J. Mens. Health **2022**; 18(1): 002
http://doi.org/10.31083/jomh.2021.051

Original Research

Identification of potential key genes associated with termination phase of rat liver regeneration through microarray analysis

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Submitted: 28 January 2021 Accepted: 12 March 2021 Published: 18 June 2021

Abstract

Background and objective: Liver regeneration (LR) is a complex process influenced by various genes and pathways, the majority of the of research on LR focus on the initiation and proliferation phase while studies on termination phase is lacking. We aimed to identify potential genes and reveal the underlying the molecular mechanisms involved in the precise regulation of liver size during the termination phase of LR. **Materials and methods**: We obtained the rat liver tissue gene datasets (GSE63742) collected following partial hepatectomy (PH) from the Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information (NCBI), from which, this study screened the late stage LR samples (7 days post-PH) using the R/Bioconductor packages for the identification of differentially expressed genes (DEGs). Afterwards, we performed enrichment analysis using the database for annotation visualization and integrated discovery (DAVID) online tool. Moreover, the Search Tool for the Retrieval of Interacting proteins (STRING) database was employed to construct protein-protein interaction (PPI) networks based on those identified DEGs; the PPI network was then used by Cytoscape software to predict hub genes and nodes. Animal experimentation (Rat PH model) was performed to acquire liver tissues which were then used for western blot analysis to verify our results. **Results**: The present study identified together 74 significant DEGs, among which, 51 showed up-regulation while 23 presented as down-regulated. As revealed by KEGG pathway enrichment analysis, DEGs were mostly related to pathways such as retinol metabolism, steroid hormone synthesis, transforming growth factor-β (TGF-β) and mitogen-activated protein kinase (MAPK) signaling. In addition, as suggested by GO enrichment analysis, DEGs were mostly related to the cyclooxygenase P450 pathway, negative regulation of Notch signaling pathway, aromatase activity, steroid hydroxylase activity, exosomes, and extracellular domain. Analyses based on STRING database and Cytoscape software identified genes like Ste2 and Btg2 as the hub genes in the termination stage LR. The obtained results were confirmed by Western blot analysis. **Conclusions**: Taken together, the microarray analysis in this study suggests that DEGs such as Ste2 and Btg2 are the hub genes, which are associated with the regulation of termination stage LR, while the molecular mechanisms are possibly related to the MAPK and TGF-β signal transduction pathways.

Keywords: Liver regeneration; Differentially expressed genes; Enrichment analysis; Protein-protein interaction networks

1. Introduction

When injury occurs, a series of signaling pathways in the liver are activated to initiate liver regeneration (LR), and the body's metabolic needs are satisfied by the rapid expansion of the remaining liver tissue. In 1931, Higgins *et al.* [1] first demonstrated its unique characteristics by performing animal experiments. After approximately 70% partial hepatectomy (PH), the remaining liver in rats could basically recover to the weight, volume and function before operation within 5–7 days, and then the cells returned to static state. It is considered that the whole process of liver regeneration includes three phases including initiation, proliferation and termination [2]. At the initiation phase, a series of cytokines including TNF-α and IL-6 act on hepatocytes to enhance their sensitivity to growth factors. Stimulation of various growth factors makes hepatocytes re-enter G1 phase and then enter proliferation phase [3]. When the function of liver tissue is sufficient to maintain the normal metabolic needs of the body, hepatocytes re-enter G0 phase and stop proliferation [4]. Currently, most of the researches on liver regeneration concentrate on the initiation signal in the early phase of regeneration, while the research on the termination signal in the late phase of regeneration remains relatively few. Therefore, an in-depth study of the negative regulatory signals and key genes involved in the late phase of liver regeneration will not only help to deepen the understanding of the process of liver regeneration, but also provide great significance in explaining the regulatory mechanism of liver tumor growth and screening potential therapeutic targets. In recent years, with the unprecedentedly rapid development of high-throughput sequencing and computer science and technology, it is possible to comprehensively utilize life science, computer and information technology to analyze the potential significance behind massive and complex biological data. In the current work, bioinformatics analysis was used to select the microarrays related to the termination of liver regeneration. Differentially expressed genes (DEGs) with statistical significance were selected and analyzed to find out the key genes and pathways that exert an important role in the process.

