Exploring the Differential Gene of CHOP-Related Factors in Hepatocellular Injury Caused by Endoplasmic Reticulum Stress

Leng-xin Duan (✉ lengxinduan@163.com)

Henan University of Science and Technology <https://orcid.org/0000-0002-8121-6940>

Man-lin Guo

Luoyang Orthopedic-Traumatological Hospital Of Henan Province(Henan Provincial Orthopedic Hospital)

Xiang-yu Ma

Henan University of Science and Technology

Yu-qing Gong

Henan University of Science and Technology

San-qiang Li

Henan University of Science and Technology

Research Article

Keywords: RNA-Seq technology, CHOP, endoplasmic reticulum stress

Posted Date: September 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-844884/v1>

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Abstract

Objective: CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP/DDIT3) is a protein activated by endoplasmic reticulum stress (ERS). However, the downstream genes of CHOP on liver damage caused by ER-stress have been unclear. Herein, we investigated the potential downstream related factors of CHOP in L-02 cells.

Methods and Material: Tunicamycin (TM) was used to induce ER-stress. Short hairpin RNA (shRNA) was used to knocked down CHOP, and the functions of differentially expressed genes (DEGs) were obtained from functional annotations. qRT-PCR was employed to validate the expression levels of candidate DEGs.

Results: 633 genes were differentially expressed between ERS L-02 cells and normal L-02 cells, and 131 genes were differentially expressed between shRNA-CHOP and shRNA-NC in ERS L-02 cells. By analyzing these results, we luckily found 8 genes including Interferon α -inducible protein 27 (IFI27), Lipocalin 2 (LCN2), Chromosome 11 Open Reading Frame 86 (C11orf86), Calmeglin (CLGN), Kelch domain-containing 7B (KLHDC7B), Niban Apoptosis Regulator 1 (Niban), T-Cell Receptor Gamma-Chain Constant Region (TARP), Lysosome associated membrane protein 3 (LAMP3) were intimately related to chop.

Conclusion: Our study might contribute to better understand how CHOP functions during ER-stress, and these results can expand databases of CHOP in GenBank or others.

1. Introduction

The liver is an essential metabolic organ in the body, and it acts a crucial role in antioxidants, storing glycogen, synthesizing secreted proteins and so on[1]. In recent years, the incidence of hepatic diseases trend to raise year by year in the world. The liver is rich in endoplasmic reticulum (ER). There is a conspicuous correlation between the hepatic diseases and dysfunction of the ER. ER is a main organelle for protein synthesis and folding to form the correct three-dimensional conformation of the proteins. Various factors would lead to the malfunctions of ER, such as oxygen deprivation, calcium metabolic disorders, oxidative stress and so on[2].

Endoplasmic reticulum stress (ERS) would be induced if the perturbation of ER is serious or persistent. The unfolded protein response (UPR) is a part of the global Endoplasmic reticulum stress (ERS) response. The unfolded and misfolded proteins accumulate in ER would result in UPR[3]. The UPR protects cell from damage by enhancing the protein folding ability, stagnating the translation of most proteins and accelerating protein degradation. Protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme-1 (IRE-1) and activating transcription factor 6 (ATF6) are important ER-localized transmembrane proteins in the three classical signaling pathways of UPR[4]. PERK/eIF2P/ATF4 pathway is necessary to regulate CHOP expression in ERS. The increasing translation of activating transcription factor 4 (ATF4) directly induces the expression of downstream gene CHOP, and then accelerates the apoptosis of cells, which suggesting that CHOP plays an important role in the apoptosis process induced by ERS[5].

CHOP, also known as GADD153/ DDIT3, is a multifunctional transcription factor that is critical in the ERS-induced apoptotic programs. It is expressed at a very low level in physiological state and is significantly up-regulated in response to a variety of cellular stresses and induces cell cycle arrest and apoptosis[6]. It also plays a dual role both as an inhibitor of CCAAT/enhancer binding protein (C/EBP) function and as an activator of other genes. CHOP plays a considerable part in ER stress-induced apoptosis, which is the main upregulated pro-apoptotic player. There are a lot of researches which focus on upstream regulatory pathway of CHOP. And few of downstream genes are associated with CHOP in public databases at present, so it is significant to find the downstream genes regulated by CHOP[7].

