Identification of Genes and Pathways Associated with Kidney Ischemia-Reperfusion Injury by Bioinformatics Analyses

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Key Words
Kidney ischemia-reperfusion injury • Differentially expressed genes • Functional enrichment analysis • Protein-protein interaction

Abstract
Background/Aims: Ischemia-reperfusion (IR) injury in the kidney is a major cause of acute kidney injury in humans. However, the molecular mechanisms responsible for the progression of kidney IR injury still need to be explored. In this study, we aimed to explore the underlying genes and pathways associated with kidney IR injury. Methods: Gene microarray of GSE27274 was downloaded from the Gene Expression Omnibus database. The differentially expressed genes (DEGs) between kidney IR injury and kidney IR rat samples were analyzed. Gene Ontology biological process (BP) and pathway enrichment analyses of DEGs were performed, followed by protein-protein interaction (PPI) network construction. Results: A total of 88 up-regulated and 102 down-regulated DEGs were identified. The up-regulated DEGs including FK506 binding protein 1A (Fkb1a) were mainly enriched in biological processes related to protein ubiquitination. The down-regulated DEGs including complement component 5 (C5) were enriched in complement and coagulation cascades pathway. Choline phosphotransferase 1 (Chpt1) was enriched in glycerophospholipid metabolism pathway. In the PPI network, heme oxygenase (decycling) 1 (Hmox1) was as a hub gene that interacted with the maximum nodes. Conclusions: DEGs of Fkb1a, C5, Chpt1, and Hmox1, as well as complement and coagulation cascades pathway, glycerophospholipid metabolism pathway, and BP terms related to protein ubiquitination may be the potential targets for diagnosis and treatment of kidney IR injury.

Introduction
Kidney disease is a major public health concern with significantly increased morbidity and mortality [1]. Ischemia-reperfusion (IR) injury in the kidney is a major cause of acute kidney injury in humans [2]. This injury occurs when blood flow is restored after an extended...
period of ischemia [3]. It is a common source of the incidence and death rates in stroke, myocardial infarction and gut ischemia. Currently, there is often no specific therapy for IR injury [4].

The pathophysiology of IR injury is complex, including molecular oxygen, neutrophils, and components of the activated complement cascade [5, 6]. Recent studies have found that cytokines, adhesion molecules, platelet-activating factors, endothelin and leukotrienes play important roles in the pathophysiology of IR injury [7, 8]. Importantly, genome-wide expression analysis of the kidney after kidney injury caused by ischemic injury has revealed that interleukin 1 beta and intercellular adhesion molecule 1 are up-regulated in kidney IR injury [9]. Additionally, kidney injury molecule-1 mRNA levels have been found elevated on the apical membrane of proximal tubular epithelial cells in most injured regions of the kidney [10]. Based on the dataset of GSE9943, Shen et al. [11] revealed that miR-29c and transient receptor potential canonical (TRPC6) were potential novel targets for IR injury using bioinformatics approach. Although significant progress has been made to understand the potential significance of genetic factors in the kidney IR injury, relatively little is known about the molecular mechanisms responsible for the development and progression of kidney IR injury.

In this study, we downloaded the gene expression profile GSE27274 which was provided by Krishnamoorthy et al. [2]. Several studies have performed bioinformatics approach to study the molecular mechanisms of kidney IR injury based on this expression profile data, however, most of them only performed differentially expressed genes (DEGs) analysis or pathway analysis [12, 13]. At present, we not only identified the differentially expressed genes (DEGs) between kidney IR injury and kidney IR samples, but also performed functional enrichment analyses and protein-protein interaction (PPI) networks analysis for the identified DEGs to further explore their functions in kidney IR injury. Findings of this study may potentially serve as biomarkers in both diagnosis and treatment of kidney IR injury.

**Materials and Methods**

**Affymetrix microarray data**

The gene expression profile data of GSE27274 were downloaded from Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) database based on the platform of Illumina ratRef-12 v1.0 expression beadchip. The dataset contains 24 samples and samples of GSM674258, GSM674259, GSM674260, GSM674264, GSM674265 and GSM674266 were analyzed in this study.

