories of KEGG pathways of the target genes ($P$ value $\leq 0.01$). (B) The top 8 significantly enriched KEGG pathways in a circle-plot style. FC – Fold Change.
ERK2 (MAPK1) can minimize H₂O₂-induced cell damage [76] and contribute to the healing of damaged renal tubular epithelial cells [77]. However, other scholars have found that decreasing the expression of ERK2 in mouse kidneys clearly diminished IRI and the production of associated inflammatory factors during renal IRI [78]. Interleukin (IL)-10 is a classic anti-inflammatory cytokine that can inhibit the production of various inflammation-related factors such as IL-1, TNF-α, IL-6, and IL-8 [79-82]. Meanwhile, in an animal study, the mRNA expression of IL-1, IL-6, TNF-α, and IL-18 was increased in mouse models of IL-10 gene knockout in the early stage of AKI [83]. Another study found that during the repair process of renal IRI, the expression of IL-10 increased, while the deletion of IL-10 gene increased the expression of pro-inflammatory factors such as TNF-α and IL-6, resulting in aggravated renal tissue injury [84]. In addition, increased expression of IL-10 has been confirmed to be effective in treatment of renal IRI [85,86].

The results of GO classification indicated that Cordyceps cicadae was involved in numerous biological processes affecting the progression of renal IRI. Taking the GO terms such as “adenosine receptor signaling pathway”, “regulation of inflammatory response”, “response to hypoxia” and “regulation of body fluid levels” as examples, the progress and treatment of renal IRI are closely associated with them. IRI is usually accompanied by strong inflammation and oxidative stress of the body to hypoxia and reperfusion, which hinders the normal functioning of organs [87]. Studies have pointed out that inhibition of inflammation and antioxidant activity can have a nephroprotective effect on IRI [87-89]. Previous evidence [16] has suggested that 4 adenosine receptors (A₁R, A₂AR, A₃R, and A₄R) are associated with ischemic AKI. A₁R is activated by binding to adenosine, activating ERK and hypoxia inducible factor 1 alpha (HIF-1α) to induce the synthesis of cytoprotective cytokine IL-11, and then IL-11 induces sphinogosine kinase-1 synthesis, which phosphorylates sphingosine into another cytoprotective molecule sphingosine-1-phosphate (S1P), and activated A₂AR also phosphorylates and induces the synthesis of cytoprotective heat shock protein 27 (HSP27) by activating p38-MAPK, thus reducing renal tubular apoptosis and inflammation. Activated A₁R and A₂AR increase the level of cyclic adenosine monophosphate (cAMP) in the cytoplasm by stimulating adenylate cyclase (AC) and protein kinase A (PKA), which leads to the translocation of cAMP response-element binding (CREB) proteins to the nucleus to produce cytoprotection. It was found that Cordyceps cicadae and its artificial extract exerted immunomodulatory, anti-inflammatory, and anti-tumor effects through the A₁R pathway [90, 91]. Meanwhile, in terms of the attenuation of renal IRI, A₂AR also plays an important role [92,93]. A previous study by our group also demonstrated that during oxidative stress, the kidney can be protected from IRI by limiting activation of NKT cells through the hypoxia/HIF-2α/A₂AR axis [37]. A₃R has also been shown to be beneficial in renal IRI [94], and its expression is also regulated by HIF-1α [95]. The exacerbation of renal IRI appears to be caused by calcium overload and apoptosis stimulated by activated A₁R [16]. Deletion of A₁R gene or inhibition of A₁R has protects against renal IRI [96,97]. The possible regulatory mechanism of AR-mediated renal IRI is summarized in Figure 5. Furthermore, according to a review, the kidney can dynamically use hyaluronic acid (HA) to regulate whole-body fluid homeostasis [98]. Another study found that Cordyceps cicadae can reduce serum HA levels in cirrhotic rats [99]. Therefore, it can be speculated that Cordyceps cicadae affects the regulation of body fluid levels by kidney through HA, thus affecting the progression of renal IRI.

