Gastroenterology and Hepatology From Bed to Bench.
2024 **RIGLD**, Research Institute for Gastroenterology and Liver Diseases

ORIGINAL ARTICLE

p-ISSN: 2008-2258 e-ISSN: 2008-4234

Introducing critical proteins related to liver ischemia/reperfusion injury

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ABSTRACT

Aim: The current study aimed to introduce the key proteins involved in liver ischemia/reperfusion (I/R) injury through protein-protein interaction (PPI) analysis.

Background: Liver transplantation (LT) is a well-known treatment for liver diseases that threaten patients with mortality. LT is a complex operation, and several risks, including liver I/R injury, affect its success. Improving LT requires detection of its molecular mechanism. Experiments have revealed that high throughput methods such as proteomics in combination with bioinformatics are useful tools for analyzing the molecular mechanism of disease.

Methods: The differentially expressed proteins (DEPs) involved in liver I/R injury were extracted from the literature. The queried DEPs plus the first 100 neighbors were included in a network through STRING database using Cytoscape software. Degree, betweenness centrality, closeness centrality, and stress were considered to determine the central nodes. The queried DEPs were assessed by action map analysis using the CluePedia application of Cytoscape software. The key proteins were identified by comparing network analysis and action map evaluation results.

Results: Six proteins, namely ALB, INS, GAPDH, CAT, IL6, and TNF, among the added first neighbors were determined as the central first neighbors. MPO, CRP, MMP9, and HMOX1 were selected as central DEPs among the queried proteins. Action map analysis confirmed the PPI findings. The final evaluation revealed that MMP9 in combination with CRP and HMOX1 plays a critical role in liver I/R injury.

Conclusion: The significant role of MMP9 in liver I/R injury was detected in this study. Two central proteins (CRP and HMOX1) were shown to have a regulatory effect on MMP9; CRP activated MMP9, while HMXO1 downregulated it.

Keywords: Liver, Ischemia/reperfusion injury, Bioinformatics, Matrix metallopeptidase 9, Network analysis.

(Please cite as: **Arjmand B, Khodadost M, Jahani Sherafat S, Rezaei Tavirani M, Ahmadi N, Rezaei Tavirani S. Introducing critical proteins related to liver ischemia/reperfusion injury. Gastroenterol Hepatol Bed Bench 2024;17(1):87-92. https://doi.org/10.22037/ghfbb.v17i1.2555).**

Introduction

Mortality due to liver disorders indicates liver transplantation, a complex process with several risk factors. Liver ischemia/reperfusion (I/R) injury significantly affects the success of transplantation (1). It is reported that hepatocyte cell death occurs during I/R injury, but the related mechanism is not clear (2). Oxidative stress is mentioned as a crucial factor related to liver I/R injury (3). Many efforts have been made to explore the molecular mechanism of liver I/R injury, and several proteins and enzymes have been identified as agents involved in it (4, 5).

Recent experiments have shown that high throughput methods, such as genomics, proteomics, and metabolomics, are useful tools for assessing the molecular mechanism of diseases. Many disorders such as cancers, neurodegenerative disorders, gastrointestinal

Received: 02 June 2023 *Accepted*: 07 August 2023
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diseases, skin diseases, and also COVID-19 infection are evaluated through high throughput methods. A complex analysis of a disease using proteomics or genomics requires the application of bioinformatics (6-8). Network analysis is an attractive method applied to explore the core of molecular changes in many diseases. Protein-protein interaction (PPI) networks represent the connections between proteins, genes, or metabolites. They are used to assess liver diseases. Nonalcoholic fatty liver disease was investigated herein to identify new, related proteins (9-11).

