



doi:10.7659/j.issn.1005-6947.2021.07.009  
http://dx.doi.org/10.7659/j.issn.1005-6947.2021.07.009  
Chinese Journal of General Surgery, 2021, 30(7):822-835.

· 基础研究 ·

# 肝脏缺血再灌注损伤的 ceRNA 网络构建和潜在治疗药物筛选

邓家琦<sup>1</sup>, 钱保林<sup>2</sup>, 张丽云<sup>3</sup>, 况容<sup>1</sup>, 李明星<sup>1</sup>

(西南医科大学附属医院 1. 超声医学科 2. 肝胆外科 3. 健康管理部, 四川 泸州 646000)

## 摘要

**背景与目的:** 肝脏缺血-再灌注 (I/R) 损伤是肝移植和肝切除手术过程中常涉及的共同病理生理变化。竞争性内源性 RNA (ceRNA) 调控网络可参与多种疾病的发生发展。然而 ceRNA 网络在肝脏 I/R 损伤中的功能仅有少量报道。本研究旨在应用生物信息学方法构建与肝脏 I/R 损伤相关的 ceRNA 网络, 同时基于差异表达基因筛选潜在治疗药物。

**方法:** 从 GEO 数据库获取肝脏 I/R 损伤的 mRNA 及 miRNA 表达芯片数据。使用 R 语言中的 limma 包进行基因差异表达分析, 并使用 ggplot2 包进行散点图、火山图和热图绘制。使用 String 数据库及 Cytoscape 软件进行蛋白互作 (PPI) 网络构建。利用 Metascape 数据库对筛选出的差异 mRNA 进行 GO/KEGG 功能富集分析。通过转录调控网络数据库分析可能调控这些差异基因的转录因子。使用 miRTarBase 数据库构建 miRNA-差异表达基因网络。通过 starBase: ceRNA 数据库构建 ceRNA 网络。使用比较毒物基因组学数据库 (CTD) 筛选对关键差异基因表达具有潜在作用的天然药物。

**结果:** 从 GEO 数据库获得 2 个肝脏 I/R 损伤 mRNA 数据集 (GSE10654 和 GSE117066) 和 1 个肝脏 I/R 损伤 miRNA 数据集 (GSE72315)。通过 limma 包及 Venn 图分析 mRNA 表达数据集, 筛选到 16 个在 I/R 组上调表达, 在缺血后适应 (IPO) 组下调表达的基因; 7 个在 I/R 组下调表达, 在 IPO 组上调表达的基因。GO/KEGG 功能富集分析结果显示差异基因主要参与细胞死亡的正调控及对细胞外刺激反应的生物学过程, 并参与 MAPK 信号通路。转录调控网络数据库分析获得 6 个转录因子 (Trp53、Cebpb、Crebbp、Fos、Nfkb1 及 SP1) 可能参与这些差异基因的调控。通过 miRTarBase 数据库分析, 并结合 GSE72315 数据集中 miRNA 在 I/R 损伤后的差异表达, 获得两个可能在肝脏 I/R 损伤中发挥重要的作用 miRNA-mRNA 轴 (mmu-miR-32-5p-Btg2 与 mmu-miR-9-5p-Mt2)。通过 starBase: ceRNA 数据库分析, 最终获得 9 条 ceRNA 网络, 分别是: XIST/MEG8/LINC00963/MALAT1-miR-32-5p-Btg2 轴、XIST/NEAT1-miR-132-3p-Btg2 轴及 HSPA9P1/RALGAPA1P1/RPS26P39-miR-9-5p-Dusp6 轴。CTD 数据库筛选到 7 种植物药 (槲皮素、白藜芦醇、染料木黄酮、香豆雌酚、姜黄素、辣椒素及东莨菪碱) 可降低关键基因的表达发挥潜在治疗作用。

**结论:** 通过生物信息学方法筛选了肝脏 I/R 损伤过程中的关键 ceRNA 网络及治疗的潜在天然药物, 为进一步研究肝脏 I/R 损伤的发病机制及治疗药物提供重要依据。

## 关键词

肝; 再灌注损伤; RNA, 竞争性内源性; 药物开发; 计算生物学

中图分类号: R657.3

**基金项目:** 西南医科大学校级科研基金资助项目 (2019ZQN013)。

**收稿日期:** 2021-04-02; **修订日期:** 2021-06-14。

**作者简介:** 邓家琦, 西南医科大学附属医院住院医师, 主要从事介入超声 (肝脏、甲状腺) 方面的研究。

**通信作者:** 李明星, Email: 1246185287@qq.com

# Construction of ceRNA network in liver ischemia/reperfusion injury and screening of the potential therapeutic agents

DENG Jiaqi<sup>1</sup>, QIAN Baolin<sup>2</sup>, ZHANG Liyun<sup>3</sup>, KUANG Rong<sup>1</sup>, LI Mingxing<sup>1</sup>

(1. Department of Ultrasound Medicine 2. Department of Hepatobiliary Surgery, 3. Health Management Department, the Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan 646000, China)

## Abstract

**Background and Aims:** Liver ischemia/reperfusion (I/R) injury is a common pathophysiological change often involved in liver transplantation and hepatectomy. Competing endogenous RNA (ceRNA) regulatory network can participate in the occurrence and development of many diseases. However, there are only a few reports on the function of ceRNA network in liver I/R injury. This study was conducted to construct a ceRNA network related to liver I/R damage via bioinformatics approaches, and meanwhile to screen potential therapeutic drugs based on the differentially expressed genes.

**Methods:** The mRNA and miRNA expression chip data of liver I/R damage were obtained from the GEO database. Gene differential expression analysis was performed using the limma package in the R language, and the scatter plots, volcano plots and heat maps were drawn using the ggplot2 package. The protein-protein interaction (PPI) network was constructed using String database and Cytoscape software. The GO/KEGG function enrichment analyses were performed on the differentially expressed mRNAs screened out using Metascape database. The transcription factors that may regulate these differentially expressed genes were analyzed through the transcriptional regulatory network database. The miRNA-differentially expressed gene network was constructed using miRTarBase database. A ceRNA network was constructed through starBase: ceRNA database. The natural medicines that have potential effects on the expressions of key differentially expressed gene were screened using the Comparative Toxicogenomics Database (CTD).

**Results:** Two liver I/R injury mRNA data sets (GSE10654 and GSE117066) and one liver I/R injury miRNA data set (GSE72315) were obtained from the GEO database. A total of 16 genes up-regulated in I/R group and down-regulated in ischemic postconditioning (IPO) group, and 7 genes down-regulated in I/R group and up-regulated in IPO group were screened out by analyzing the mRNA expression data set using limma package and Venn diagram. The results of GO/KEGG functional enrichment analyses showed that the differential genes were mainly involved in the positive regulation of cell death and the biological process of response to extracellular stimuli, and participated in the MAPK signaling pathway. Transcription regulatory network database analysis revealed that six transcription factors (Trp53, Cebpb, Crebbp, Fos, Nfkb1 and SP1) may be involved in the regulation of these differentially expressed genes., two miRNA-mRNA axes (mmu-miR-32-5p-Btg2 and mmu-miR-9-5p-Mt2) may play an important role in liver I/R injury were obtained through miRTarBase database analysis and combined with the differential expression of miRNAs after I/R injury in the GSE72315 data set. Through starBase: ceRNA database analysis, 9 ceRNA networks were finally obtained, namely, the XIST/MEG8/LINC00963/MALAT1-miR-32-5p-Btg2 axis, XIST/NEAT1-miR-132-3p-Btg2 axis and HSPA9P1/RALGAPA1P1/RPS26P39-miR-9-5p-Dusp6 axis. Seven botanicals (quercetin, resveratrol, genistein, couestrol, curcumin, capsaicin, and scopolamine) that could exert potential therapeutic effects by reducing the expressions of key genes were screened out in the CTD database.

**Conclusion:** The important ceRNA networks in the process of hepatic I/R injury and potential therapeutic drugs are screened through bioinformatics analysis, which provide an important basis for further research of the pathogenesis of liver I/R injury and its therapeutic drugs.

## Key words

Liver; Reperfusion Injury; RNA, Competing Endogenous; Drug Development; Computational Biology

**CLC number:** R657.3

肝脏缺血-再灌注 (ischemia/reperfusion, I/R) 损伤是肝组织经过一段时间缺血后, 再恢复血液灌注时触发一系列复杂级联反应导致肝损伤进一步加重的病理生理过程<sup>[1]</sup>。临床上肝脏 I/R 损伤常发生于肝移植和肝切除等肝脏手术过程中。肝脏在缺血状态下血流再灌注后, 常引起术后肝功能短时间内急剧下降、凝血系统障碍等多种并发症, 同时会产生肝脏乃至全身的一系列代谢紊乱, 若未能得到及时处理, 还可能引发肝功能衰竭, 严重者甚至导致患者死亡, 大大降低肝切除或肝移植手术成功率及阻碍患者术后康复, 影响患者预后<sup>[2]</sup>。目前临床上通常使用全入肝血流阻断法 (Pringle 法) 进行术中肝脏血流的阻断, 正常的肝实质连续性 Pringle 法阻断的安全时限可达 90 min, 而间歇性 Pringle 法阻断 (每次阻断 10~15 min, 间歇 5 min 后重复) 的累计缺血安全时限可长达 120 min<sup>[3-4]</sup>。如果超过这一时限可能导致肝坏死、肝衰竭的发生。