According to the results of KEGG analysis, MAPK (hsa04010), cAMP (hsa04024), PPAR (hsa03320), Rap1 (hsa04015), and HIF-1 (hsa04066) signaling pathways are the primary pathways of Cordyceps cicadae in treating renal IRI. As one of the classical pathways involved in inflammation and apoptosis [100], activation of the MAPK signaling pathway is the elementary factor controlling the release of pro-inflammatory cytokines and apoptosis [101]. The MAPK pathway can be activated by activation of MAPKs, which produce oxidative stress and TNF-α in renal tissues [40,102], and its activation is also closely associated with the upregulation of NF-kB [103,104]. Inhibiting the activation of MAPK and NF-kB pathways was proved by animal experiments to moderate IR-induced renal inflammatory injury and apoptosis [100,105]. In addition, Cordyceps cicadae was suggested to ameliorate cisplatin-induced AKI by inhibiting the NF-kB/MAPK pathways [106]. Therefore, it is reasonable to speculate that the MAPK pathway is an important therapeutic target for Cordyceps cicadae in repairing renal IRI. HIF-1 is a heterodimer composed of active subunit HIF-1α and structural subunit HIF-1β, and as a transcription factor, HIF-1 participates in the occurrence and development of many clinical diseases [106]. Studies demonstrated that ischemia and hypoxia can induce copious expression of HIF-1α, which can exert a protective effect on the kidney by activating downstream related genes [107-109]. It was reported that Cordyceps could alleviate the renal IR-induced AKI by upregulating the expression of HIF-1α in renal tissues [106]. It can be inferred that Cordyceps cicadae may exert an anti-renal IRI effect through the HIF-1 signaling pathway. cAMP (adenosine 3’,5’ cyclic monophosphate), as the second messenger in cells, participates in many different biological processes [110]. There is evidence that regulating the level of cAMP and relevant signaling pathways is an effective strategy for the treatment of AKI caused by renal IR [111]. Furthermore, experimental studies revealed that Cordyceps cicadae extract or its pure compound cordycepin has the effect of controlling cell secretion and substance synthesis and inducing apoptosis of cancer cells through the cAMP signaling pathway [91,112-114]. PPAR is a family of nuclear receptors, that is, peroxisome-proliferator activated
receptors, which are significant regulators of IR-induced vascular inflammation [115-117]. The activation of PPAR is involved in many metabolic processes, such as vascular inflammation regulation, lipid β-oxidation, adipogenesis, and cell proliferation/differentiation [118]. Experimental evidence suggests that Cordyceps extract or cordycepin can protect against UVB-induced injury and resist adipogenesis through various PPAR signal pathways [119,120]. Rap, a subfamily of the Ras family, can be activated by Epac-1 or Epac-selective cAMP analogs [121]. The activation of Rap1 acts on various cellular processes such as cellular proliferation/migration/metastasis, cytoskeletal remodeling, and cellular metabolism by regulating its downstream pathways such as ERK, FAK, Wnt, and AKT signaling pathways [122]. In addition, a recent study revealed that targeting the Epac-1/Rap-1 signaling pathway can play a therapeutic role in renal IR, as does adenosine receptor [123]. The results of GO and KEGG obtained by our analysis strongly agree with the results presented above, which indicates that *Cordyceps cicadae* plays a protective role in renal IR through a variety of related pathways.