In scale-free networks, a limited number of nodes (known as central nodes) play critical roles in the construction of the studied network. Hubs that are characterized with higher values of degree are central nodes, and they are applied to explore the molecular mechanisms of many diseases. The second type of central nodes used in the network analysis of various disorders, known as bottlenecks, are identified by higher values of betweenness centrality. Common hubs and bottlenecks (hub-bottlenecks) are known as a potent central node that plays a critical role in the integration of the studied network (12, 13). Closeness centrality and stress are two other centrality parameters that discriminate central nodes from other nodes in the network (14, 15). PPI network analysis was applied to discover the molecular mechanism of liver ischemia/reperfusion injury. SLC8A3, CYP3A7, TNFRSF8, P2RY6, HRH1, LRP2, HKDC1, SGO1, and IL20RB are introduced as hubs of the analyzed network (16). In the present study, the dysregulated proteins related to human liver I/R injury were extracted from the literature, and the significant individuals were included in a PPI network to determine the central nodes. Using action map, the central nodes were screened and the critical proteins related to liver I/R injury were introduced.

Methods

In their paper entitled “Global proteome profiling of human livers upon ischemia/reperfusion treatment,” Haijian Cai et al. reported the findings of their investigation of the protein expression ratios of liver

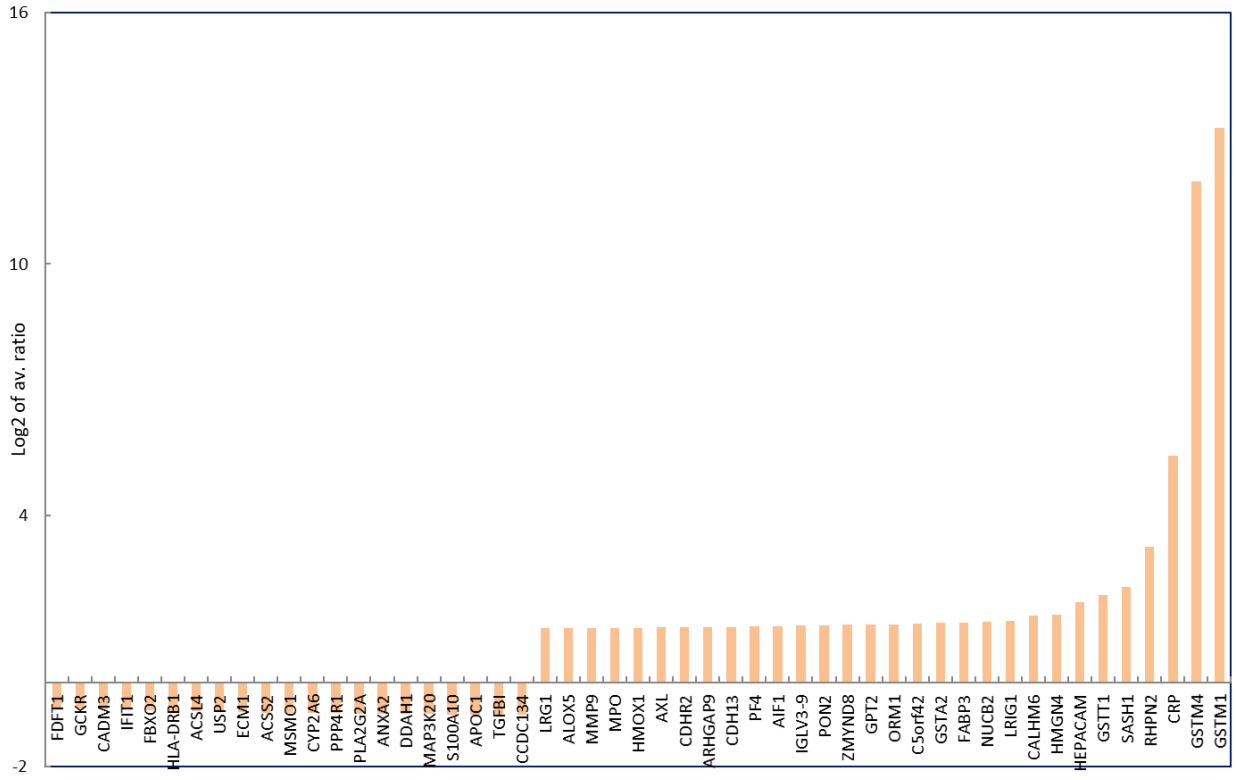

Figure 1. Log2 of average ratio (average ratio of ischemia/reperfusion group expression value versus control group expression amount) of the down- and upregulated DEPs.

samples from six patients with hepatic hemangioma who underwent hepatectomy before receiving the Pringle maneuver which caused hepatic ischemia reperfusion injury (as the control group) and 10 min after restoration of blood supply (as ischemia/reperfusion group) (17). Based on an average ratio > 1.5 and a p-value < 0.01, significant DEPs were selected from their data. The average protein expression ratios for the samples relative to the controls were compared and illustrated.