Nowadays, the next-generation of sequencing technology has been widely used, and it has become a powerful tool for simultaneous sequencing of millions of genomic features and identification of specific genes in sample tissues. High-throughput RNA sequencing is superior to expression profiles from the microarray technology. The high sensitivity of RNA-Seq data could provide a qualitative and quantitative description of gene expression[8]. In this study, we used RNA-seq to obtain mRNA expression data from L-02 cells, and to identify Differentially expressed genes between ERS L-02 cells and normal L-02 cells and between shRNA-CHOP and shRNA-NC in ERS L-02 cells. Our aim was to discover the potential downstream related factors for CHOP.

2. Materials And Methods

2.1. Cell culture

L-02 cells were from Procell Life Science & Technology Co., Ltd (Wuhan, China). L-02 cells were cultured in 1640 medium containing 10% fetal bovine serum, 100 units/ml penicillin G and 100 µg/ml streptomycin sulfate. The culture medium was kept replacing every 2 days, and cells were maintained in the logarithmic growth phase.

2.2. Knockdown of CHOP by shRNA and treatment

Knockdown of CHOP was performed using short hairpin RNA (shRNA) in L-02 cells. Briefly, The L-02 cells were divided into four groups (A: The normal L-02 cells, B: The L-02 cells were treated with TM for 24 h in a dose of 60 µg/mL, C: The L-02 cells were firstly transfected shRNA-CHOP for 24 h, then were treated with TM for 24 h in a dose of 60 µg/mL, D: The L-02 cells were firstly transfected shRNA-NC for 24 h, then were treated with TM for 24 h in a dose of 60 µg/mL). Western blotting was used to identify the knockdown efficiency. The most efficient shRNA sequences were as following: shRNA 1: 5'-GCGCATG AAGGAG- AAAGAACAGG; shRNA2: 5'-CCGAGCTCTGATTGACCGAATGG[9].

2.3. Extraction and construction of RNA-seq library

Total RNA of each group was extracted by using TRIzol reagent (Invitrogen, USA). The library preparation followed the standard procedures of Baerveldt glaucoma implant (BGI). Oligo (dT) magnetic beads are used to select mRNA with polyA tail from total RNA, and the mRNA was cleaved into short fragments, and reverse transcribed into double-strand cDNA (dscDNA) by N6 random primer. The ends of the dscDNA

were filled in, then the 5' end was phosphorylated, and a sticky end protruding from A was formed at the 3' end, followed by a bubbling junction with a protruding 'T' at the 3' end. After polymerase chain reaction (PCR) amplification with two specific primers, the enriched cDNA libraries were sequenced with a BGISEQ-500 (BGI, Inc., Wu Han, China).

2.4. Differentially expressed genes analysis

Differentially expressed genes were identified by using the Noise Sensitivity Questionnaire (NOISeq) method, which could screen differentially expressed genes between two groups, showing a good performance compared with other differential expression methods[10]. we screen differentially expressed genes according to the following default criteria: fold change ≥ 2 and diverge probability ≥ 0.8 .

2.5. *Functional annotation of differentially expressed genes.*

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were used to predict the biological functions of DEGs. GO is an international standard gene functional classification system. It firstly mapped all DEGs to GO terms in the database (<http://www.geneontology.org/>), calculating gene numbers for every term, then using the hypergeometric test to find significantly enriched GO terms in the input list of DEGs. KEGG was used to perform pathway enrichment analysis of DEGs. This analysis identified by significantly enriched metabolic pathways or signal transduction pathways in DEGs comparing with the whole genome[11]. P-value ≤ 0.05 were set as the threshold for selecting significantly enriched functional GO terms and KEGG pathway enrichment.