*Cordyceps cicadae* has been reported to have no obvious adverse reactions or few adverse reactions [124-127]. Chen et al [124] repeatedly exposed rats to freeze-dried submerged *Cordyceps cicadae* mycelium culture for 90 days to evaluate its possible toxicity. No animal deaths occurred, and no clinical signs related to treatment were observed during the study period. There were also no treatment-related abnormalities found in various examination indicators, necropsy, and histopathological examination. Zhang et al [125] reviewed the potential adverse effects of Cordyceps and found diarrhea, constipation, and discomfort in some participants after taking Cordyceps-related products. Nonetheless, the exact relationship between these adverse reactions and Cordyceps has not been established. Chen et al [126] found that some known compounds in Cordyceps, such as adenosine analogs cordycepin, pentostatin, benzoquinone oosporein, and 2-pyridone alkaloid tenellin-like compounds have gastrointestinal toxicity, bone marrow toxicity, neurotoxicity, dose-dependent cytotoxicity, and/or toxicological effects on humans and animals, and the possibility of mycotoxin produced by Cordyceps fungi has not been completely ruled out yet. However, these toxic effects have rarely been reported in clinical practice. In addition, Cordyceps derivatives have been clinically approved for the treatment of various diseases such as cancer, cardiovascular disease, diabetes, inflammation, and nervous system diseases [127]. Taking into account its broad application in different diseases, its toxicity and appropriate therapeutic dose should be evaluated by more studies, and the standardized preparation methods should be explored to optimize therapeutic efficacy and minimize adverse reactions, along with benefiting more patients.

There are some deficiencies in the current research. First, the bioactive components actually absorbed by patients with renal IR may differ from those identified. Second, suppressor target genes and activated genes are difficult to distinguish. Furthermore, not all predictions were experimentally verified. Therefore, further research is needed to validate this TCM therapy.

**Conclusions**

The strategy of network pharmacology gives a new prediction method to mine the evidence of TCMs treatment mechanisms from an integrative point of view. Through target fishing and I-T network analysis, 3 active components of *Cordyceps cicadae* acting on 81 target genes associated with renal IR were forecast, implying that *Cordyceps cicadae* can repair renal IRI through multi-targets action. Adenosine, which was linked to the most target genes, was predicted to be the primary synergistic component of *Cordyceps cicadae*. Five genes (TGM2, ERBB4, PTGIS, IGF1R, and SIRT3) are associated with 3 active components, suggesting that these genes may be important for the repair of renal IRI. Additionally, PPI network analysis showed that ALB, GAPDH, CASP3, MAPK1, FN1, and IL-10 were the core hubs. In addition, the enrichment analyses showed that the 3 components identified in this study had latent pharmacological effects on renal IRI through MAPK, cAMP, PPAR, Rap1, and HIF-1 pathways. However, further experiments are needed to verify the theoretical prediction.

**Declaration of Figures’ Authenticity**

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.
**Supplementary Materials**

**Supplementary Table 1.** Information of *Cordyceps cicadae* and Renal IR-related targets. By combining the related targets of active ingredients of *Cordyceps cicadae* and the disease related targets, 81 overlapping targets were selected as the key targets in the treatment of renal IRI.