The significant DEPs plus 100 first neighbors from STRING database were interacted by Cytoscape software v 3.7.2. The formed network was analyzed by "NetworkAnalyzer," and the queried hubs were determined among the top 20 nodes based on degree value. Like the hubs, the other central nodes were identified based on betweenness centrality, closeness centrality, and stress. Common central DEPs were identified, and this process was repeated for the first neighbors.

Action map analysis was done for the 51 queried DEPs regarding expression, activation, inhibition, reaction, and binding actions. The clusters of nodes as well as the isolated individuals were determined.

Results

Based on an average ratio > 1.5 and a p-value < 0.01, 51 significant DEPs, comprising 21 down-regulated proteins and 30 up-regulated individuals, were selected from among the 60 introduced DEPs (Figure 1). The 51 significant DEPs plus 100 first neighbors were included in the "protein query" of STRING database to make an interactome. A total of 49 DEPs from the 51 queried proteins were recognized by STRING database. The network was created using the 49 recognized DEPs and the 100 added first neighbors. The main connected component, two paired proteins, and eight isolated individuals formed all the components of the constructed network. The main connected component was formed from 139 nodes that were connected by 3062 non-directed edges.

As shown in Tables 1 and 2, network analysis led to the introduction of six central first neighbors, namely ALB, IL6, TNF, INS, GAPDH, and CAT, and four central queried DEGs as well as MPO, HMOX1, CRP, and MMP9.

Figure 2 presents the results of action map analysis. Five actions, i.e., counting expression, activation, inhibition, reaction, and binding, were investigated for the relationship between the 51 significant DEPs. As Figure 2 shows, 33 individuals were isolated, and the other 18 proteins were distributed in the five components. Inhibition action did not appear as a relation between the queried DEPs.

Discussion

Down- and upregulation of several proteins and microRNA in liver I/R injury are reported by researchers (18-20). As described in the methods, a proteomic analysis of I/R injury was conducted by Haijian Cai et al. (17). The distribution of the significant dysregulated proteins from this investigation is presented in Figure 1. A total of 31 upregulated proteins and 20 downregulated

Table 1. Central first neighbor proteins of analyzed PPI network related to liver ischemia/reperfusion injury

No.	Display name	Degree	Betweenness centrality	Closeness centrality	Stress
1	ALB	108	1.00	1.00	12698
2	IL6	98	0.35	0.57	9018
3	TNF	97	0.17	0.53	8578
4	INS	92	0.26	0.33	8540
5	GAPDH	88	0.00	0.18	7442
6	CAT	85	0.33	0.00	9188

Table 2. Central queried DEPs of analyzed PPI network related to liver ischemia/reperfusion injury

No.	display name	Degree	Betweenness Centrality	Closeness Centrality	Stress
1	MPO	81	0.17	0.71	4236
2	HMOX1	80	1.00	1.00	5474
3	CRP	76	0.00	0.00	3402
4	MMP9	75	0.005	0.680	2472

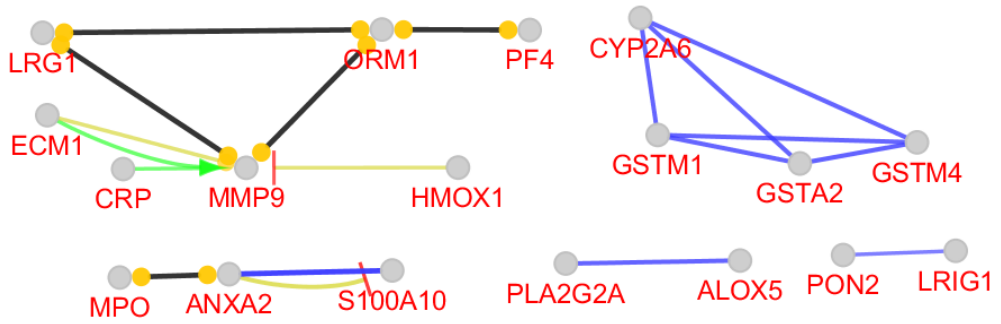

Figure 2. Action map analysis of 51 significant DEPs. The yellow, green, black, and blue colors refer to expression, activation, reaction, and binding, respectively. A total of 33 individuals were isolated.