2.6. Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain (qRT-PCR) analysis was used to validate the DGEs selected from the RNA-seq analysis. According to the manufacturer's instructions (Invitrogen, USA), total RNA was extracted using TRIzol reagent. The primers pairs designed using Primer Premier 5.0 were listed in Table 1. The HiScript® 1st Strand cDNA Synthesis Kit (Vazyme Biotech, Nanjing, China) was used to synthesize the cDNA. AceQ® qPCR SYBR® Green Master Mix (Vazyme Biotech, Nanjing, China) was used to perform qRT-PCR reactions on a Bio-Rad CFX96 Real-Time PCR machine (Bio-Rad, USA). These reactions were used the following cycle parameters: 95°C for 30 seconds, followed by 40 cycles of 95°C for 5 seconds, 60°C for 30 seconds, and then 72°C for 30 seconds. The relative expression of target genes was calculated by the $2^{-\Delta\Delta CT}$ equation[12]. The GraphPad Prism version 5.01 software was used to output figures.

Table 1
Primer sequences of qPCR

Gene	Primers Sequence(5'-3')
CHOP	Forward CTGCTTCTCTGGCTTGGCTGAC
	Reverse TTGGTCTTCCTCCTCTTCCTCCTG
CLGN	Forward TAGTGGAAGGTTGGCTGGAT
	Reverse CCAGTCCTCTGTCACCAGGT
LCN2	Forward GGTCCCTCTGCAGCAGAACT
	Reverse GTGGCATAACATCTTTTGCGG
LAMP3	Forward CAGTTCATCAACCGTCAGCC
	Reverse TCTGACCGGTTGTGCTTTTG
KLHDC7B	Forward ACGGCCCTCTTCTTCAGTG
	Reverse ATCCGTTGGTCTCGGAGCTT
FAM129A	Forward ATCCACCCCGACAACTGTGA
	Reverse TACCTCTGGAAATCCCCCA
IFI27	Forward CGTCCTCCATAGCAGCCAAG
	Reverse TGCTCCCAGTGACTGCAGAG
C11orf86	Forward ACAGAGCAGCTGATCCAAGC
	Reverse GGCTGACTCAGAGTCACGCT
GAPDH	Forward ACCCAGAAGACTGTGGATGG
	Reverse TTCTAGACGGCAGGTCAGGT

2.7. Statistical analysis

Experimental data were indicated by mean $\pm$ standard deviation. The software of SPSS version 17.0 for Windows was used for statistical analysis. t-test was used to analyze statistical significance between groups. P -values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Efficient CHOP knockdown and RNA-seq analysis

Western blotting showed that CHOP was knocked down in L-02 cells by short hairpin RNA (shRNA), as shown in Fig. 1. These results identified an efficient decrease of the CHOP protein level in sh-RNA-3, which

was used to subsequent experiments[13].

RNA-seq was performed on the L-02 samples of 8 subjects (normal L-02 cells, ERS L-02 cells groups, shRNA-CHOP in ERS L-02 cells and shRNA-NC in ERS L-02 cells, each group has two biological repeats). Approximately, averagely generating 24137291 raw sequencing reads and then 23980654 clean reads after filtering low quality. All of the clean reads were aligned to the UCSC human reference genome (hg.19) using HISAT/ Bowtie2 tool. The average mapping ratio with reference gene is 80.77% and the average genome mapping ratio is 95.87%. Specific information of sequencing data for each sample is shown in Table 2.