| Ingredient | Gene | Name | Target class |
|------------|------|------|--------------|
| **Cordycepin** | NCAM1 | Neural cell adhesion molecule 1 | -- |
| | HBEGF | Proheparin-binding EGF-like growth factor | Complex (toxin/growth factor) |
| | COL20A1 | Collagen alpha-1(XX) chain | Signaling protein |
| | FN1 | Fibronectin | -- |
| | CYP2C8 | Cytochrome P450 2C8 | -- |
| | COL20A1 | Collagen alpha-1(XX) chain | -- |
| | FN1 | Fibronectin | -- |
| | CYP2C8 | Cytochrome P450 2C8 | -- |
| | PDE4A | cAMP-specific 3',5'-cyclic phosphodiesterase 4A | Hydrolase |
| | C3 | Complement C3 | -- |
| **Cordycepic acid** | CCL26 | C-C motif chemokine 26 | Cytokine |
| | INSR | Insulin receptor | Transferase/signaling protein |
| | ALB | Serum albumin | Transport protein |
| | PROC | Vitamin K-dependent protein C | -- |
| | CDK5R1 | Cyclin-dependent kinase 5 activator 1 | Complex (kinase/activator) |
| | NR1H3 | LXR-alpha | Nuclear receptor |
| | SHH | Sonic hedgehog protein (by homology) | Unclassified protein |
| | ESR1 | Estrogen receptor alpha | Nuclear receptor |
| | CASP3 | Caspase-3 | Protease |
| | PTGER2 | Prostanoid EP2 receptor | Family A G protein-coupled receptor |
| | CHRNA7 | Neuronal acetylcholine receptor protein alpha-7 subunit | Ligand-gated ion channel |
| | ACE | Angiotensin-converting enzyme | Protease |
| | PTGS1 | Cyclooxygenase-1 | Oxidoreductase |
| | MGAM | Maltase-glucoamylase | Hydrolase |
| | SI | Sucrase-isomaltase | Enzyme |
| | SLC22A6 | Solute carrier family 22 member 6 (by homology) | Electrochemical transporter |
| | FGF2 | Basic fibroblast growth factor | Secreted protein |
| | LAP3 | Leucine aminopeptidase | Protease |
| | G6PD | Glucose-6-phosphate 1-dehydrogenase | Enzyme |
| | PPARA | Peroxisome proliferator-activated receptor alpha | Nuclear receptor |
| | FABP3 | Fatty acid binding protein | Fatty acid binding protein family |
| | PPARD | Peroxisome proliferator-activated receptor delta | Nuclear receptor |
| | FABP2 | Fatty acid binding protein intestinal | Fatty acid binding protein family |
| | HMGR | HMG-CoA reductase | Oxidoreductase |
| Ingredient | Gene   | Name                                           | Target class                                      |
|------------|--------|------------------------------------------------|--------------------------------------------------|
| Adenosine  | VWF    | von Willebrand factor                          | Immune system                                    |
|            | MMP9   | Matrix metalloproteinase-9                     | Hydrolase                                         |
|            | HLA-G  | HLA class I histocompatibility antigen, alpha chain G | Immune system                                    |
|            | AGC3   | Membrane primary amine oxidase                 |                                                  |
|            | ADORA1 | Adenosine A1 receptor                          | Family A G protein-coupled receptor              |
|            | ADORA2A| Adenosine A2a receptor                          | Family A G protein-coupled receptor              |
|            | ADK    | Adenosine kinase                               | Enzyme                                           |
|            | ADORA3 | Adenosine A3 receptor                          | Family A G protein-coupled receptor              |
|            | HSPA8  | Heat shock cognate 71 kDa protein              | Other cytosolic protein                          |
|            | HSPA5  | 78 kDa glucose-regulated protein               | Unclassified protein                             |
|            | ADA    | Adenosine deaminase                            | Hydrolase                                        |
|            | GAPDH  | Glyceraldehyde-3-phosphate dehydrogenase liver | Oxidoreductase                                    |
|            | AMD1   | S-adenosylmethionine decarboxylase              |                                                  |
|            | SRC    | Tyrosine-protein kinase SRC                     | Kinase                                           |
|            | ADORA2B| Adenosine A2b receptor                          | Family A G protein-coupled receptor              |
|            | MAPK1  | MAP kinase ERK2                                 | Kinase                                           |
|            | SLC29A1| Equilibrative nucleoside transporter 1          | Electrochemical transporter                       |
|            | PNP    | Purine nucleoside phosphorylase                | Enzyme                                           |
|            | P2RY1  | Purinergic receptor P2Y1                        | Family A G protein-coupled receptor              |
|            | P2RY11 | Purinergic receptor P2Y11                       | Family A G protein-coupled receptor              |
|            | EZH2   | EZH2/SUZ12/EED/RBBP7/RBBP4                     | Writer                                           |
|            | MAPKAPK2| MAP kinase-activated protein kinase 2           | Kinase                                           |
|            | HSPA1A | Heat shock 70 kDa protein 1                    | Other cytosolic protein                          |
|            | PMI1   | Serine/threonine-protein kinase PMI1           | Writer                                           |
|            | PARG   | Poly(ADP-ribose) glycohydrolase                | Enzyme                                           |
|            | GSK3B  | Glycogen synthase kinase-3 beta                | Kinase                                           |
|            | DAO    | D-amino-acid oxidase                           | Enzyme                                           |
|            | CTSL   | Cathepsin L                                    | Protease                                         |
|            | F2     | Thrombin                                       | Protease                                         |
|            | IDO1   | Indoleamine 2,3-dioxygenase                    | Enzyme                                           |
|            | HK2    | Hexokinase type II                             | Enzyme                                           |
|            | ROCK2  | Rho-associated protein kinase 2                | Kinase                                           |
|            | PRKACA | cAMP-dependent protein kinase alpha-catalytic subunit | Kinase                          |
|            | ADCY10 | Adenylate cyclase type 10                      | Enzyme                                           |
|            | TYR    | Tyrosinase                                      | Oxidoreductase                                    |
|            | P2RY12 | Purinergic receptor P2Y12                      | Family A G protein-coupled receptor              |
### Ingredient