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2. Microarray analysis

2.1 Data sources

The gene expression data set (GSE63742) of rat liver tissue after PH operation was selected and downloaded by gene expression omnibus (GEO), a high-throughput gene expression database of the National Center for Biotechnology Information (NCBI), with "liver regeneration" as the key word. The data set contained altogether 57 samples at 9 time points after PH operation. In the present study, 6 samples (3 in sham-operation group and 3 in PH late phase group) were selected as the original data of gene expression at the termination phase of liver regeneration in rats.

2.2 Analysis methods

2.2.1 Quality verification

The "affyPLM" package of R language (https://www.r-project.org/) was employed to verify the quality of regression calculation of original data. Meanwhile, the results were evaluated and displayed using Relative log expression (RLE) box plot, normalized unscaled standard errors (NUSE) box plot and RNA degradation plot [5].

2.2.2 Data processing and differential gene screening

The "affy" and "limma" [6,7] packages of R language were used to process the original chip data as follows: ① Background correction and quantile homogenization; ② Screening differently expressed gene (DEGs) under the following conditions: Absolute value of Log2 (fold change, FC) >1.5 and adjusted $p < 0.001$; ③ Match the probe ID to gene name through the chip platform file. The analysis results were visualized by employing "ggplot2" package of R language.

2.2.3 KEGG and GO enrichment analysis

On-line tool DAVID database (https://david.ncifcrf.gov/home.jsp) [8] was adopted to analyze the enrichment of DEGs by Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene ontology (GO) [9,10]. GO enrichment analysis included Biological process (BP), Cellular component (CC) and Molecular function (MF). In addition, the analysis results were visualized by "ggplot2" package of R language.

2.2.4 Construction of protein-protein interaction network and screening of key genes

The DEGs obtained in the above steps was input into the STRING database (https://string-db.org/). At the same time, the protein-protein interaction network (PPI) was constructed on the condition of medium confidence of 0.400. PPI network information was downloaded and imported into the Cytoscape software. The key nodes (hub nodes) and key genes (hub genes) in the network were analyzed and predicted by using the cytohubba plug-in [11,12].

3. Animal experiment models and western blot assay verification

3.1 Animal experiment models

A total of 6 Rats (male, 8-week-old, 260–280 g, SD) acquired from Experimental Animal Center of Chongqing Medical university (Chongqing, China) and were maintained in specific pathogen free (SPF) barrier (sterile polycarbonate cages, sustained at 24 ± 2 °C with a 12-h light/dark cycle) with free access to water and food. Animal Management Rules of the Ministry of Health of the People's Republic of China were compiled and experimental models were approved by the Animal Care and Use Committee of Second affiliated Hospital of Chongqing Medical university.

PH Group underwent the procedure described by Higgins *et al.* [1]. Firstly, rats were given intraperitoneal injection of sodium pentobarbital (50 mg/kg) for anesthesia, then the left and middle lobes of the liver were ligated and resected (approx. 70% of rat liver weight). The same protocol was performed on the sham groups but without undergoing PH. At 7 days postoperatively (late phase of liver regeneration), rats were anesthetized and sacrificed, then the liver tissues were collected for Western blotting analysis.

3.2 Western blot analysis

The acquired liver tissues were lysed (RIPA lysis buffer and PMSF (Beyotime, Shanghai)), which were quantified using BCA protein quantitative kit (Beyotime, Shanghai). Protein samples was separated by electrophoresis of SDS-PAGE and then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were first blocked in 5% skim milk (37 °C, 1 h), incubated with primary antibodies against A1GB, (Invitrogen Product # PA5-88154) and CYP2C11 (Abcam product # ab3571) (4 °C, overnight) and then washed before being incubated with secondary antibodies (room temp, 1 h). Band visualization was obtained through the use of an enhanced chemiluminescence system (Thermo Scientific, USA). Comparisons between groups were undertaken on the basis of the unpaired Student *t* test. Results are expressed as means ± SD. $p < 0.05$ was considered statistically significant.