individuals were identified. As Figure 1 shows, upregulation regarding the number of proteins and amounts of fold change is a prominent process relative to downregulation. GSTM1 and GSTM4 as the two super-expressed proteins are highlighted in Figure 1. Dysregulation of GSTM1 in liver I/R injury was reported by a previous investigation (21).

Among the added first neighbors, six proteins (ALB, IL6, TNF, INS, GAPDH, and CAT) were highlighted as central first neighbors. A significant reduction in albumin levels in chronic liver disease patients has been confirmed by reports of investigators (22). Jorge As et al. published evidence of the involvement of IL6 and TNFα in non-alcoholic fatty liver disease (23). Furthermore, there is an evidence that insulin-like growth factor-1-binding protein-3 plays an important role in the hepatic response to ischemia/reperfusion (24). Akateh Clifford et al. showed that direct intrahepatic administration of pegylated catalase (PEG-CAT) provides noteworthy protection against I/R injury in rat (25).

Four central queried DEPs (MPO, HMOX1, CRP, and MMP9) were determined through network analysis. As shown in Figure 1, these 4 central proteins are upregulated. C-reactive protein (CRP) is the most upregulated one. The PPI network findings were evaluated using action map analysis. Among the 51 queried proteins, 18 DEPs were connected through 5 components. Clusters are identified as two paired nodes (clusters 1-2), a triple unit (cluster 3), a component of 4 proteins (cluster 4), and a main connected component including 7 DEPs (cluster 5). Three members of cluster 4 (GSTM1, GSTM4, and GSTA2) belong to the glutathione S-transferase protein family. The important clusters are clusters 3 and 5 which are formed with various types of nodes and edges. As illustrated in Figure 2, the 4 central DEPs are organized in clusters 3 and 5 (MPO in cluster 3 and HMOX1, CRP, and MMP9 in cluster 5). Action map analysis confirmed the PPI results and provided useful additional information. It can be concluded that cluster 5 is the most highlighted cluster, but the connections between HMOX1, CRP, and MMP9 are crucial links. As depicted in Figure 2, CRP activates MMP9, while HMOX1 downregulates it. This consistency between the PPI network results and the action map findings indicates that the introduced hubs are key proteins related to liver ischemia/reperfusion injury.

Matrix metallopeptidase 9 (MMP9) is known as a proteinase enzyme that plays a role in invadopodium maturation and metastasis through extracellular matrix degradation (26). Invadopodia are described as actin-rich structures that are present in invasive cancer cells. These structures are enzymatically active and promote degradation of the adjacent extracellular matrix to simplify invasion (27). The described function for MMP9 is a useful answer for upregulation as well as its highlighted role as a central protein in liver I/R injury. It seems that the destructive role of MMP9 in the damages of liver I/R injury is a prominent point. This function can be considered a defense response to the products which accumulate after ischemia. Activation of MMP9 by C-reactive protein (as an inflammatory protein) confirms this hypothesis. The negative co-expression between heme oxygenase-1 (HMOX1) and MMP9 is shown in Figure 2. Investigations indicate that HMOX1 is involved in cell protection against stresses (28).

Conclusion

MPO, HMOX1, CRP, and MMP9 are the central proteins that play critical roles in liver I/R injury. The significant role of MMP9 as a matrix metallopeptidase in response to ischemia was highlighted. CRP and HMOX1 regulate MMP9 in different ways. It may be the inhibition of MMP9 that leads to a reduction in the damages of liver I/R injury; however, regulation of MPO, HMOX1, and CRP may be the key process in protecting the liver from I/R injury.

Acknowledgment

This project is supported by Shahid Beheshti University of Medical Sciences.

Conflict of interests

The authors declare no conflict of interest.

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