| Ingredient              | Gene  | Name                          | Target class    |
|-------------------------|-------|-------------------------------|-----------------|
| Cordycepin & Adenosine  | IL10  | Interleukin-10                 | Transferase     |
| Cordycepic acid & Adenosine | TGM3     | Protein-glutamine gamma-glutamyltransferase E | Immune system |
|                         | MAOA   | Amine oxidase [flavin-containing] A |                |
|                         | SMURF2 | E3 ubiquitin-protein ligase SMURF2 |                |
|                         | DPP4   | Dipeptidyl peptidase IV       | Protease        |
|                         | CA2    | Carbonic anhydrase II         | Lyase           |
|                         | CA1    | Carbonic anhydrase I          | Lyase           |
|                         | CA9    | Carbonic anhydrase IX         | Lyase           |
| Cordycepin & Cordycepic acid & Adenosine | TGM2     | Protein-glutamine gamma-glutamyltransferase 2 | Transferase   |
|                         | PTGIS  | Prostacyclin synthase         | Isomerase       |
|                         | IGF1R  | Insulin-like growth factor 1 receptor | Hormone receptor |
|                         | SIRT3  | NAD-dependent deacetylase sirtuin-3, mitochondrial |                |

### Supplementary Table 2

Gene ontology (GO) terms of therapeutic target genes and their corresponding Counts, P value, and q-value. Through GO enrichment of the key targets, the remarkably (P value ≤ 0.01) enriched GO terms were obtained, indicating that some targets were involved in the development of renal IRI.

| Term                      | Pathway                              | Count | p-value       | q-value       |
|---------------------------|--------------------------------------|-------|---------------|---------------|
| hsa05205                  | Proteoglycans in cancer              | 14    | 6.27E-09      | 9.24E-07      |
| hsa04611                  | Platelet activation                  | 9     | 2.52E-06      | 0.000185883   |
| hsa01522                  | Endocrine resistance                 | 5     | 1.86E-05      | 0.00913186    |
| hsa04915                  | Estrogen signaling pathway           | 7     | 3.93E-05      | 0.001436564   |
| hsa04024                  | cAMP signaling pathway               | 8     | 4.87E-05      | 0.001436564   |
| hsa04213                  | Longevity regulating pathway - multiple species | 9 | 0.000208478 | 0.005120512   |
| hsa04010                  | MAPK signaling pathway               | 10    | 0.000482468   | 0.007751953   |
| hsa00910                  | Nitrogen metabolism                  | 5     | 0.000526025   | 0.007751953   |
| hsa05219                  | Bladder cancer                       | 4     | 0.000608781   | 0.008155917   |
| hsa03320                  | PPAR signaling pathway               | 6     | 0.000792088   | 0.008556796   |