4. Results

4.1 Quality verification

As shown in Fig. 1, the RLE values in each group were relatively uniform and close to 0, while the NUSE values in each group were relatively uniform and close to 1. Typically, a greater deviation from the corresponding center implies a larger variability of supposed sample, thus meaning that the sample was invalid for further analysis. RNA degradation plot indicated that the degradation of 5' end was more significant than that of 3' end in each group, and the trend among each group was relatively parallel. All the above results demonstrated that the chip had excellent quality and stable detection results, which provided reliable

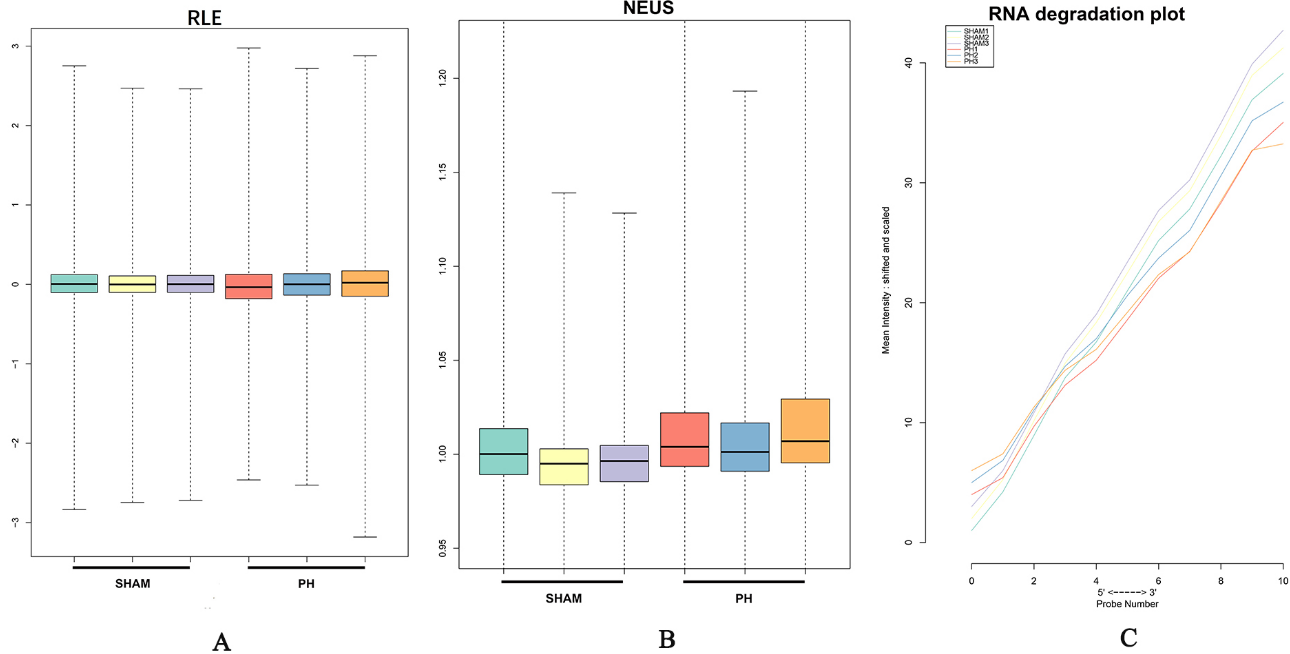

Fig. 1. Quality verification of Microarray. (A) In the RLE box plot, the boxes centered at 0 low quality arrays had great variability with large deviation from 0. (B) In the NEUS box plot, median standard errors from the PLM scaled to 1, the boxes centered at 1, median standard error >1 in low quality arrays. (C) RNA degradation plot of the microarray samples used.

data for subsequent analysis.