Table 2
RNA-seq analysis

Sample	Total Raw Reads	Total Clean Reads	Clean Reads Rate (%)	Total Mapped Clean Reads with reference gene (%)	Total Mapped Clean Reads with reference genome (%)
A1	24137294	23717132	98.25	81.07	95.62
A2	24137224	23861103	98.85	81.21	95.82
B1	24137369	24038383	99.58	80.18	95.90
B2	24137278	24050273	99.63	80.62	95.93
C1	24137185	23950019	99.22	78.84	95.92
C2	24137223	23941693	99.18	82.13	95.88
H1	24137351	24002279	99.44	78.64	96.10
H2	24137291	23897662	99.00	78.78	96.06
A: normal L-02 cells; B: ERS L-02 cells; C: shRNA-CHOP in ERS L-02 cells; H: shRNA-NC in ERS L-02 cells					

3.2. Analysis of the differentially expressed genes

CHOP was up-regulated in ERS L-02 cells compared with normal L-02 cells and down-regulated in shRNA-CHOP compared with shRNA-NC in ERS L-02 cells, which was in line with our expectations. 633 genes were differentially expressed by more than two fold between ERS L-02 cells and normal L-02 cells, of which 303 genes were up-regulated and 330 genes were down-regulated in ERS L-02 cells compared with normal L-02 cells. At the same time, 131 DEGs were identified between shRNA-CHOP and shRNA-NC in ERS L-02 cells, of which 65 genes were up-regulated and 66 genes were down-regulated in shRNA-CHOP ERS L-02 cells compared with shRNA-NC ERS L-02 cells (Fig. 2). We used cluster and Java TreeView software to perform clustering analysis of DEGs, the result as the following Fig. 3A and Fig. 3B. We were interested in the DEGs which were highly changed between the two compared groups. According to the data of DEGs, we found eight genes were significantly altered, Calmegin (CLGN), Lipocalin 2 (LCN2), The

family of lysosome-associated membrane protein 3 (LAMP3), Kelch domain-containing 7B (KLHDC7B) and family with sequence similarity 129 member A (FAM129A) were up- regulated when ERS occurred and were down-regulated when CHOP was knockdown. Conversely, Interferon a-inducible protein 27 (IFI27), T-cell receptor gamma chain alternate reading frame protein (TARP) and Chromosome 11 Open Reading Frame 86 (C11orf86) were down regulated when ERS occurred and were up-regulated when CHOP was knockdown. The specific information of DEGs we selected were listed in Table 3.

Table 3
The potential DEGs related to CHOP

Gene ID	Gene Symbol	B vs A			C vs H		
		Up/Down	log ₂ FC	Probability	Up/Down	log ₂ FC	Probability
1649	DDIT3	Up	1.963842	1	Down	-1.14236	1
1047	CLGN	Up	4.506032	1	Down	-1.26628	0.9888389
3934	LCN2	Up	4.260299	1	Down	-1.60746	1
27074	LAMP3	Up	1.836967	0.999774	Down	-1.48398	0.989275
113730	KLHDC7B	Up	3.390823	1	Down	-2.08504	0.9967607
116496	FAM129A	Up	2.798175	1	Down	-1.08559	1
3429	IFI27	Down	-1.469	0.999819	Up	1.219566	0.938389
445347	TARP	Down	-1.06839	0.979598	Up	1.827731	0.999911
254439	C11orf86	Down	-2.21122	1	Up	1.233879	0.998804
A: normal L-02 cells; B: ERS L-02 cells; C: shRNA-CHOP in ERS L-02 cells; H: shRNA-NC in ERS L-02 cells							

3.3. Gene Ontology (GO) annotation of DEGs

633 DEGs between ERS L-02 cells and normal L-02 cells and 131 DEGs between shRNA-CHOP and shRNA-NC ERS L-02 cells were performed to GO annotation to obtain the biological roles. GO terms with FDR < 0.05 were considered as significant enrichment. Cellular process (GO: 0009987), single-organism process (GO: 0044699) and metabolic process (GO: 0008152) were the most significant enrichments of biological process; binding (GO: 0005488), catalytic activity (GO: 0003824) and transporter activity (GO: 0005215) were the highest enrichment of molecular function; cell (GO: 0005623), cell part (GO: 0044464) and organelle (GO:0043226) were the highest enrichment of cellular component, as Fig. 4 and Fig. 5 shown.