### Supplementary Table 2 available from the corresponding author on request.

### Supplementary Table 3

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of therapeutic target genes and their corresponding counts, P value, and q-value. Through KEGG enrichment of the key targets, the remarkably (P value ≤ 0.01) enriched pathways were obtained, indicating that numerous targets were involved in the occurrence and development of renal IRI.
| Term                          | Pathway                          | Count | p-value       | q-value     |
|-------------------------------|----------------------------------|-------|---------------|-------------|
| hsa04015                      | Rap1 signaling pathway           | 8     | 0.00086555    | 0.008556796 |
| hsa04612                      | Antigen processing and presentation | 5   | 0.000876731   | 0.008556796 |
| hsa01521                      | EGFR tyrosine kinase inhibitor resistance | 5   | 0.000929024   | 0.008556796 |
| hsa05133                      | Yersinia infection               | 6     | 0.000990009    | 0.008556796 |
| hsa04012                      | ErbB signaling pathway           | 5     | 0.001296977    | 0.008556796 |
| hsa00330                      | Arginine and proline metabolism  | 4     | 0.001296977    | 0.008556796 |
| hsa04340                      | Hedgehog signaling pathway       | 4     | 0.001296977    | 0.008556796 |
| hsa05163                      | Human cytomegalovirus infection  | 8     | 0.001351732    | 0.008556796 |
| hsa04080                      | Neuroactive ligand-receptor interaction | 10  | 0.001439263    | 0.00940995  |
| hsa04923                      | Regulation of lipolysis in adipocytes | 4   | 0.001852435    | 0.011869146 |
| hsa05134                      | Legionellosis                    | 4     | 0.002114676    | 0.012984852 |
| hsa04510                      | Focal adhesion                   | 7     | 0.002894723    | 0.017063628 |
| hsa05020                      | Prion diseases                   | 5     | 0.004701593    | 0.020402295 |
| hsa00350                      | Glycine, serine and threonine metabolism | 3   | 0.005985333    | 0.023851911 |
| hsa00260                      | Glycine, serine and threonine metabolism | 3   | 0.00652359     | 0.025299241 |
| hsa00230                      | Purine metabolism                | 5     | 0.0080581938   | 0.030593053 |
| hsa00500                      | Starch and sucrose metabolism    | 2     | 0.00812305     | 0.020402295 |
| hsa04270                      | Vascular smooth muscle contraction | 5   | 0.0080581938   | 0.030593053 |
| hsa04610                      | Complement and coagulation cascades | 4   | 0.008858907    | 0.031083883 |
| hsa05205                      | Proteoglycans in cancer          | 14    | 6.27E-09       | 9.2E-07     |
| hsa04611                      | Platelet activation              | 9     | 2.52E-06       | 0.000185883 |
| hsa05020                      | Prion diseases                   | 5     | 1.86E-05       | 0.000913186 |
| hsa01522                      | Endocrine resistance             | 7     | 3.93E-05       | 0.001436564 |
| hsa04915                      | Estrogen signaling pathway       | 8     | 4.87E-05       | 0.001436564 |
| hsa04024                      | cAMP signaling pathway           | 9     | 0.00208678     | 0.005120512 |