4.2 DEGs screening

Principal component analysis (PCA) was performed on the chip samples, and the results showed a significant difference between the two groups, with different characteristics (Fig. 2A). Cluster analysis of DEGs showed that the differences within the group were small and the repeatability was good. The gene expression pattern in PH group was significantly different from that in SHAM group. After normalizing the chip data, 51 genes were up-regulated and 23 genes were down-regulated, with a total of 74 DEGs. The results were displayed by volcano map (Fig. 2B). Gene expression across the samples can be visualized through the heatmap (Fig. 2C), green denotes up-regulation and red signifies down-regulation. Full details of the 74 expressed genes along with their fold change and ID given can be observed in the **Supplementary Table 1** along with p-values, average expression and gene title (**Supplementary Table 1**).

4.3 Enrichment analysis of KEGG and GO pathways of DEGs

The results of KEGG visualization analysis of DEGs (Fig. 3) showed that a large number of DEGs in LR termination phase were closely related to seven signaling and metabolic pathways, including steroid hormone synthesis, retinol metabolism, mitogen activated protein kinase signaling pathway and transforming growth factor-β. The results of GO enrichment analysis (Fig. 3) showed that in terms of biological process, DEGs was mainly associated with the negative regulation of Notch signaling pathway, and the positive regulation of cyclooxygenase P450 pathway and IL-8 secretion. In terms of cell components, it was mainly related to the extracellular region, basolateral membrane, exosomes and organelle membranes. Regarding molecular function, it was mainly related to aromatic enzyme activity, small molecule binding, arachidonic acid epoxidase activity and steroid hydroxylase activity.

4.4 PPI network construction and analysis

PPI network constructed by STRING database and analysis by Cytoscape software could further clarify the protein interactions involved in DEGs and the key genes involved in regulation. The results showed that (Fig. 4) the PPI network contained 46 relationships of 67 node proteins, among which most proteins had interaction relationships. The cytohubba plug-in was used to screen key nodes and genes in the PPI network. The top 10 key genes included Cyp2c13, Ste2, Mup5, LOC259244, Rup2, Hsd3b5, UST4r, Btg2, Cyp2c11 and Myc.

4.5 Western blot verification

A total of 74 genes were identified as differentially expressed through microarray analysis. Two genes, cytochrome P450, subfamily 2, polypeptide 11 (Cyp2c11) , alpha-1-B glycoprotein (A1bg) were chosen, due to the fact that they were the most up and down-regulated genes respectively in our analysis. In the western blot analysis, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. Western blotting showed that