3.4. *Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of DEGs*

KEGG is a knowledge database for the systematic functional analysis of genes to connect genomic information with higher functional information by computerizing current knowledge on cellular processes and standardizing gene annotations. Thus, KEGG analyses were performed to assign pathways and functionally classify genes. $FDR < 0.05$ was defined as significant KEGG pathway enrichment. As shown in Table 4, pathways in protein processing, mTOR signaling, oxidative phosphorylation, PI3K-Akt signaling pathway, Rap1 signaling pathway, MAPK signaling pathway and others were significantly enriched KEGG pathways between ERS L-02 cells and normal L-02 cells. The signaling pathways of DEGs between shRNA-CHOP and shRNA-NC in ERS L-02 cells were not available in the KEGG database.

Table 4
KEGG analysis of DEGs between ERS L-02 cells and normal L-02 cells

Pathway ID	Pathway	number of genes in pathway	Differentially expressed genes
ko04141	Protein processing in endoplasmic reticulum	37	<i>DERL2, HSP90B1, HSPA1A, SSR3, DNAJB11, CALR, DDOST, HYOU1, DNAJC10, PDIA6, DNAJC3, RAD23A, SEL1L, DERL3, SIL1, 29927_SEC61A1, PDIA4, HSPA1B, SSR4, EDEM2, SEC24D, YOD1, HSPA5, ERLEC1, RPN1, DDIT3, PDIA3, P4HB, SYVN1, 9709_HERPUD1, CANX, UGGT1, TRAM1, OS9, VIMP, EDEM1, STT3A</i>
ko04350	TGF-beta signaling pathway	9	<i>TGFB2, CHRD, ID2, ID3, SMAD6, ID1, TGFB1, FST, DCN</i>
ko04151	PI3K-Akt signaling pathway	23	<i>EPHA2, HSP90B1, GNG11, COMP, VTN, IL4R, CREB3L4, KITLG, IL6, COL1A1, NR4A1, CCND1, CASP9, SGK3, COL4A6, ITGA6, COL4A1, PRKAA2, SGK1, 2247_FGF2, MST1, OSMR, ITGB4</i>
ko00590	Arachidonic acid metabolism	7	<i>PTGDS, PLA2G4B, PTGS1, PTGS2, LTC4S, LCN2, ALOX15</i>
ko04668	TNF signaling pathway	9	<i>JUNB, CREB3L4, IL6, PTGS2, TNFAIP3, SOCS3, BCL3, ISOC2, FOS</i>
ko04015	Rap1 signaling pathway	16	<i>EPHA2, RGS14, SIPA1L1, KITLG, RAP1GAP, RASGEF1C, TLN2, ACTL10, ID1, CALML6, ARVCF, ISOC2, RASSF5, FGF2, MST1, TARP</i>
ko04010	MAPK signaling pathway	16	<i>HSPA1A, TGFB2, DUSP2, PLA2G4B, DUSP4, NALCN, BDNF, DUSP9, NR4A1, HSPA1B, DDIT3, DUSP5, TGFB1, ISOC2, FOS, FGF2</i>
ko05202	Transcriptional misregulation in cancer	19	<i>ANKRD37, ETV4, DUSP2, DUSP4, ETV5, HOXD9, IL6, ZCWPW1, ID2, HPGD, PLEKHA7, ETV1, ATP6AP1L, DDIT3, HIST1H3E, BCL6, DUSP5, PLEKHA2, TARP</i>
ko04115	p53 signaling pathway	7	<i>STEAP3, TNFRSF10C, CCND1, CASP9, CNTD2, SERPINB5, PMAIP1</i>
ko04064	NF-kappa B signaling pathway	6	<i>PTGS2, TNFAIP3, CARD14, PLCG2, PEG10, TARP</i>
ko04370	VEGF signaling pathway	4	<i>PLA2G4B, PTGS2, CASP9, PLCG2</i>
ko04014	Ras signaling pathway	12	<i>EPHA2, GNG11, PLA2G4B, UPK3B, KITLG, ABL2, PLCG2, CALML6, RASSF5, FGF2, MST1, TARP</i>
ko04210	Apoptosis	8	<i>ITPR1, TNFRSF10C, CASP9, DDIT3, ACTL10, PEG10, FOS, PMAIP1</i>