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| Term               | Pathway                                           | Count | p-value       | q-value       |
|-------------------|---------------------------------------------------|-------|---------------|---------------|
| hsa00980          | Metabolism of xenobiotics by cytochrome P450       | 6     | 6.79E-05      | 0.000132      |
| hsa05133          | Pertussis                                         | 6     | 6.79E-05      | 0.000132      |
| hsa05220          | Chronic myeloid leukemia                          | 6     | 6.79E-05      | 0.000132      |
| hsa05145          | Toxoplasmosis                                     | 7     | 7.81E-05      | 0.000128      |
| hsa05204          | Chemical carcinogenesis                           | 6     | 0.000104      | 0.000194      |
| hsa05165          | Human papillomavirus infection                    | 11    | 0.000207      | 0.000376      |
| hsa04657          | IL-17 signaling pathway                           | 6     | 0.000209      | 0.000376      |
| hsa05152          | Tuberculosis                                      | 8     | 0.000234      | 0.000408      |
| hsa05146          | Amoebiasis                                        | 6     | 0.000235      | 0.000408      |
| hsa04910          | Insulin signaling pathway                         | 7     | 0.000261      | 0.000445      |
| hsa04014          | Ras signaling pathway                             | 9     | 0.000271      | 0.000455      |
| hsa05231          | Choline metabolism in cancer                      | 6     | 0.000294      | 0.000485      |
| hsa05221          | Acute myeloid leukemia                            | 5     | 0.000345      | 0.000566      |
| hsa05216          | Thyroid cancer                                    | 4     | 0.000358      | 0.000571      |
| hsa04625          | C-type lectin receptor signaling pathway          | 6     | 0.000384      | 0.000603      |
| hsa04928          | Parathyroid hormone synthesis, secretion and action| 6     | 0.000426      | 0.000658      |
| hsa04917          | Prolactin signaling pathway                       | 5     | 0.000454      | 0.000691      |
| hsa04931          | Insulin resistance                                | 6     | 0.000476      | 0.000705      |
| hsa04921          | Oxytocin signaling pathway                        | 7     | 0.000511      | 0.000754      |
| hsa04630          | JAK-STAT signaling pathway                        | 7     | 0.000719      | 0.001046      |
| hsa04071          | Sphingolipid signaling pathway                    | 6     | 0.000787      | 0.001129      |
| hsa04912          | AMPK signaling pathway                             | 6     | 0.000823      | 0.001163      |
| hsa05144          | Malaria                                           | 4     | 0.001055      | 0.00147       |
| hsa04683          | FoxO signaling pathway                            | 6     | 0.001351      | 0.001857      |
| hsa05202          | Transcriptional misregulation in cancer            | 7     | 0.001612      | 0.002185      |
| hsa05142          | Chagas disease (American trypanosomiasis)         | 5     | 0.002592      | 0.003466      |
| hsa04620          | Toll-like receptor signaling pathway              | 5     | 0.002703      | 0.003567      |
| hsa01523          | Antifolate resistance                             | 3     | 0.002861      | 0.003724      |
| hsa04920          | Adipocytokine signaling pathway                   | 4     | 0.003745      | 0.004813      |
| hsa05120          | Epithelial cell signaling in Helicobacter pylori infection | 4 | 0.003945 | 0.005004 |
| hsa04726          | Serotonergic synapse                              | 5     | 0.004171      | 0.005224      |
| hsa00982          | Drug metabolism – cytochrome P450                 | 4     | 0.004365      | 0.005332      |
| hsa05218          | Melanoma                                          | 4     | 0.004365      | 0.005332      |
| hsa05140          | Leishmaniasis                                     | 4     | 0.004815      | 0.00581       |
| hsa04110          | Cell cycle                                        | 5     | 0.005738      | 0.00684       |
Table

| Term                        | Pathway                                   | Count | p-value      | q-value      |
|-----------------------------|-------------------------------------------|-------|--------------|--------------|
| hsa00983                    | Drug metabolism – other enzymes           | 4     | 0.006073     | 0.007153     |
| hsa04140                    | Autophagy – animal                        | 5     | 0.006553     | 0.007628     |
| hsa04662                    | B cell receptor signaling pathway         | 4     | 0.006924     | 0.007966     |
| hsa04930                    | Type II diabetes mellitus                 | 3     | 0.008748     | 0.009893     |
| hsa04020                    | Calcium signaling pathway                 | 6     | 0.008799     | 0.009893     |
| hsa05923                    | Rheumatoid arthritis                      | 4     | 0.009941     | 0.011052     |

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