肝脏 I/R 损伤的发生机制十分复杂, 可能与活性氧产生、细胞凋亡、钙离子超载及大量炎性细胞浸润导致的炎症反应有关, 这些机制在肝脏缺血的过程中开始出现, 并在血液再灌注后进一步加重<sup>[5]</sup>。lncRNA 作为一类长度超过 200 个核苷酸的非编码 RNA, 具有复杂的种类和多样化的功能。近年来随着对 lncRNA 研究的深入, 发现 lncRNA、mRNA、miRNA 三者可通过竞争性内源性 RNA (competing endogenous RNA, ceRNA) 机制相互作用, 形成 ceRNA 调控网络, 干扰许多因子的表达影响多种疾病的整体进程<sup>[6]</sup>。然而 ceRNA 网络在肝脏 I/R 损伤中的功能仅有少量报道。Ying 等<sup>[7]</sup>研究发现 lncRNA Gm4419/miR-455/SOX6 轴通过促进缺氧复氧处理的 BRL-3A 细胞凋亡, 加速肝脏 I/R 损伤。Dai 等<sup>[8]</sup>研究表明 lncRNA AK054386/miR-199 通过激活内质网应激通路, 加速肝脏 I/R 损伤。Tang 等<sup>[9]</sup>发现 lncRNA HOTAIR / miR-20b-5p/ATG7 轴可通过激活细胞自噬, 加速肝脏 I/R 损伤。Huang 等<sup>[10]</sup>证明 lncRNA MEG3/miR-34a/NRF2 网络通过抑制细胞凋亡, 降低血清 ALT 和 AST 的表达, 保护肝脏 I/R 损伤。本研究旨在利用生物信息学方法从基因表达数据库中挖掘在肝脏 I/R 损伤中可能发挥重要作用的基因, 并构建潜在 ceRNA 网络, 为后续机制研究提供数据支持。同时基于这些差异基因从药物数据库筛选出潜在天然药物, 以期减轻肝脏 I/R

损伤为肝脏手术后的恢复创造有利条件。

## 1 材料与方法

### 1.1 材料

从 GEO (Gene Expression Omnibus) 数据库中按照以下标准获取肝脏 I/R 损伤的基因芯片数据: 以“hepatic ischemia/reperfusion injury”为关键词; 选择物种为“Mus musculus”、研究类型为“expression profiling by array”两个过滤条件进一步筛选数据; 最后选定 I/R 组织样本数、缺血后适应 (IPO) 组织样本数与假手术对照组织样本数均  $\geq 3$  的芯片。根据筛选结果, 获得 Zhang 等<sup>[11]</sup>2019 年提交的 GSE117066 数据集: I/R 组通过夹闭左门静脉和中门静脉分支行 70% 肝脏缺血, 60 min 后重新恢复血供, 并再灌注 4 h 后处死小鼠。IPO 组通过 70% 肝脏缺血 60 min, 然后用连续 35 s 周期的再灌注治疗之后, 持续的再灌注 4 h。采集肝脏标本进行基因表达谱测序。GSE117066 数据集 (平台: GPL21103) 有 9 个样本: 3 个正常样本 (样本编号: GSM3269879、GSM3269880 和 GSM3269881), 3 个 I/R 样本 (样本编号: GSM3269882、GSM3269883 和 GSM3269884), 3 个 IPO 样本 (样本编号: GSM3269885、GSM3269886 和 GSM3269887)。获得 Huber 等<sup>[12]</sup>2008 年提交的 GSE10654 数据集: I/R 组通过 70% 肝脏缺血, 90 min 后重新恢复血供, 并再灌注 1 h 后处死小鼠。采集肝脏标本进行基因表达谱测序。GSE10654 数据集 (平台: GPL5759) 有 6 个样本: 3 个正常样本 (样本编号: GSM269536、GSM269537 和 GSM269538), 3 个 I/R 样本 (样本编号: GSM269560、GSM269561 和 GSM269562)。获得 Zheng 等<sup>[13]</sup>2015 年分析的 GSE72315 数据集差异 miRNA 结果 (PMID: 26859886)。

### 1.2 方法

**1.2.1 差异表达基因的筛选** 使用 GEO 数据库下载表达谱数据, 并使用 R 语言中的 limma 包进行数据分析。删去数据集中没有注释的探针, 并对一个基因的多个探针取最大值。使用 R 语言中的 ggplot2 包进行总基因散点图和火山图绘制。以  $|\logFC| > 1.0$ 、 $P < 0.05$  为筛选条件进行差异 mRNA 的筛选, 使用 R 语言中的 ggplot2 包进行差异 mRNA 热图绘制。

### 1.2.2 对差异 mRNA 进行 GO/KEGG 功能富集分



析 利用 Metascape 数据库对筛选出的差异 mRNA 进行 GO/KEGG 功能富集分析, 得到这些差异 mRNA 主要参与的生物学过程,  $P < 0.05$  为有统计学意义。

**1.2.3 对差异 mRNA 进行蛋白互作网络构建及分析** 使用 String 数据库及 Cytoscape 软件进行蛋白互作 (protein-protein interaction, PPI) 网络构建。首先使用 String 数据库 (<https://string-db.org/>) 对差异 mRNA 进行构建蛋白互作网络, 选取 combined score  $> 0.4$  的蛋白互作关系导入 Cytoscape 3.8.0 软件进行网络可视化。

**1.2.4 miRNA 及 lncRNA 的预测分析** 使用 miRTarBase 数据库 (<http://mirtarbase.cuhk.edu.cn/php/index.php>) 检索靶向关键差异基因的 miRNA。使用 starBase: ceRNA 数据库 (<http://starbase.sysu.edu.cn/ceRNA.php?source=mRNA>) 构建 ceRNA 网络。

**1.2.5 比较毒物基因组学数据库筛选关键差异基因的候选药物** 使用比较毒物基因组学数据库 (Comparative Toxicogenomics Database, CTD, <http://ctdbase.org/>) 筛选对关键差异基因表达具有潜在作用的药物。参数设定如下: Analyze 选择 Batch Query; input type 选择 Genes; Chemical-gene interactions 选择 expression。

## 2 结果

### 2.1 GEO 表达谱数据筛选差异表达基因

GSE10654 数据集共包括 14 685 个蛋白编码基因芯片数据, GSE117066 数据集共包括 10 976 个蛋白编码基因芯片数据。总基因散点图结果显示 I/R 组与假手术组相比, GSE10654 数据集中明显上调基因有 52 个, 下调基因有 62 个 ( $\log_2FC > 1.0$ ,  $P < 0.05$ ) (图 1A), GSE117066 数据集中明显上调基因有 162 个, 下调基因有 195 个 ( $\log_2FC > 1.0$ ,  $P < 0.05$ ) (图 1B)。进一步通过基因表达热图可直观显示出 GSE10654 与 GSE117066 数据集中差异表达基因的变化情况 (图 1C-D), 利于下一步的功能分析。为获得两个数据集中共同上调或下调基因, 通过 Venn 图分析 (图 2A), 获得 18 个在 I/R 组共同上调表达的基因, 分别是: Atf3、Btg2、Chka、Cxcl2、Dusp1、Dusp6、Fos、Fosb、G6pc、Hspa1a、Ier2、Ifrd1、Jun、Klf6、Nr4a1、Pdk4、RhoB 和 Slc25a25; 7 个在 I/R 组共同下调表达的基因, 分别是: Cyp2b10、Il1r1、Lyve1、Mt1、Mt2、Saa4 和 Scara5,

其变化趋势如图 2B 所示。

### 2.2 与 I/R 损伤相关的差异基因 PPI 网络可视化分析

为确定与假手术组相比, I/R 组差异表达基因的功能, 进一步分析了 GSE117066 数据集中 IPO 组与 I/R 组中基因的表达变化。总基因散点图结果显示 IPO 组与 I/R 组相比, GSE117066 数据集中显著上调基因有 283 个, 下调基因有 242 个 ( $\log_2FC > 1.0$ ,  $P < 0.05$ ) (图 3A)。通过基因表达热图可直观显示出 GSE117066 数据集中差异表达基因的变化情况 (图 3B)。通过 Venn 图分析 (图 3C), 获得 16 个在 I/R 组上调表达, 在 IPO 组下调表达的基因, 分别是: Atf3、Btg2、Chka、Dusp1、Dusp6、Fos、Fosb、G6pc、Hspa1a、Ier2、Ifrd1、Jun、Nr4a1、Pdk4、RhoB 和 Slc25a25; 7 个在 I/R 组下调表达, 在 IPO 组上调表达的基因, 分别是: Cyp2b10、Il1r1、Lyve1、Mt1、Mt2、Saa4 和 Scara5。进一步通过 String 数据库构建 PPI 网络, 该网络中共有 18 个差异表达基因, 分别是: Atf3、Btg2、Cyp2b10、Dusp1、Dusp6、Fos、Fosb、G6pc、Hspa1a、Ier2、Ifrd1、Il1r1、Jun、Mt1、Mt2、Nr4a1、Pdk4 和 RhoB (图 3D), 提示这些基因可能是 I/R 损伤中的关键基因。

### 2.3 与 I/R 损伤相关的差异基因功能富集分析

利用 Metascape 对 PPI 网络中的 18 个差异表达基因进行 GO/KEGG 功能富集分析, GO 功能富集分析结果显示, 在生物学过程 (biological processes) 方面, 主要参与细胞死亡的正调控及对细胞外刺激的反应。KEGG 通路富集结果显示: 差异基因主要参与 MAPK 信号通路。GO/KEGG 富集分析网络图如图 4A 所示。进一步转录调控网络数据库 (transcriptional regulatory relationships unraveled by sentence-based text mining, TRRUST) 分析可能调控这些差异基因的转录因子, 发现有 6 个转录因子参与对这些靶基因的调控, 分别是: Trp53、Cebpb、Crebbp、Fos、Nfkb1 及 SP1 (图 4B)。

### 2.4 筛选与 I/R 损伤相关的差异 miRNA

PPI 网络中的关键基因在体内的异常表达可能是肝脏 I/R 损伤发生发展的重要因素。基因的表达水平在真核生物体中主要受转录后调控, 而 miRNA 作为一类由内源基因编码的长度约为 22 个核苷酸的非编码单链 RNA 分子, 其可通过靶向 mRNA 的 3'UTR (非编码区) 区抑制 mRNA 翻译或

使其发生降解从而发挥转录后调控作用。miRTarBase 数据库作为一个专门收集有实验证据支持的 miRNA-mRNA 靶向关系的数据库，通过对 PPI 网络中的 18 个差异表达基因进行预测分析，获得与 I/R 损伤相关基因互作的 miRNA (图 5A)。为进一步分析这些 miRNA 在 I/R 损伤中的功能，分析了 GSE72315 数据集中 miRNA 在 I/R 损伤后的差异表