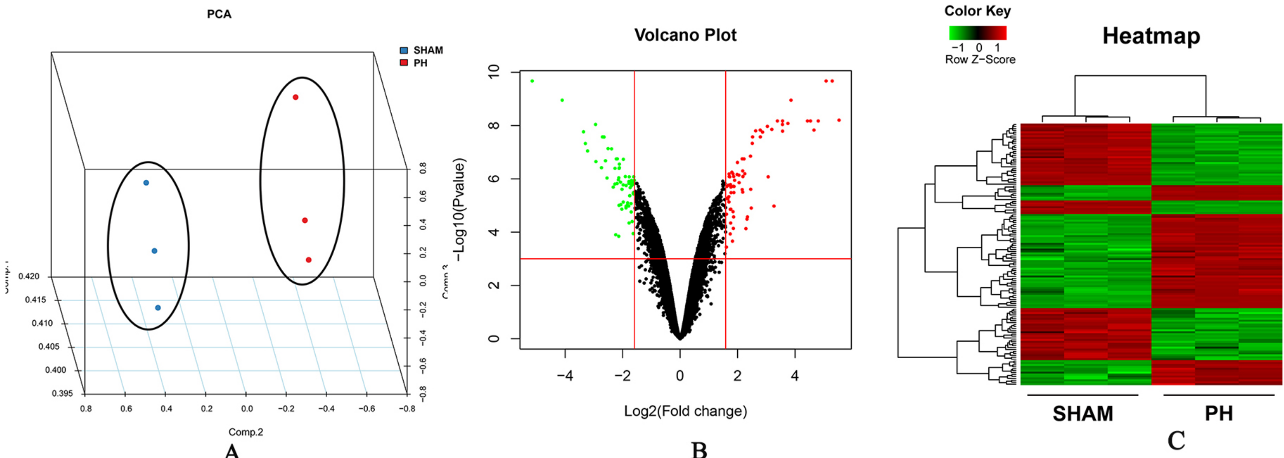

Fig. 2. PCA and DEGs screening at the termination phase of liver regeneration and in sham operation control group. (A) PCA on sham-operation group and PH group. (B) Volcano plot. Black represents non-DEGs, red stands for up-regulated DEGs, and green represents down-regulated DEGs. (C) Hierarchical clustering heatmap of the 74 up- and down-regulated DEGs screened in the GSE63742 dataset. Each row represents one gene, the right column represents for PH samples, the left column represents SHAM samples.