Pathway ID	Pathway	number of genes in pathway	Differentially expressed genes
ko00190	Oxidative phosphorylation	4	<i>ATP6V0E2, PEAR1, ATP6V1C2, ATP6V0A1</i>
ko04024	cAMP signaling pathway	7	<i>CREB3L4, BDNF, MYL9, CALML6, SOX9, ATP2A2, FOS</i>
ko04310	Wnt signaling pathway	10	<i>SPACA9, FOSL1, TCF7, LOC400927-CSNK1E, DKK1, AXIN2, CCND1, PRSS23, FZD10, NKD2</i>
ko04142	Lysosome	4	<i>ATP6V0A1, CLN3, NEU1, LAMP3</i>
ko04660	T cell receptor signaling pathway	3	<i>CARD14, FOS, TARP</i>
ko04150	mTOR signaling pathway	7	<i>SPACA9, ATP6V1C2, SLC3A2, PRSS23, FZD10, PRKAA2, SGK1</i>
ko03018	RNA degradation	9	<i>PAN2, C4orf26, TMEM50B, SLFN5, NCOA7, FAM84B, PABPC4L, SMIM11A, ENO3</i>

3.5. Verification of the expression level of DEGs

The expression levels of DEGs between ERS L-02 cells groups and normal L-02 cells groups and between shRNA-CHOP in ERS L-02 cells groups and shRNA-NC in ERS L-02 cells groups were quantified by qRT-PCR. As shown in Fig. 3, DDIT3, CLGN, LCN2, LAMP3, KLHDC7B and FAM129A were significantly up-regulated in ERS L-02 cells compared with normal controls ($P < 0.05$), and they were down-regulated after we knocked down DDIT3. In Fig. 6, IFI27, TARP and C11orf86 shown the exact opposite situations, they were significantly down-regulated in ERS L-02 cells compared with normal controls ($P < 0.05$), and they were up-regulated after we knocked down CHOP ($P < 0.05$). The qRT-PCR results were all in accordance with our RNA-seq data.

4. Discussion

There are numerous researches on the expression CHOP involved in ER-stress at present. CHOP plays a considerable role in many biological processes such as autophagy and apoptosis. In this study, we used RNA-seq to analyze these potential genes located downstream of CHOP gene, which showed that knockdown of CHOP resulted in an enormous impact on other genes whose role remains unknown in ER-stress. These results indicated that CHOP contributed to important functions in ER-stress signaling pathways and others. After contrasting with cell-to-cell variation in both L-02 cells and normal L-02 cells, and that change between shRNA-CHOP and shRNA-NC in ERS L-02 cells, we identified that considerable genes with obvious changes after CHOP knockdown. To our knowledge, this study is the first attempt to investigate the gene regulatory network after CHOP knockdown. Our study advances the understanding

of CHOP function in ER-stress and more importantly, initially clarified the relevant factors and pathways of CHOP. Interestingly, we identified eight genes showing a close relationship with CHOP gene which have been rarely reported. We further noticed that a kind of unknown regulatory mechanism may exist between these genes and CHOP gene[14].