达，如图 5B 所示。结合构建的 miRNA 网络我们构建了在 I/R 损伤过程中差异表达的 miRNA-mRNA 网络，如图 5C 所示。基于 miRNA 对靶基因的负调控作用，发现两个 miRNA-mRNA 轴 (mmu-miR-32-5p-Btg2 与 mmu-miR-9-5p-Mt2) 可能在肝脏 I/R 损伤中发挥重要的作用。

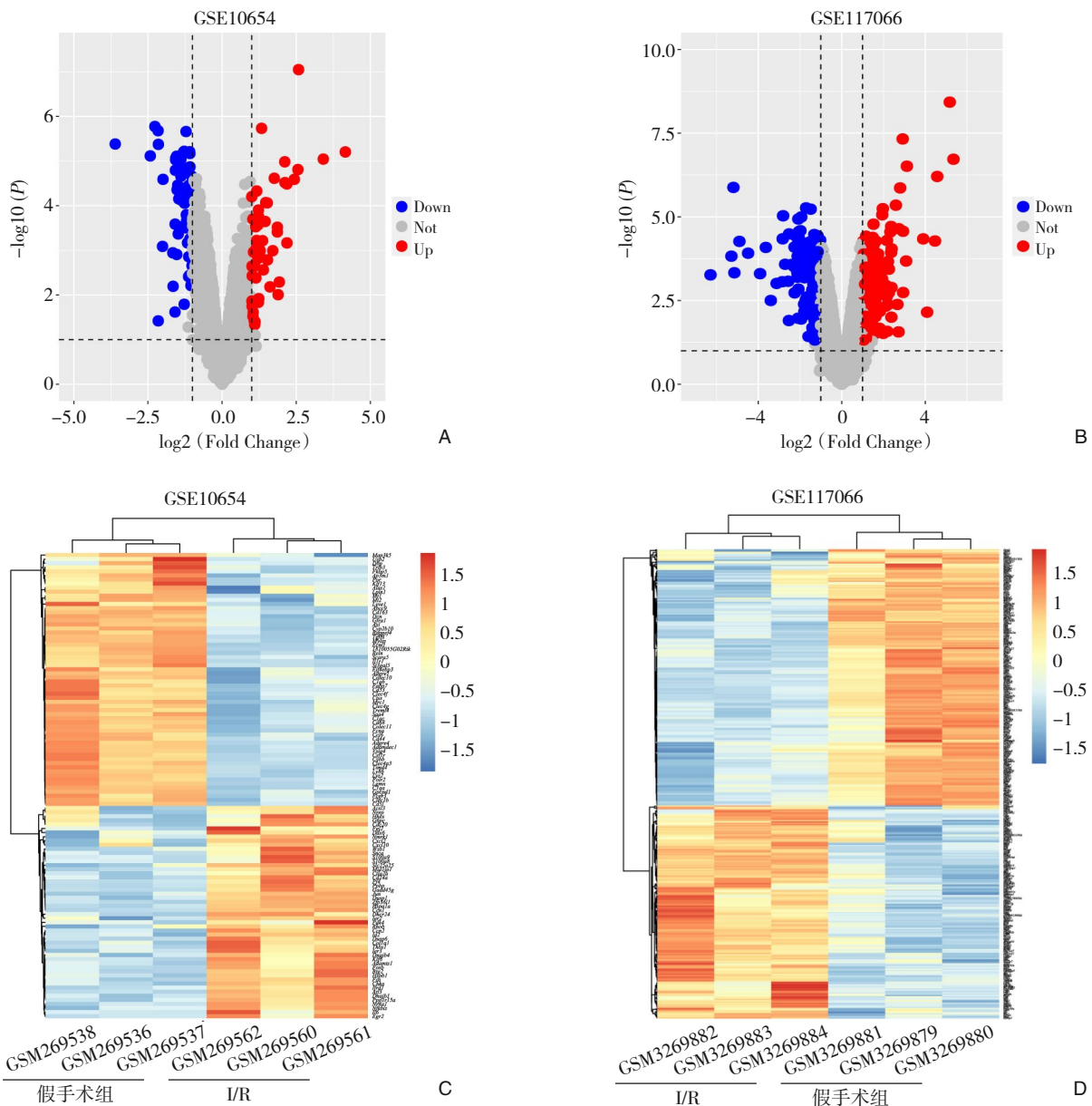


图1 GSE10654与GSE117066数据集基因表达情况 A-B: GSE10654与GSE117066数据集总基因散点图(红色表示基因上调,蓝色表示基因下调);C-D: GSE10654与GSE117066数据集总基因火山图

Figure 1 Gene expressions in GSE10654 and GSE117066 datasets A-B: Scatter plot of total genes in GSE10654 and GSE117066 datasets (red color standing for up-regulation of genes, blue color standing for down-regulation of genes); C-D: Total gene volcano map of GSE10654 and GSE117066 datasets

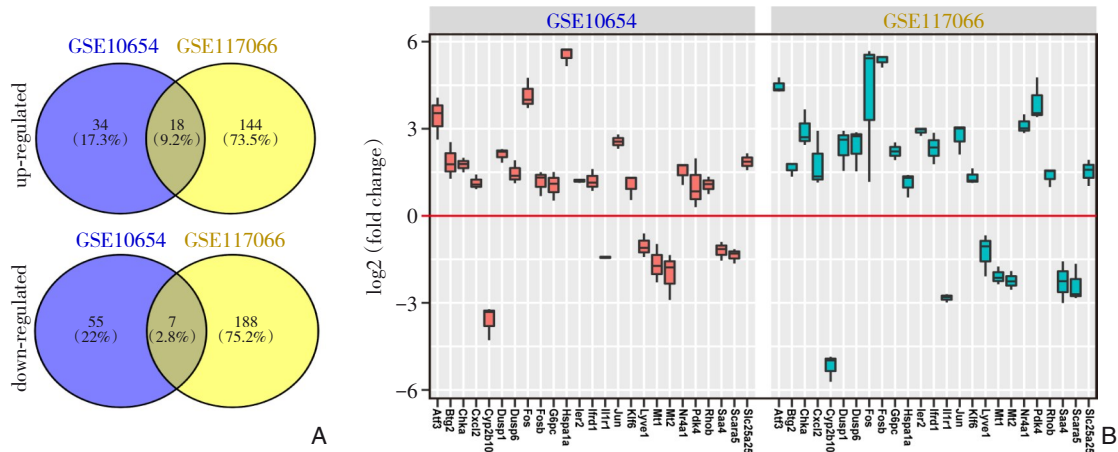


图 2 GSE10654 与 GSE117066 数据集差异基因的表达情况 A: GSE10654 与 GSE117066 上调或下调基因的 Venn 图分析; B: GSE10654 与 GSE117066 数据集共同上调或下调基因的表达情况

Figure 2 The expressions of differential genes between GSE10654 and GSE117066 datasets A: Venn diagram analysis of up-regulated or down-regulated genes between GSE10654 and GSE117066; B: The expression of co-up-regulated or co-down-regulated genes in the GSE10654 and GSE117066 datasets

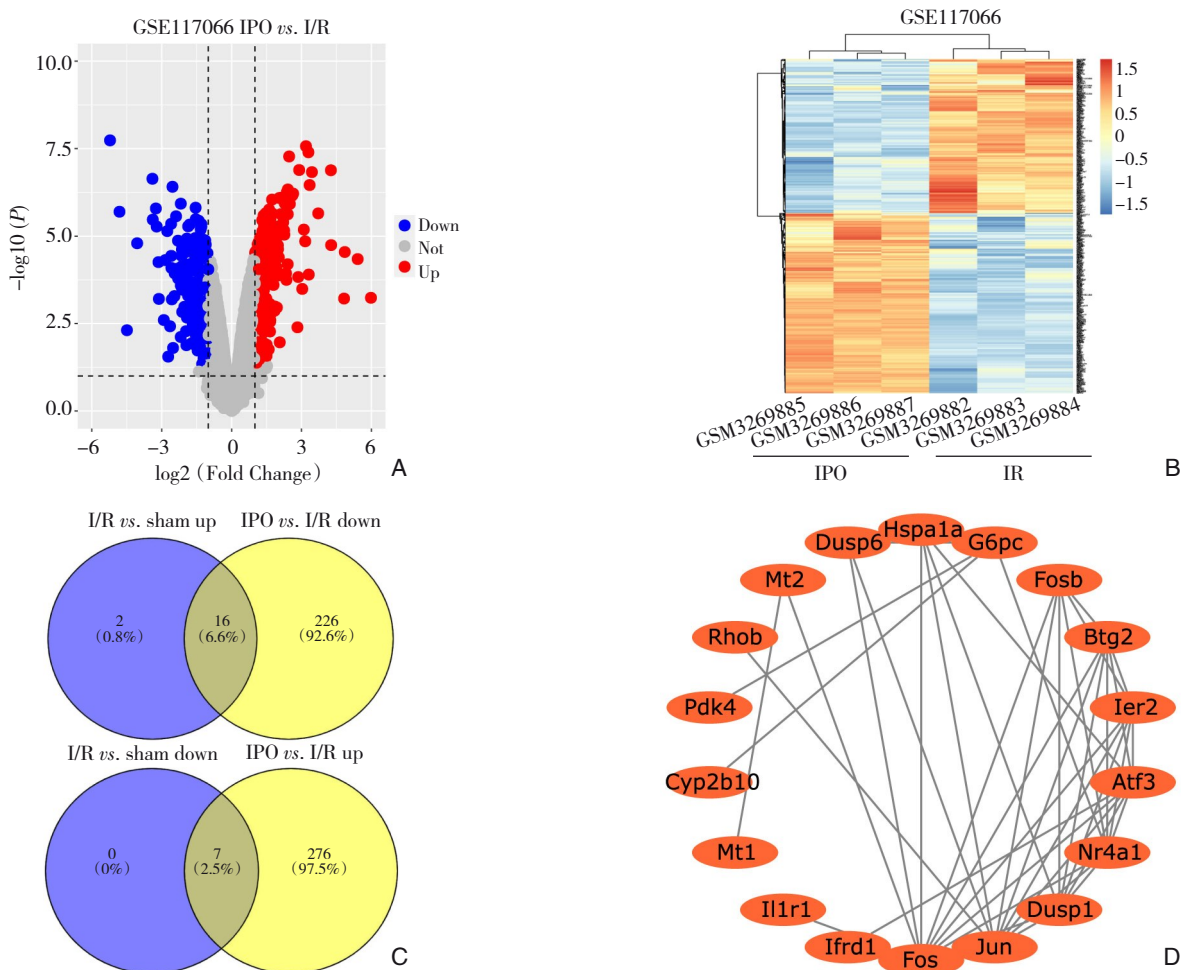


图 3 与 I/R 损伤相关的差异基因 PPI 网络可视化分析 A: GSE117066 数据集总基因散点图 (红色表示基因上调, 蓝色表示基因下调); B: GSE117066 数据集总基因火山图; C: Venn 图分析; D: PPI 网络可视化分析