**Data preprocessing and differential expression analysis**

The original array data were downloaded and normalized. The expression values of multiple probes for a given gene were reduced to a single value by taking the average expression value. The limma [14] package in R language was used to identify the DEGs. The log$_2$-fold change (log$_2$FC) and false discovery rate (FDR) were calculated. |log$_2$FC| > 0.5 and FDR < 0.05 were considered as the cutoffs.

**Functional enrichment analyses**

The Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov/knowledgebase/) [15] is a comprehensive set of functional annotation tools, which has been developed for relating the functional terms with gene lists by clustering algorithm. In this study, we performed Gene Ontology (GO) biological process (BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses for the DEGs using the DAVID online tool. The p-value < 0.01 was set as threshold.

**PPI network construction**

The Search Tool for the Retrieval of Interacting Genes (STRING, http://string-db.org/) [16] database is designed to evaluate the PPI information. In this study, STRING online tool was applied to analyze the PPI of DEGs and only those experimentally validated interactions with a combined score > 0.4 were selected as significant interactions.
Results

Identification of DEGs

For the dataset GSE27274, a total of 190 DEGs were obtained after data preprocessing. Among them, 88 were down-regulated and 102 were up-regulated in the renal IR injury group.

Functional enrichment analyses

The top 5 BP terms enriched by up-regulated and down-regulated DEGs were shown in Table 1. As shown in the table, the up-regulated DEGs were enriched in BP terms related to protein ubiquitination, molecular function, and cellular protein metabolic process, such as FK506 binding protein 1A (Fkb1a) and Proteasome (Prosome, Macropain) 26S Subunit, ATPase, 2 (Psmc2). BP terms enriched by down-regulated DEGs were mainly associated with response to mercury ion, cell proliferation, and coenzyme metabolic process. The up-regulated DEGs were enriched in 2 pathways including proteasome and one carbon pool by folate. The down-regulated DEGs were enriched in 3 pathways including glycerophospholipid metabolism (choline phosphotransferase 1 (Chpt1)), complement and coagulation cascades (complement component 5 (C5)), and phenylalanine metabolism (Table 2).

PPI network construction

Based on STRING database, the PPI network was constructed (Fig. 1). The figure showed that heme oxygenase (decycling) 1 (Hmox1) was as a hub gene that interacted with the maximum nodes.

Table 1. Gene Ontology (GO) functional enrichment analysis for the up- and down-regulated differentially expressed genes (DEGs) (top 5)

| ID       | Term                                         | P-value   |
|----------|----------------------------------------------|-----------|
| GO:0031398 | positive regulation of protein ubiquitination | 4.46E-04  |
| GO:0044092 | negative regulation of molecular function    | 6.75E-04  |
| GO:0031396 | regulation of protein ubiquitination         | 1.06E-03  |
| GO:0043086 | negative regulation of catalytic activity    | 1.23E-03  |
| GO:0032269 | negative regulation of cellular protein metabolic process | 1.62E-03 |

| ID       | Term                                         | P-value   |
|----------|----------------------------------------------|-----------|
| GO:0046689 | response to mercury ion                      | 4.66E-02  |
| GO:0008285 | negative regulation of cell proliferation   | 5.10E-02  |
| GO:0006732 | coenzyme metabolic process                   | 5.27E-02  |

Table 2. Pathway enrichment analysis for the up- and down-regulated differentially expressed genes (DEGs)

| Category | Term                  | P-value |
|----------|-----------------------|---------|
| Up-regulated DEGs | Proteasome              | 0.0387  |
|           | One carbon pool by folate | 0.0900  |
| Down-regulated DEGs | Glycerophospholipid metabolism | 0.0356 |
|           | Complement and coagulation cascades            | 0.0444  |
|           | Phenylalanine metabolism                        | 0.0746  |
Discussion

Our study found that 190 DEGs between kidney IR injury and kidney IR samples were obtained after data processing. The up-regulated DEGs were significantly enriched in BP terms related to protein ubiquitination. On the other hand, the down-regulated DEGs were enriched in complement and coagulation cascades pathway, and metabolism related pathways. In addition, in the PPI network, Hmox1 was as a hub gene which interacted with the maximum nodes. The results suggested that these genes and pathways may play important roles in the progression of kidney IR injury.