These genes are further explained in detail as follows: LCN2 belongs to the lipocalin family. The protein encoded by this gene is a neutrophil gelatinase-associated lipocalin (NGAL) and participates actively in a lot of physiological activities, such as innate immunity, inflammation, apoptosis, proliferation and differentiation of cells and so on[15, 16]. A number of studies discovered that up-regulation of LCN2 is associated with the occurrence and development of many liver diseases, suppression or knockdown of LCN2 could alleviate various liver diseases, such as liver fibrosis[17], alcoholic fatty liver[18], liver failure[19], inflammation and infection[20]. In addition, Thyroid hormone (T3)/thyroid hormone receptor (TR) could regulate LCN2 via the Met/FAK cascade, which suggested that LCN2 may be effectively utilized as a novel marker and therapeutic target in hepatocellular carcinoma (HCC)[21]. What's more, the urinary neutrophil gelatinase-associated lipocalin (NGAL) may be a sensitive biomarker of acute kidney injury (AKI) in serious complication after liver transplantation[22]. In this study, we found that LCN2 was up-regulation in ERS L-02 cells and down-regulation when we knockdown CHOP, suggested that it had an intimate connection with ERS[23].

The family of lysosome-associated membrane proteins (LAMPs) included three members, LAMP1, LAMP2 and LAMP3. The function of LAMP1 and LAMP2 is mainly to maintain the stability of the lysosomal membrane, while LAMP3 is closely related to all kinds of pathological processes, such as ovarian cancer[24], breast cancer[25], oral squamous cell carcinoma[26], uterine cervical cancer[27] et al. Several studies have reported that LAMP3 could be induced up-regulated by PERK-eIF2 α -ATF4 signaling pathways and promote the invasion and metastasis of tumors[28]. In patients with esophageal squamous cell carcinoma, the high expression of LAMP3 may serve as a novel biomarker of poor prognosis[29]. It is also a downstream target gene of tumor suppressor TP53[30]. In addition, The coding for LAMP3 is considered to be a risk factor for Parkinson's disease (PD)[31]. CHOP is a downstream target gene of PERK-eIF2 α -ATF4 signaling pathways. The results of our dates showed that LAMP3 could be regulated by CHOP. The mechanism needs further studies.

FAM129A, also known as Niban, is highly expressed in several cancer cells, and it is closely related to changes in mitochondrial function during preneoplastic and neoplastic processes[32]. Previous research has shown that FAM129A mRNA and protein were induced up-regulated by tunicamycin, which was in conformance with our results, and it could modulate cell death signaling by regulating translation, such as the phosphorylation of eIF2 α and S6 subunit kinase (S6K) 1/4E-binding protein (4E-BP) 1[33]. It could also promote the interaction of MDM2-p53 and subsequent p53 degradation, thereby providing an antiapoptotic effect[34]. What's more, overexpression of zinc-finger protein 777 (ZNF777) inhibits proliferation at low cell density through down- regulation of FAM129A[35]. There are only 32 literature reports on this factor at present. Our study first identified a regulatory mechanism between FAM129A and chop, which provided a new ideas to explore this factor.

CLGN, a homologue of endoplasmic reticulum (ER) residing lectin chaperone calnexin, is required for the generation of normal spermatozoa[36]. Previous studies have shown that CLGN was transcriptionally regulated by histone deacetylase (HDAC) and the cytosine-phosphate- guanine (CpG) methyltransferase[37]. In APAs, CLGN served as an ER- associated gene, which was highly expressed and got involved in aldosterone production by translational regulation of CYP11B2[38]. So far, almost no literature has reported the relationship between CLGN and the liver. In this study, we found that CLGN had a close relationship with CHOP in L-02 cells. The further regulative mechanism needs a more in-depth research.

IFI27, a highly inducible proteins, has been suggested to be a putative cell proliferation marker, IFI27 knockdown resulted in S-phase arrest that was found to be associated with increased Tyrosine-15 (Tyr15) phosphorylation of cyclin-dependent kinase 1 (CDK1), reduced cell division cycle 25B (CDC25B) and reduced formation of cyclin A/CDK1 complex[39]. It is also supposed to be biological markers of variety of diseases. In respiratory infection, it could be an immune biomarker to discriminate influenza and bacterial infections in patients[40]. It could serve as a noninvasive diagnostic marker for chronic glomerulonephritis using peripheral blood[41]. It was also supposed to be a novel marker of epithelial stromal tumor[42]. Our research found for the first time that IFI27 is expressed in L-02 cells. In addition, it may be a stress-induced genes which lies downstream of CHOP.