Figure 3 Visualized analysis of PPI network of differential genes related to I/R injury A: Scatter plot of the total genes in the GSE117066 dataset (red color standing for up-regulation of genes, blue color standing for down-regulation of genes); B: Total gene volcano map of GSE117066 dataset; C: Venn diagram analysis; D: PPI network visualization analysis

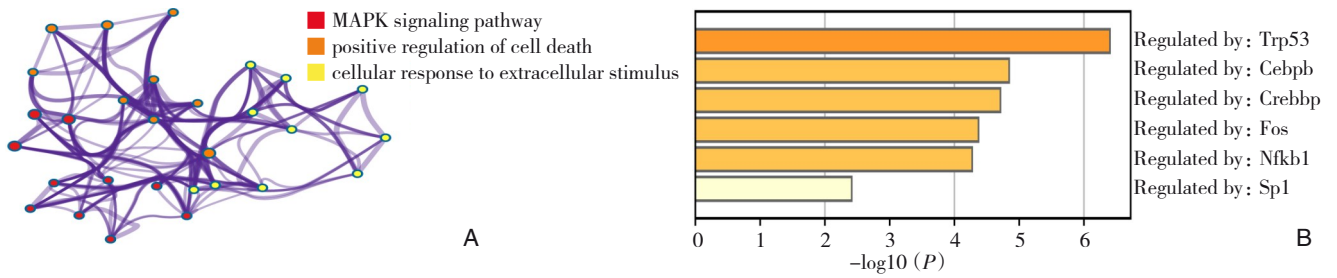


图4 与I/R损伤相关的差异基因功能富集分析 A: GO/KEGG功能富集分析; B: 转录因子富集分析

Figure 4 Function enrichment analysis of differentially expressed genes related to I/R injury A: GO/KEGG function enrichment analysis; B: Transcription factor enrichment analysis

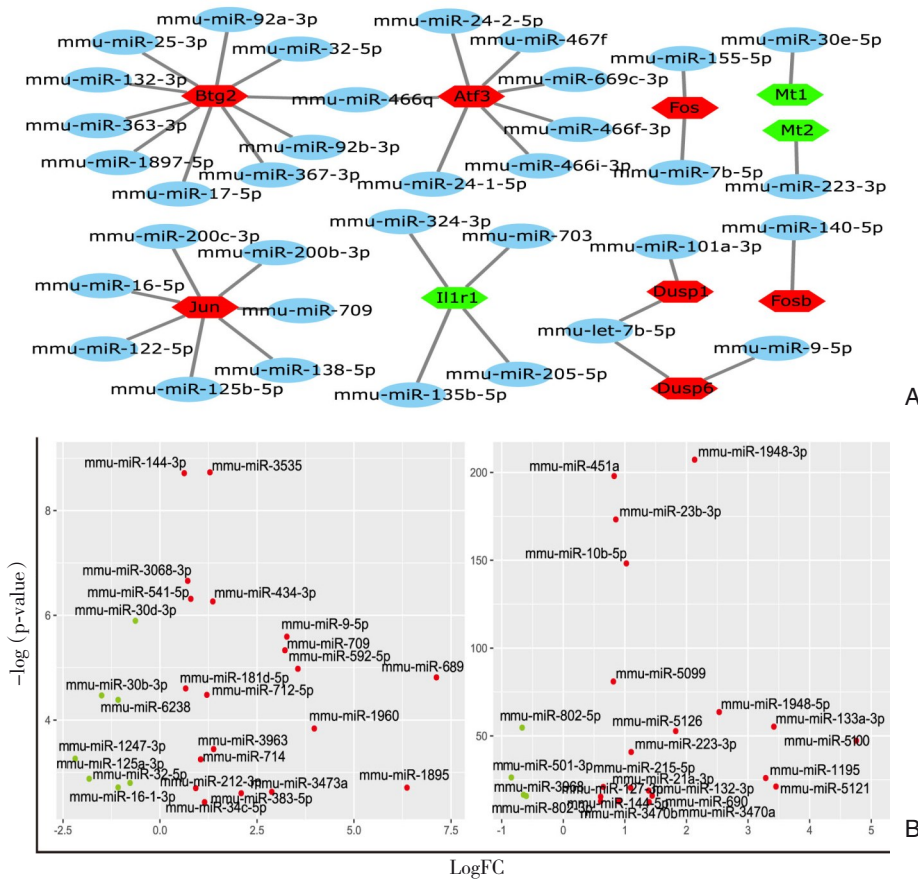


图5 与I/R损伤相关的差异miRNA A: 与差异基因结合的miRNA网络分析; B: miRNA在I/R组中的表达; C: 与I/R损伤相关miRNA网络

Figure 5 Differentially expressed miRNAs related to I/R injury A: Analysis of miRNA networks combined with differentially expressed genes; B: Expressions of miRNA in I/R group; C: The miRNA network associated with I/R injury



### 2.5 构建与I/R损伤相关的ceRNA网络

长链非编码RNA (lncRNA) 是长度>200 bp, 不编码蛋白质的内源性RNA分子。近年来的研究表明, lncRNA可以作为一种ceRNA吸附miRNA, 参与靶基因的表达调控。鉴于miR-132-3p、miR-223-3p、miR-32-5p、miR-790及miR-9-5p在人与小鼠中序列的保守性, 通过starBase: ceRNA数据库分析可调控PPI网络中的18个差异基因表达的ceRNA网络, 获得9条ceRNA网络, 分别是: XIST/MEG8/LINC00963/MALAT1-miR-32-5p-Btg2、XIST/NEAT1-miR-132-3p-Btg2、HSPA9P1/RALGAPA1P1/RPS26P39-miR-9-5p-Dusp6 (图6)。

### 2.6 肝脏I/R损伤的潜在治疗药物筛选

在比较毒物基因组学数据库 (comparative toxicogenomics database, CTD) 中检索能够减少PPI网络中关键基因表达的天然植物药, 并构建药物分子和靶点之间网络关系, 共获得7种天然药物分别是槲皮素 (quercetin, ID: D011794)、白藜芦醇 (resveratrol, ID: D000077185)、染料木黄酮 (genistein, ID: D019833)、香豆雌酚 (coumestrol, ID: D003375)、姜黄素 (curcumin, ID: D003474)、辣椒素 (capsaicin, ID: D002211) 及东莨菪碱 (scopolamine, ID: D012601) (图7)。

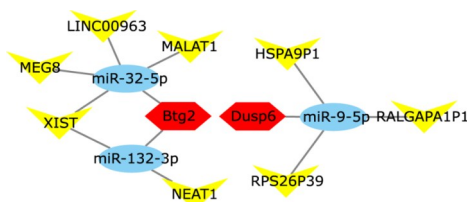


图6 与I/R损伤相关的ceRNA网络

Figure 6 The ceRNA network associated with I/R injury

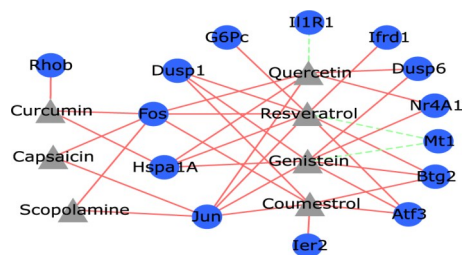


图7 与I/R损伤治疗相关的药物-靶点网络 (▲表示药物, ○表示靶点, 红色直线表示下调表达, 绿色虚线表示上调表达)

Figure 7 Drug-target network related to I/R injury treatment (▲ standing for drug, ○ standing for target, the red straight line standing for the down-regulated expression, and the green dashed line standing for up-regulated expression)

## 3 讨论

肝脏I/R损伤是多因素共同导致的复杂病理生理过程, 可引起肝功能损害甚至衰竭, 影响肝移植和肝切除等肝脏手术的预后<sup>[14]</sup>。ceRNA作为一种调控机制, 可参与多种疾病的进展, 具有重要的生物学意义。lncRNA和circRNA作为常见的参与ceRNA调控的RNA分子, 在肝脏I/R损伤中的功能越来越受到重视。目前基于circRNA的ceRNA网络已有研究, Zhang等<sup>[15]</sup>的研究首次表明circRNA与肝脏I/R损伤密切相关, 并发现靶向mmu\_circRNA\_005186-miR-124-3p-Epha2通路可能减轻肝脏I/R损伤。然而基于lncRNA的ceRNA网络在肝脏I/R损伤的研究还鲜有报道, 因此应用生物信息学方法从基因表达数据库中挖掘差异基因, 构建基于lncRNA的ceRNA调控网络, 探索肝脏I/R损伤的发生机制, 同时基于这些差异基因从药物数据库筛选出潜在的治疗药物具有重要意义。