Studies have suggested that the ubiquitin-proteasome system mediates the degradation of most intracellular proteins in cells, including ubiquitination of protein molecules and degradation of the ubiquitinated protein by the proteasome [17, 18]. Ubiquitinated proteins usually accumulate in cells when the function of proteasome is insufficient [19]. Tian et al. [17] have found a global increase of ubiquitinated proteins in IR hearts. In the present study, BP terms related to protein ubiquitination were enriched by the up-regulated DEGs including Fkb1a. Fkb1a encodes a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes involving protein folding and trafficking [20]. Accumulation and activation of immune system cells in the kidney during IR is a major mediator of kidney injury. Animal models experiments have revealed that both the innate and adaptive immune systems mediate IR injury in the kidney [21]. Importantly, ischemic damage is the most common cause of delayed graft function in accompany with tissue inflammation [22]. Boros and Bromberg [23] also reported that IR injury is a multi-factorial antigen-independent inflammatory condition. Therefore, the up-regulation of Fkb1a in kidney IR injury may be associated with the inflammation of this injury.

In addition, the down-regulated DEGs including C5 were found to be enriched in the pathway of complement and coagulation cascades. The complement system, consisting of multiple cascades, is a component of the immune response, which plays an important role in the regulation of the inflammatory response [24]. Study has found that the complement cascade plays a key role in numerous systemic organ IR models of tissue damage [25-27]. Shen et al. [11] have also suggested that complement and coagulation cascades pathway is a significant pathway in IR injury. For the enriched DEG of C5, it has been suggested to play an important role in inflammatory and cell killing processes. Importantly, the generation of C5 has also been implicated as playing a potential role in IR injury models of rat myocardium, rat liver, and baboon myocardium [28-30]. Taken together, the results suggest a potential
place for complement and coagulation cascades pathway and C5 in the treatment of kidney IR injury.

The main function of kidney is to excrete the metabolic wastes; the loss of kidney function can induce significant changes in various metabolites in the body [31]. Qingqing et al. [32] have reported that renal IR produce strong changes of metabolites in kidney cortex, kidney medulla, and plasma over time. In our study, the down-regulated DEGs were also enriched in metabolism related pathways. For instance, Chpt1 was enriched in glycerophospholipid metabolism, which was in accordance with the findings of Cadenas et al. [33], that was, the down-regulation of Chpt1 was responsible for glycerophospholipid and cholesterol metabolism. Therefore, we speculated that the changes of metabolism related pathways in kidney IR injury might be due to the down-regulation of Chpt1.

Furthermore, in the PPI network, Hmox1 was as a hub gene which interacted with the maximum nodes. Hmox1 is a protective gene with anti-inflammatory and anti-apoptotic actions [34]. Hmox1 induction protects cellular against injury caused by the reactive oxygen species (ROS) [35]. The pathophysiology of IR injury includes oxidant stress that contribute to various degrees to the overall organ damage. Formation of ROS has been demonstrated to be the most invoked disease mechanisms in IR injury [36]. Thus, Hmox1 is able to restore tissue architecture and organ function after injury. Many studies have demonstrated its role in IR injury in organs including kidney [37]. Interestingly, in the PPI network, Hmox1 interacted with NAD(P)H dehydrogenase, quinone 1 (Nqo1) which was also an antioxidant protein that regulated ROS generation [38]. Importantly, Gang et al. [39] have suggested that Nqo1 has a protective role against renal injury induced by IR. Therefore, Hmox1 and Nqo1 may play crucial roles in IR injury of kidney.

Conclusion

Our data provided a comprehensive bioinformatics analysis of DEGs and pathways which might be involved in kidney IR injury. DEGs such as Fkb1a, C5, Chpt1, Hmox1 and Nqo1, as well as complement and coagulation cascades pathway, glycerophospholipid metabolism pathway, and BP terms related to protein ubiquitinatione have the potential to be used as targets for the diagnosis and treatment of kidney IR injury. The findings in current study may contribute to our understanding of the underlying molecular mechanisms of this disorder.

However, this study had some limitations. Firstly, the sample size for microarray analysis was small which might cause a high rate of false positive results. Secondly, this study lacked experimental verification. Therefore further genetic and experimental studies with large sample sizes should be considered to confirm this findings.

Disclosure Statement

The authors declare no competing interests.

Acknowledgments

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