TARP is the first mitochondrial membrane protein found in human prostate[43]. Several studies have found that TARP could serve as a new target for immunotherapy[44]. It could act as a molecular prognostic marker since it is highly expressed in prostate cancer, breast cancer, endometrial carcinomas and so on[45]. Surprisingly, we found that TARP may play a role in liver injury caused by ER-stress in this experiment, and its regulatory mechanism of this process may be related to CHOP.

There are almost no reports about KLHDC7B and C11orf86. In this study, the up-regulated of CHOP would cause the increase of KLHDC7B and the decrease of C11orf86, and they both had the opposite changes when CHOP was knockdown. Therefore, we predict that they may be regulated by CHOP, but this conclusion remains to be further studied. Exploring the upstream and downstream genes of CHOP in ER-stress will help us better understand the regulatory mechanism of ER-stress and provide a new perspective and strategy for the prevention and treatment of diseases related to liver injury.

5. Conclusion

We totally identified 633 DEGs between ERS L-02 cells and normal L-02 cells, 131 DEGs between shRNA-CHOP and shRNA-NC in ERS L-02 cells. GO and KEGG enrichment were used to analysis the potential functions of dysregulated genes. We found 9 genes were both changed between ERS L-02 cells and normal L-02 cells and between shRNA-CHOP and shRNA-NC in ERS L-02 cells. They were DDIT3, CLGN, LCN2, LAMP3, KLHDC7B, FAM129A, IFI27, TARP and C11orf86. Their expression levels were also detected through qRT-PCR. At present, the relationship between these DEGs and DDIT3 was rarely reported and in our future work, the biological functions of these DEGs will be further researches. Our findings might

provide a foundation for the intense research of biological functions CHOP. In addition, according to these genes may provide new intervention targets for the treatment of liver diseases, and contribute to new design ideas for molecular targeted drugs used to treat liver injury.

Declarations

Conflicts of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. U1204826).

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Figures

Figure 1

Western blot analysis of CHOP. Note: A: control group B model group C: shRNA-1 D: shRNA-2 E: shRNA-3 F sh-NC group. Compared with control group: **P<0.01, Compared with model group: ##P<0.01.

Figure 2

General analyses of DEGs in control vs model, sh-CHOP vs sh-NC. Number of up-regulated and down-regulated DEGs.

Figure 3

General analyses of DEGs in model vs control, sh-CHOP vs sh-NC. (A) Intersection heatmap of DEGs for each cluster plan. (B) Union heatmap of DEGs for each cluster plan. Note:Each line represents a differential gene and different types of differential genes are indicated by different colors. Red indicates up-regulation and blue indicates down-regulation.

Figure 4

General analyses of DEGs in model vs control, EJ vs model groups. Note:X axis means number of DEGs (the number is presented by its square root value). Y axis represents GO terms. All GO terms are grouped

in to three ontologies: blue is for biological process, green is for cellular component and red is for molecular function.

Figure 5

The GO Terms enrichment of DEGs between shRNA-CHOP and shRNA-NC in ERS L-02 cells. Note: X axis means number of DEGs (the number is presented by its square root value). Y axis represents GO terms. All GO terms are grouped in to three ontologies: blue is for biological process, green is for cellular component and red is for molecular function.

Figure 6

Relative mRNA levels of representative DEGs. Note: A: control group B: model group C: sh-CHOP group D: sh-NC group Relative mRNA levels of representative DEGs (DDIT3, CLGN, LCN2, LAMP3, KLHDC7B, FAM129A, C11orf86, IFI27, TARP) were quantified using q-PCR. Compared with control group: * $p < 0.05$, ** $p < 0.01$. Compared with model group: ## $p < 0.01$.