目前报道的肝脏I/R损伤发生机制包括: 无氧代谢、钙离子超载、氧化应激反应、线粒体结构功能的损坏、库普细胞激活和中性粒细胞的活化聚集、细胞因子作用、细胞凋亡与细胞自噬等<sup>[16-17]</sup>。在本研究中通过对差异基因的GO/KEGG功能富集分析发现, 差异基因主要参与细胞死亡的正调控及对细胞外刺激反应的生物学过程, 并参与MAPK信号通路的调控。这与目前的Zhang等<sup>[11]</sup>和Amersi等<sup>[18]</sup>的研究报道是一致的。ceRNA调控网络作为一种精细而又复杂的基因表达调控模式, 可参与多种疾病的发生发展。本研究最终获得9条ceRNA网络, 分别是: XIST/MEG8/LINC00963/MALAT1-miR-32-5p-Btg2、XIST/NEAT1-miR-132-3p-Btg2、HSPA9P1/RALGAPA1P1/RPS26P39-miR-9-5p-Dusp6。研究表明, MALAT1及NEAT1可能通过调节细胞凋亡和炎症而加重肝脏I/R损伤<sup>[19-20]</sup>。miR-9-5p通过下调N-κB亚基p65的表达, 保护肝脏免受I/R损伤<sup>[21]</sup>。Zhang等<sup>[11]</sup>的实验数据表明, 与对照组相比, Dusp6在I/R组显著上调, 在IPO组显著下调。然而鉴定出的ceRNA网络在肝脏I/R中的功能仍需进一步的研究。

ceRNA调控网络可在转录后水平实现对肝脏I/R损伤关键mRNA的调控, 但这些关键mRNA的表达同样受转录水平的调控。转录因子作为一类DNA结合蛋白, 可与基因启动子结合, 实现对靶基因的调控。在本研究中, 通过TRRUST转录调控网络



数据库分析获得6个与肝脏I/R损伤相关的转录因子,分别是:Trp53、Cebpb、Crebbp、Fos、Nfkb1及SP1。其中Trp53也称为p53,是一种重要的肿瘤抑制基因,其介导的细胞信号转导途径在调节细胞正常生命活动中起重要作用。Fos蛋白作为一类核蛋白转录因子,在调控细胞生长、分裂、增殖、分化乃至程序性死亡等方面具有重要的作用。Nfkb1也称为p50,与RelA(p65)形成p50/RelA二聚体,参与NF- $\kappa$ B经典信号通路的激活。在肝脏I/R损伤中的研究表明,Trp53、Fos及Nfkb1可调控细胞凋亡、自噬及炎症等参与肝脏I/R损伤<sup>[22-24]</sup>。转录因子Sp1在肿瘤组织中的异常表达和活化可参与调控肿瘤的增殖、血管生成和转移潜能,Cebpb(作为一种含有亮氨酸拉链结构域的转录因子)与Crebbp(CREB结合蛋白)在血液系统肿瘤中发挥重要作用,但它们在肝脏I/R损伤中功能仍有待研究。

天然药物作为一种自然界中存在的有药理活性的天然产物,一般地讲,其副作用比人工合成的化学药物要小的多,但也有些天然药物毒性较大。目前,多种天然药物及化学药物已报道可显著改善肝脏I/R损伤<sup>[25-26]</sup>。如表1所示,目前已报道有26种天然药物可显著改善肝脏I/R损伤<sup>[27-53]</sup>。同时如表2所示,有22种化学药物被报道可减轻肝脏I/R损伤<sup>[54-78]</sup>。两种药物的作用机制相似,主要通过抑制炎症反应、氧化应激和细胞凋亡等发挥作用。为进一步获得潜在的可改善肝脏I/R损伤

的天然药物,本研究利用CTD数据库分析筛选出7种天然药物(槲皮素、白藜芦醇、染料木黄酮、香豆雌酚、姜黄素、辣椒素及东莨菪碱)可能对肝脏I/R损伤具有一定治疗作用。其中槲皮素、白藜芦醇、染料木黄酮及姜黄素对于肝脏I/R损伤的治疗作用已有证实。Wu等<sup>[42]</sup>的研究表明,槲皮素预处理通过抑制ERK/NF- $\kappa$ B途径减轻肝I/R诱导的细胞凋亡和自噬;He等<sup>[48]</sup>的研究发现,白藜芦醇可通过抑制TLR4/NF- $\kappa$ B通路减弱炎症反应和细胞凋亡,从而改善肝脏I/R损伤;Akinci等<sup>[53]</sup>证明,染料木黄酮通过减少炎症因子TNF- $\alpha$ 及IL-6的表达保护肝脏I/R损伤;Wang等<sup>[37]</sup>的研究表明,姜黄素通过抑制TLR4/NF- $\kappa$ B途径来预防肝I/R损伤。香豆雌酚、辣椒素及东莨菪碱已报道对脑I/R损伤具有保护作用。Castro等<sup>[79]</sup>的研究认为,香豆雌醇发挥神经保护作用的潜在机制可能与雌激素受体激活、抗氧化活性和其它介导雌二醇神经保护作用的膜受体的激活有关。Huang等<sup>[80]</sup>发现辣椒素对皮质神经元的神经保护作用是TRPV1依赖性的,NMDA受体表达和功能的下调有助于辣椒素提供的保护作用。Wang等<sup>[81]</sup>的研究表明东莨菪碱作为一种治疗心脏骤停的药物,通过减轻脑组织水肿和炎症反应,对心肺复苏后急性脑I/R损伤具有保护作用。但香豆雌酚、辣椒素及东莨菪碱在肝脏I/R损伤的作用暂无报道,这为今后肝脏I/R损伤的治疗提供新的潜在治疗药物。

表1 改善肝脏I/R损伤的天然药物汇总

Table 1 Summary of natural medicines to improve liver I/R injury

文献	天然药物	中文名	作用机制
Jang, 等 <sup>[27]</sup>	eupatilin	异泽兰黄素	减少炎症和细胞凋亡
Pan, 等 <sup>[28]</sup>	alpinetin	山姜素	抑制NF- $\kappa$ B/MAPK通路
Zhang, 等 <sup>[29]</sup>	aucubin	桃叶珊瑚苷	抑制HMGB1/TLR-4/NF- $\kappa$ B通路,抑制氧化应激和细胞凋亡
Lin, 等 <sup>[30]</sup>	ginsenoside rg1	人参皂苷Rg1	抑制细胞凋亡
Du, 等 <sup>[31]</sup>	aloin	芦荟苷	抑制TLR4/MyD88/NF- $\kappa$ B通路
Park, 等 <sup>[32]</sup>	hesperidin	橘皮苷	抑制超氧化物和羟基自由基
Dusabimana, 等 <sup>[33]</sup>	nobiletin	川陈皮素	激活细胞自噬和线粒体生物发生
Hua, 等 <sup>[34]</sup>	glycyrrhizin	甘草酸	通过抑制库普弗细胞焦亡
Zaki, 等 <sup>[35]</sup>	plumbagin	白花丹素	抑制了炎症反应和细胞凋亡
Lu, 等 <sup>[36]</sup>	asiatic acid	积雪草酸	抑制氧化应激和恢复线粒体功能
Wang, 等 <sup>[37]</sup>	curcumin	姜黄素	抑制TLR4/NF- $\kappa$ B通路
Atef, 等 <sup>[38]</sup>	cardamonin	小豆蔻素	改善氧化应激和炎症
Li, 等 <sup>[39]</sup>	fucoidan	岩藻多糖	激活的JAK2/STAT1通路减弱凋亡和自噬

表1 改善肝脏I/R损伤的天然药物汇总(续)

Table 1 Summary of natural medicines to improve liver I/R injury (continued)

文献	天然药物	中文名	作用机制
Feng, 等 <sup>[40]</sup> Cai, 等 <sup>[41]</sup>	salidroside	红景天苷	抑制 MAPK 通路, 激活 GSK-3B/NRF2 改善炎症反应和细胞凋亡
Wu, 等 <sup>[42]</sup>	quercetin	槲皮素	抑制 ERK/NF- $\kappa$ B, 抑制细胞凋亡和自噬
Akbari, 等 <sup>[43]</sup>	crocin	藏红花素	增加抗氧化酶的活性, 提高血清肝酶水平
Zheng, 等 <sup>[44]</sup>	schisantherin a	五味子酯甲	抑制 MAPK 通路减弱炎症反应和细胞凋亡
Wu, 等 <sup>[45]</sup>	nobiletin	川皮苷	抑制库普弗细胞炎症反应和 TLR4/NF- $\kappa$ B 通路活性
Lin, 等 <sup>[46]</sup>	berberine	黄连素	激活 Sirt1/FoxO3 $\alpha$ 诱导的细胞自噬
Tsaroucha, 等 <sup>[47]</sup>	apigenin	芹菜素	抑制细胞凋亡
He, 等 <sup>[48]</sup>	resveratrol	白藜芦醇	抑制 TLR4/NF- $\kappa$ B 通路减弱炎症反应和细胞凋亡
Chi, 等 <sup>[49]</sup>	sulforaphane	萝卜硫素	激活 NRF2/ARE 通路减弱细胞凋亡和氧化应激
Sun, 等 <sup>[50]</sup>	cryptotanshinone	隐丹参酮	抑制 JNK 与 MAPK 通路, 抑制细胞凋亡
Mahmoud, 等 <sup>[51]</sup>	limonin	柠檬苦素	抑制 TLR 通路减弱氧化反应和炎症反应
Zhang, 等 <sup>[52]</sup>	matrine	苦参碱	抑制炎症和氧化应激反应
Akinci, 等 <sup>[53]</sup>	genistein	染料木黄酮	减少炎症因子的表达

表2 改善肝脏I/R损伤的化学药物汇总

Table 2 Summary of chemical drugs to improve liver I/R injury

文献	化学药物	中文名	生物学功能
Zhang, 等 <sup>[54]</sup>	fenofibrate	非诺贝特	减轻炎症反应并抑制细胞凋亡和自噬
Ma, 等 <sup>[55]</sup>	propofol	丙泊酚	抑制 Bnip3 介导的氧化应激
Đurašević, 等 <sup>[56]</sup>	meldonium	米屈肼	抑制氧化应激和炎症反应
Xiong, 等 <sup>[57]</sup>	mycophenolate mofetil	霉酚酸酯	诱导自噬, 减少细胞凋亡和炎症反应
Liu, 等 <sup>[58]</sup>			抑制活性氧的产生和改善微循环障碍
Zhou, 等 <sup>[59]</sup>	remifentanyl	瑞芬太尼	上调 HIF1 $\alpha$ 表达抑制细胞凋亡
Liu, 等 <sup>[60]</sup>			抑制白细胞介素 18 介导的信号传导
Wang, 等 <sup>[61]</sup>	suberoylanilide hydroxamic acid	伏立诺他	抑制 AKT/GSK3 $\beta$ /NF- $\kappa$ B 通路来减少库普弗细胞的 M1 极化, 抑制 AKT/mTOR 通路促进库普弗细胞自噬
Kamel, 等 <sup>[62]</sup>	perindopril	培哚普利	减少氧化应激并改善炎症反应
Liu, 等 <sup>[63]</sup>	spermidine	亚精胺	抑制 AMPK-mTOR-ULK1 通路上调自噬
Sherif, 等 <sup>[64]</sup>	vildagliptin	维达列汀	下调 TLR4/NF- $\kappa$ B/HMGB1 和 caspase-3 表达
Liu, 等 <sup>[65-66]</sup>	simvastatin	辛伐他汀	上调 KLF2 及其保护性靶基因(eNOS、TM 和 HO-1)的表达, 减少氧化应激, 抑制炎症反应, 抑制肝细胞凋亡
Li, 等 <sup>[67]</sup>	ketamine	氯胺酮	抑制库普弗细胞活化, 降低肝细胞凋亡
Shin, 等 <sup>[68]</sup>	genipin	京尼平	缓解肝细胞氧化损伤及线粒体功能障碍
Wang, 等 <sup>[69]</sup>	dexmedetomidine	右旋美托咪啶	抑制 TLR4/NF- $\kappa$ B 通路, 降低炎症反应
Lee, 等 <sup>[70]</sup>	everolimus	依维莫司	激活肝细胞自噬
Shen, 等 <sup>[71]</sup>	ethyl pyruvate	丙酮酸乙酯	下调 HMGB1/TLR4/NF- $\kappa$ B 轴, 抑制细胞凋亡和自噬
Koh, 等 <sup>[72]</sup>	losartan	氯沙坦	激活 PPAR- $\gamma$ , 下调晚期糖基化终产物
Tüfek, 等 <sup>[73]</sup>	dexmedetomidine	右美托咪啶	降低氧化应激反应
Fu, 等 <sup>[74]</sup>	flurbiprofen	氟比洛芬	抑制 GSK-3 $\beta$ 信号传导和线粒体通透性
Ocuin, 等 <sup>[75]</sup>	nilotinib	尼罗替尼	抑制肝细胞 JNK 和非实质细胞 MAPK
Dai, 等 <sup>[76]</sup>	tetrahydrobiopterin	四氢生物蝶呤	减弱活性氧和增加 NO 合成
Fondevila, 等 <sup>[77]</sup>	biliverdin	胆绿素	促进抗凋亡分子的表达
Settaf, 等 <sup>[78]</sup>	trimetazidine	曲美他嗪	降低 ASAT 和 ALAT 活性, 上调 ATP 含量和胆汁流量

综上所述,本研究通过生物信息学方法构建肝脏 I/R 损伤过程中的关键 ceRNA 网络,并筛选潜在治疗药物。同时本研究也具有一定的局限性,主要基于鼠类肝脏缺血再灌注的相关数据进行挖掘,与人类基因的相关表达可能有较大差异,同时获得的 ceRNA 网络及潜在天然药物缺乏临床数据及实验数据的验证。但本研究可对未来进一步深入了解肝脏 I/R 损伤的分子机制提供重要指导作用,并对未来临床治疗提供更多依据。

### 参考文献

- [1] Soares ROS, Losada DM, Jordani MC, et al. Ischemia/reperfusion injury revisited: an overview of the latest pharmacological strategies[J]. *Int J Mol Sci*, 2019, 20(20): 5034. doi: 10.3390/ijms20205034.
- [2] Cannistrà M, Ruggiero M, Zullo A, et al. Hepatic ischemia reperfusion injury: A systematic review of literature and the role of current drugs and biomarkers[J]. *Int J Surg*, 2016, 33(Suppl 1):S57-70. doi: 10.1016/j.ijssu.2016.05.050.
- [3] van Riel WG, van Golen RF, Reiniers MJ, et al. How much ischemia can the liver tolerate during resection?[J]. *Hepatobiliary Surg Nutr*, 2016, 5(1): 58-71. doi: 10.3978/j.issn.2304-3881.2015.07.05.
- [4] Smyrniotis VE, Kostopanagiotou GG, Contis JC, et al. Selective hepatic vascular exclusion versus Pringle maneuver in major liver resections: prospective study[J]. *World J Surg*, 2003, 27(7): 765-769. doi: 10.1007/s00268-003-6978-8.
- [5] Abu-Amara M, Yang SY, Tapuria N, et al. Liver ischemia/reperfusion injury: processes in inflammatory networks--a review[J]. *Liver Transpl*, 2010, 16(9): 1016-1032. doi: 10.1002/lt.22117.
- [6] 周艳,陈超,杨竹林,等.长链非编码RNA在肝细胞性肝癌中的作用[J].*中国普通外科杂志*, 2021, 30(3):349-356. doi:10.7659/j.issn.1005-6947.2021.03.014.  
Zhou Y, Chen C, Yang ZL, et al. The role of long non-coding RNAs in hepatocellular carcinoma[J]. *Chinese Journal of General Surgery*, 2021, 30(3): 349-356. doi: 10.7659/j.issn.1005-6947.2021.03.014.
- [7] Ying D, Zhou X, Ruan Y, et al. LncRNA Gm4419 induces cell apoptosis in hepatic ischemia - reperfusion injury via regulating the miR-455-SOX6 axis[J]. *Biochem Cell Biol*, 2020, 98(4):474-483. doi: 10.1139/bcb-2019-0331.
- [8] Dai B, Qiao L, Zhang M, et al. lncRNA AK054386 functions as a ceRNA to sequester miR-199 and induce sustained endoplasmic reticulum stress in hepatic reperfusion injury[J]. *Oxid Med Cell Longev*, 2019, 2019:8189079. doi: 10.1155/2019/8189079.
- [9] Tang B, Bao N, He G, et al. Long noncoding RNA HOTAIR regulates autophagy via the miR-20b-5p/ATG7 axis in hepatic ischemia/reperfusion injury[J]. *Gene*, 2019, 686: 56-62. doi: 10.1016/j.gene.2018.10.059.
- [10] Huang X, Gao Y, Qin J, et al. The mechanism of long non-coding RNA MEG3 for hepatic ischemia-reperfusion: Mediated by miR-34a/Nrf2 signaling pathway[J]. *J Cell Biochem*, 2018, 119(1):1163-1172. doi: 10.1002/jcb.26286.
- [11] Zhang P, Ming Y, Cheng K, et al. Gene Expression profiling in ischemic postconditioning to alleviate mouse liver ischemia/reperfusion injury[J]. *Int J Med Sci*, 2019, 16(2): 343-354. doi: 10.7150/ijms.29393.
- [12] Huber N, Sakai N, Eismann T, et al. Age-related decrease in proteasome expression contributes to defective nuclear factor- $\kappa$ B activation during hepatic ischemia/reperfusion[J]. *Hepatology*, 2009, 49(5):1718-1728. doi: 10.1002/hep.22840.
- [13] Zheng W, Men H, Li J, et al. Global microRNA expression profiling of mouse livers following ischemia-reperfusion injury at different stages[J]. *PLoS One*, 2016, 11(2):e0148677. doi: 10.1371/journal.pone.0148677.
- [14] Nakazato PCG, Victorino JP, Fina CF, et al. Liver ischemia and reperfusion injury. Pathophysiology and new horizons in preconditioning and therapy[J]. *Acta Cir Bras*, 2018, 33(8): 723-735. doi: 10.1590/s0102-865020180080000008.
- [15] Zhang P, Ming Y, Ye Q, et al. Comprehensive circRNA expression profile during ischemic postconditioning attenuating hepatic ischemia/reperfusion injury[J]. *Sci Rep*, 2019, 9(1): 264. doi: 10.1038/s41598-018-36443-8.
- [16] Guan LY, Fu PY, Li PD, et al. Mechanisms of hepatic ischemia-reperfusion injury and protective effects of nitric oxide[J]. *World J Gastrointest Surg*, 2014, 6(7): 122-128. doi: 10.4240/wjgs. v6. i7.122.
- [17] Go KL, Lee S, Zendejas I, et al. Mitochondrial Dysfunction and Autophagy in Hepatic Ischemia/Reperfusion Injury[J]. *Biomed Res Int*, 2015, 2015:183469. doi: 10.1155/2015/183469.
- [18] Amersi F, Shen XD, Anselmo D, et al. Ex vivo exposure to carbon monoxide prevents hepatic ischemia/reperfusion injury through p38 MAP kinase pathway[J]. *Hepatology*, 2002, 35(4): 815-823. doi: 10.1053/jhep.2002.32467.
- [19] Zhang Y, Zhang H, Zhang Z, et al. LncRNA MALAT1 cessation antagonizes hypoxia/reoxygenation injury in hepatocytes by inhibiting apoptosis and inflammation via the HMGB1-TLR4 axis[J]. *Mol Immunol*, 2019, 112: 22-29. doi: 10.1016/j.molimm. 2019. 04.015.



- [20] Wang L, Qu P, Yin W, et al. Lnc-NEAT1 induces cell apoptosis and inflammation but inhibits proliferation in a cellular model of hepatic ischemia/reperfusion injury[J]. *J Int Med Res*, 2021, 49(3): 300060519887251. doi: 10.1177/0300060519887251.
- [21] Liao X, Zhou S, Zong J, et al. Sevoflurane exerts protective effects on liver ischemia/reperfusion injury by regulating NFKB3 expression via miR-9-5p[J]. *Exp Ther Med*, 2019, 17(4): 2632–2640. doi: 10.3892/etm.2019.7272.
- [22] Wu H, Bai H, Duan S, et al. Downregulating Serine Hydroxymethyltransferase 2 Deteriorates Hepatic Ischemia-Reperfusion Injury through ROS/JNK/P53 Signaling in Mice[J]. *Biomed Res Int*, 2019, 2019:2712185. doi: 10.1155/2019/2712185.
- [23] Zabala V, Boylan J M, Thevenot P, et al. Transcriptional changes during hepatic ischemia-reperfusion in the rat[J]. *PLoS One*, 2019, 14(12):e0227038. doi: 10.1371/journal.pone.0227038.
- [24] Zhu J, Zhu F, Song W, et al. Altered miR-370 expression in hepatic ischemia-reperfusion injury correlates with the level of nuclear kappa B (NF- $\kappa$ B) related factors[J]. *Gene*, 2017, 607:23–30. doi: 10.1016/j.gene.2016.12.026.
- [25] 高伟东, 冯赞杰, 彭慈军, 等. 杜仲不同部位及不同提取方法的提取物对大鼠肝脏缺血再灌注损伤的保护作用[J]. *中国普通外科杂志*, 2020, 29(8):958–965. doi: 10.7659/j.issn.1005–6947.2020.08.006.
- Gao WD, Feng ZJ, Peng CJ, et al. Protective effects of extracts from different parts of *Eucommia ulmoides* and by different extraction methods against hepatic ischemia-reperfusion injury in rats[J]. *Chinese Journal of General Surgery*, 2020, 29(8):958–965. doi:10.7659/j.issn.1005–6947.2020.08.006.
- [26] 白宁, 王栋, 欧阳锡武, 等. 苦参碱对大鼠肝缺血再灌注损伤的抑制作用及机制[J]. *中国普通外科杂志*, 2018, 27(1):81–86. doi: 10.3978/j.issn.1005–6947.2018.01.013.
- Bai N, Wang D, Ouyang XW, et al. Inhibitory effect of matrine against hepatic ischemia-reperfusion injury in rats and its mechanism[J]. *Chinese Journal of General Surgery*, 2018, 27(1):81–86. doi:10.3978/j.issn.1005–6947.2018.01.013.
- [27] Jang HJ, Oh MY. Eupatilin pretreatment ameliorates hepatic ischemia-reperfusion injury in mice[J]. *Ann Hepatobiliary Pancreat Surg*, 2021, 25(Suppl 1):S179. doi: 10.14701/ahbps.LV-PP-1–4.
- [28] Pan J, Chen S, Guo W, et al. Alpinetin protects against hepatic ischemia/reperfusion injury in mice by inhibiting the NF- $\kappa$ B/MAPK signaling pathways[J]. *Int Immunopharmacol*, 2021, 95: 107527. doi: 10.1016/j.intimp.2021.107527.
- [29] Zhang S, Feng Z, Gao W, et al. Aucubin attenuates liver ischemia-reperfusion injury by inhibiting the HMGB1/TLR-4/NF- $\kappa$ B signaling pathway, oxidative stress, and apoptosis[J]. *Front Pharmacol*, 2020, 11:544124. doi: 10.3389/fphar.2020.544124.
- [30] Lin J, Huang H, Yang S, et al. The effect of Ginsenoside Rg1 in hepatic ischemia reperfusion (I/R) injury ameliorates ischemia-reperfusion-induced liver injury by inhibiting apoptosis[J]. *Biomed Pharmacother*, 2020, 129: 110398. doi: 10.1016/j.biopha.2020.110398.
- [31] Du Y, Qian B, Gao L, et al. Aloin preconditioning attenuates hepatic ischemia/reperfusion injury via inhibiting TLR4/MyD88/NF- $\kappa$ B signal pathway in vivo and in vitro[J]. *Oxid Med Cell Longev*, 2019, 2019:3765898. doi: 10.1155/2019/3765898.
- [32] Park HK, Kang SW, Park MS. Hesperidin ameliorates hepatic ischemia-reperfusion injury in Sprague-Dawley rats[J]. *Transplant Proc*, 2019, 51(8):2828–2832. doi: 10.1016/j.transproceed.2019.02.059.
- [33] Dusabimana T, Kim SR, Kim HJ, et al. Nobiletin ameliorates hepatic ischemia and reperfusion injury through the activation of SIRT-1/FOXO3a-mediated autophagy and mitochondrial biogenesis [J]. *Exp Mol Med*, 2019, 51(4):1–16. doi: 10.1038/s12276–019–0245–z.
- [34] Hua S, Ma M, Fei X, et al. Glycyrrhizin attenuates hepatic ischemia-reperfusion injury by suppressing HMGB1-dependent GSDMD-mediated kupffer cells pyroptosis[J]. *Int Immunopharmacol*, 2019, 68:145–155. doi: 10.1016/j.intimp.2019.01.002.
- [35] Zaki AM, El-Tanbouly DM, Abdelsalam RM, et al. Plumbagin ameliorates hepatic ischemia-reperfusion injury in rats: role of high mobility group box 1 in inflammation, oxidative stress and apoptosis[J]. *Biomed Pharmacother*, 2018, 106: 785–793. doi: 10.1016/j.biopha.2018.07.004..
- [36] Lu Y, Kan H, Wang Y, et al. Asiatic acid ameliorates hepatic ischemia/reperfusion injury in rats via mitochondria-targeted protective mechanism[J]. *Toxicol Appl Pharmacol*, 2018, 338:214–223. doi: 10.1016/j.taap.2017.11.023.
- [37] Wang L, Li N, Lin D, et al. Curcumin protects against hepatic ischemia/reperfusion induced injury through inhibiting TLR4/NF- $\kappa$ B pathway[J]. *Oncotarget*, 2017, 8(39): 65414. doi: 10.18632/oncotarget.18676.
- [38] Atef Y, El-Fayoumi HM, Abdel-Mottaleb Y, et al. Effect of cardamonin on hepatic ischemia reperfusion induced in rats: role of nitric oxide[J]. *Eur J Pharmacol*, 2017, 815:446–453. doi: 10.1016/j.ejphar.2017.09.037.
- [39] Li J, Zhang QH, Li S, et al. The natural product fucoidan ameliorates hepatic ischemia-reperfusion injury in mice[J]. *Biomed Pharmacother*, 2017, 94: 687–696. doi: 10.1016/j.biopha.2017.07.109.
- [40] Feng J, Zhang Q, Mo W, et al. Salidroside pretreatment attenuates apoptosis and autophagy during hepatic ischemia-reperfusion

- injury by inhibiting the mitogen-activated protein kinase pathway in mice[J]. *Drug Des Devel Ther*, 2017, 11: 1989–2006. doi: 10.2147/DDDT.S136792.
- [41] Cai L, Li Y, Zhang Q, et al. Salidroside protects rat liver against ischemia/reperfusion injury by regulating the GSK-3 $\beta$ /Nrf2-dependent antioxidant response and mitochondrial permeability transition[J]. *Eur J Pharmacol*, 2017, 806: 32–42. doi: 10.1016/j.ejphar.2017.04.011.
- [42] Wu L, Zhang Q, Dai W, et al. Quercetin pretreatment attenuates hepatic ischemia reperfusion-induced apoptosis and autophagy by inhibiting ERK/NF- $\kappa$ B pathway[J]. *Gastroenterol Res Pract*, 2017, 2017:9724217. doi: 10.1155/2017/9724217.
- [43] Akbari G, Mard SA, Dianat M, et al. The hepatoprotective and microRNAs downregulatory effects of crocin following hepatic ischemia-reperfusion injury in rats[J]. *Oxid Med Cell Longev*, 2017, 2017:1702967. doi: 10.1155/2017/1702967.
- [44] Zheng N, Liu F, Lu H, et al. Schisantherin A protects against liver ischemia-reperfusion injury via inhibition of mitogen-activated protein kinase pathway[J]. *Int Immunopharmacol*, 2017, 47:28–37. doi: 10.1016/j.intimp.2017.03.019.
- [45] Wu Y, Zhang W, Li M, et al. Nobiletin ameliorates ischemia-reperfusion injury by suppressing the function of Kupffer cells after liver transplantation in rats[J]. *Biomed Pharmacother*, 2017, 89: 732–741. doi: 10.1016/j.biopha.2017.02.087.
- [46] Lin Y, Sheng M, Weng Y, et al. Berberine protects against ischemia/reperfusion injury after orthotopic liver transplantation via activating Sirt1/FoxO3 $\alpha$  induced autophagy[J]. *Biochem Biophys Res Commun*, 2017, 483(2): 885–891. doi: 10.1016/j.bbrc. 2017. 01.028.
- [47] Tsaroucha AK, Tsiaousidou A, Ouzounidis N, et al. Intraperitoneal administration of apigenin in liver ischemia/reperfusion injury protective effects[J]. *Saudi J Gastroenterol*, 2016, 22(6): 415–422. doi: 10.4103/1319–3767.195556.
- [48] He D, Guo Z, Pu J L, et al. Resveratrol preconditioning protects hepatocytes against hepatic ischemia reperfusion injury via Toll-like receptor 4/nuclear factor- $\kappa$ B signaling pathway in vitro and in vivo[J]. *Int Immunopharmacol*, 2016, 35:201–209. doi: 10.1016/j.intimp.2016.03.032.
- [49] Chi X, Zhang R, Shen N, et al. Sulforaphane reduces apoptosis and oncosis along with protecting liver injury-induced ischemic reperfusion by activating the Nrf2/ARE pathway[J]. *Hepatol Int*, 2015, 9(2):321–329. doi: 10.1007/s12072–014–9604–y.
- [50] Sun P, Yuan F, Xu J, et al. Cryptotanshinone ameliorates hepatic normothermic ischemia and reperfusion injury in rats by anti-mitochondrial apoptosis[J]. *Biol Pharm Bull*, 2014, 37(11): 1758–1765. doi: 10.1248/bpb.b14–00389.
- [51] Mahmoud MF, Gamal S, El-Fayoumi HM. Limonin attenuates hepatocellular injury following liver ischemia and reperfusion in rats via toll-like receptor dependent pathway[J]. *Eur J Pharmacol*, 2014, 740:676–682. doi: 10.1016/j.ejphar.2014.06.010.
- [52] Zhang F, Wang X, Tong L, et al. Matrine attenuates endotoxin-induced acute liver injury after hepatic ischemia/reperfusion in rats[J]. *Surg Today*, 2011, 41(8): 1075–1084. doi: 10.1007/s00595–010–4423–9.
- [53] Akinci O, Durgun V, Kepil N, et al. The role of genistein in experimental hepatic ischemia-reperfusion model in rats[J]. *Bratisl Lek Listy*, 2019, 120(8):558–562. doi: 10.4149/BLL\_2019\_090.
- [54] Zhang J, Cheng P, Dai W, et al. Fenofibrate Ameliorates Hepatic Ischemia/Reperfusion Injury in Mice: Involvements of Apoptosis, Autophagy, and PPAR- $\alpha$  Activation[J]. *PPAR Res*, 2021, 2021: 6658944. doi: 10.1155/2021/6658944.
- [55] Ma H, Liu Y, Li Z, et al. Propofol Protects Against Hepatic Ischemia Reperfusion Injury via Inhibiting Bnip3-Mediated Oxidative Stress[J]. *Inflammation*, 2021, 44(4): 1288–1301. doi: 10.1007/s10753–021–01416–z.
- [56] Đurašević S, Stojković M, Sopta J, et al. The effects of meldonium on the acute ischemia/reperfusion liver injury in rats[J]. *Sci Rep*, 2021, 11(1):1305. doi: 10.1038/s41598–020–80011–y.
- [57] Xiong C, Du Z, Zhu Y, et al. Mycophenolate mofetil preconditioning protects mouse liver against ischemia/reperfusion injury in wild type and toll-like receptor 4 knockout mice[J]. *Transplant Immunology*, 2021, 65: 101357. doi: 10.1016/j.trim.2020.101357.
- [58] Liu YX, Jin LM, Zhou L, et al. Mycophenolate mofetil attenuates liver ischemia/reperfusion injury in rats[J]. *Transpl Int*, 2009, 22(7): 747–756. doi: 10.1111/j.1432–2277.2009.00866.x.
- [59] Zhou R, Li S, Mei X, et al. Remifentanyl up-regulates HIF1 $\alpha$  expression to ameliorate hepatic ischaemia/reperfusion injury via the ZEB1/LIF axis[J]. *J Cell Mol Med*, 2020, 24(22):13196–13207. doi: 10.1111/jcmm.15929.
- [60] Liu X, Pan Z, Su D, et al. Remifentanyl ameliorates liver ischemia-reperfusion injury through inhibition of interleukin-18 signaling[J]. *Transplantation*, 2015, 99(10): 2109–2117. doi: 10.1097/TP.00000-0000000737.
- [61] Wang J, Deng M, Wu H, et al. Suberoylanilide hydroxamic acid alleviates orthotopic liver transplantation-induced hepatic ischemia-reperfusion injury by regulating the AKT/GSK3 $\beta$ /NF- $\kappa$ B and AKT/mTOR pathways in rat Kupffer cells[J]. *Int J Mol Med*, 2020, 45(6):1875–1887. doi: 10.3892/ijmm.2020.4551.
- [62] Kamel EO, Hassanein EHM, Ahmed MA, et al. Perindopril Ameliorates Hepatic Ischemia Reperfusion Injury Via Regulation of NF- $\kappa$ B-p65/TLR-4, JAK1/STAT-3, Nrf-2, and PI3K/Akt/mTOR

- Signaling Pathways[J]. *Anat Rec (Hoboken)*, 2020, 303(7): 1935–1949. doi: 10.1002/ar.24292.
- [63] Liu H, Dong J, Song S, et al. Spermidine ameliorates liver ischaemia-reperfusion injury through the regulation of autophagy by the AMPK-mTOR-ULK1 signalling pathway[J]. *Biochem Biophys Res Commun*, 2019, 519(2): 227–233. doi: 10.1016/j.bbrc.2019.08.162.
- [64] Sherif IO, Al-Shaalan NH. Vildagliptin attenuates hepatic ischemia/reperfusion injury via the TLR4/NF- $\kappa$ B signaling pathway[J]. *Oxid Med Cell Longev*, 2018, 2018: 3509091. doi: 10.1155/2018/3509091.
- [65] Liu Z, Lai C, Zhang X, et al. Simvastatin ameliorates total liver ischemia/reperfusion injury via KLF2-mediated mechanism in rats[J]. *Clin Res Hepatol Gastroenterol*, 2019, 43(2): 171–178. doi: 10.1016/j.clinre.2018.08.014.
- [66] Liu Z, Zhang X, Xiao Q, et al. Pretreatment donors after circulatory death with simvastatin alleviates liver ischemia reperfusion injury through a KLF2-dependent mechanism in rat[J]. *Oxid Med Cell Longev*, 2017, 2017:3861914. doi: 10.1155/2017/3861914.
- [67] Li X, Wang J, Song X, et al. Ketamine ameliorates ischemia-reperfusion injury after liver autotransplantation by suppressing activation of Kupffer cells in rats[J]. *Can J Physiol Pharmacol*, 2018, 96(9):886–892. doi: 10.1139/cjpp-2018-0046.
- [68] Shin JK, Lee SM. Genipin protects the liver from ischemia/reperfusion injury by modulating mitochondrial quality control[J]. *Toxicol Appl Pharmacol*, 2017, 328: 25–33. doi: 10.1016/j.taap.2017.05.002.
- [69] Wang Y, Wu S, Yu X, et al. Dexmedetomidine protects rat liver against ischemia-reperfusion injury partly by the  $\alpha$ 2A-adrenoceptor subtype and the mechanism is associated with the TLR4/NF- $\kappa$ B pathway[J]. *Int J Mol Sci*, 2016, 17(7): 995. doi: 10.3390/ijms17070995.
- [70] Lee SC, Kim KH, Kim OH, et al. Activation of autophagy by everolimus confers hepatoprotection against ischemia-reperfusion injury[J]. *Am J Transplant*, 2016, 16(7): 2042–2054. doi: 10.1111/ajt.13729.
- [71] Shen M, Lu J, Dai W, et al. Ethyl pyruvate ameliorates hepatic ischemia-reperfusion injury by inhibiting intrinsic pathway of apoptosis and autophagy[J]. *Mediators Inflamm*, 2013, 2013: 461536. doi: 10.1155/2013/461536.
- [72] Koh EJ, Yoon SJ, Lee SM. Losartan protects liver against ischaemia/reperfusion injury through PPAR- $\gamma$  activation and receptor for advanced glycation end-products down-regulation[J]. *Br J Pharmacol*, 2013, 169(6):1404–1416. doi: 10.1111/bph.12229.
- [73] Tüfek A, Tokgöz O, Aliosmanoglu İ, et al. The protective effects of dexmedetomidine on the liver and remote organs against hepatic ischemia reperfusion injury in rats[J]. *Int J Surg*, 2013, 11(1):96–100. doi: 10.1016/j.ijssu.2012.12.003.
- [74] Fu H, Chen H, Wang C, et al. Flurbiprofen, a cyclooxygenase inhibitor, protects mice from hepatic ischemia/reperfusion injury by inhibiting GSK-3 $\beta$  signaling and mitochondrial permeability transition[J]. *Mol Med*, 2012, 18(1): 1128–1135. doi: 10.2119/molmed.2012.00088.
- [75] Ocuin LM, Zeng S, Cavnar MJ, et al. Nilotinib protects the murine liver from ischemia/reperfusion injury[J]. *J Hepatol*, 2012, 57(4): 766–773. doi: 10.1016/j.jhep.2012.05.012.
- [76] Dai Y, Cui J, Cun Y, et al. Tetrahydrobiopterin ameliorates hepatic ischemia-reperfusion Injury by coupling with eNOS in mice[J]. *J Surg Res*, 2012, 176(2):e65–71. doi: 10.1016/j.jss.2011.12.034.
- [77] Fondevila C, Shen XD, Tsuchiyashi S, et al. Biliverdin therapy protects rat livers from ischemia and reperfusion injury[J]. *Hepatology*, 2004, 40(6):1333–1341. doi: 10.1002/hep.20480.
- [78] Settaf A, Morin D, Lamchouri F, et al. Trimetazidine ameliorates the hepatic injury associated with ischaemia-reperfusion in rats[J]. *Pharmacol Res*, 1999, 39(3):211–216. doi: 10.1006/phrs.1998.0427.
- [79] Castro CC, Pagnussat AS, Orlandi L, et al. Coumestrol has neuroprotective effects before and after global cerebral ischemia in female rats[J]. *Brain Res*, 2012, 1474: 82–90. doi: 10.1016/j.brainres.2012.07.025.
- [80] Huang M, Cheng G, Tan H, et al. Capsaicin protects cortical neurons against ischemia/reperfusion injury via down-regulating NMDA receptors[J]. *Exp Neurol*, 2017, 295:66–76. doi: 10.1016/j.expneurol.2017.05.001.
- [81] Wang D, Jiang Q, Du X. Protective effects of scopolamine and penehyclidine hydrochloride on acute cerebral ischemia-reperfusion injury after cardiopulmonary resuscitation and effects on cytokines[J]. *Exp Ther Med*, 2018, 15(2): 2027–2031. doi: 10.3892/etm.2017.5646.

( 本文编辑 宋涛 )

本文引用格式: 邓家琦, 钱保林, 张丽云, 等. 肝脏缺血再灌注损伤的ceRNA网络构建和潜在治疗药物筛选[J]. 中国普通外科杂志, 2021, 30(7):822–835. doi:10.7659/j.issn.1005-6947.2021.07.009

Cite this article as: Deng JQ, Qian BL, Zhang LY, et al. Construction of ceRNA network in liver ischemia/reperfusion injury and screening of the potential therapeutic agents[J]. *Chin J Gen Surg*, 2021, 30(7):822–835. doi:10.7659/j.issn.1005-6947.2021.07.009