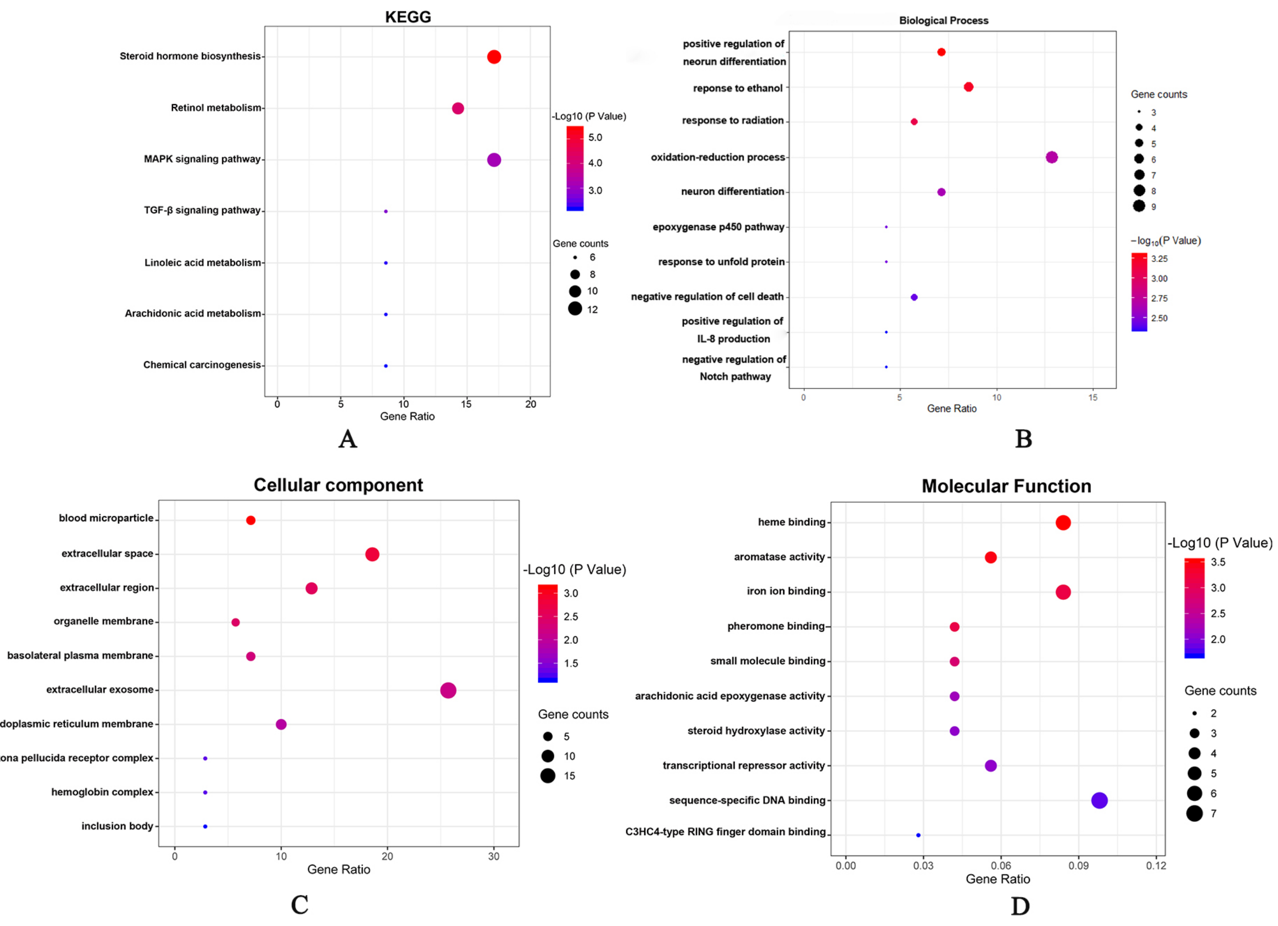

Fig. 3. KEGG and GO enrichment analyses on DEGs. (A) Distribution of DEGs associated with the termination phase of LR in different KEGG pathways. (B) Distribution of DEGs associated with the termination phase of LR in biological processes (BP) of GO category. (C) Distribution of DEGs associated with the termination phase of LR in the cell component (CC) of GO category. (D) Distribution of DEGs associated with the termination phase of LR in molecular function (MF) of GO category.

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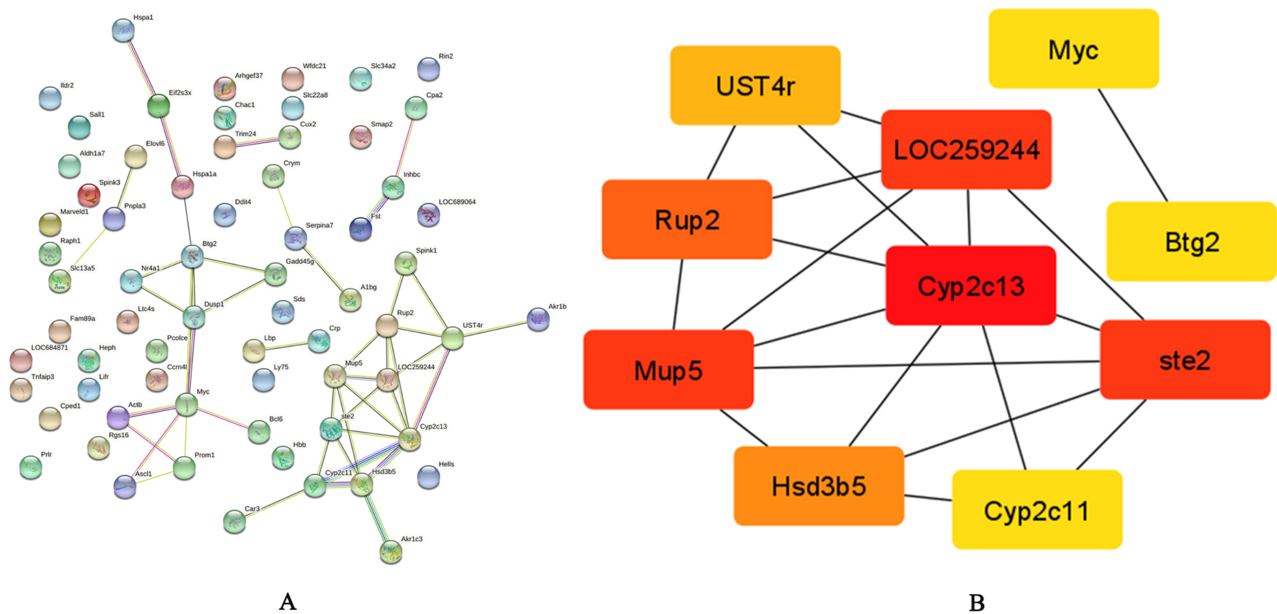

Fig. 4. Construction of protein-protein interaction (PPI) network and hub gene analysis on DEGs associated with the termination phase of liver regeneration. (A) Construction of PPI network using the STRING tool (medium confidence of 0.400. PPI). (B) The top 10 hub genes predicted using the Cytoscape software by adopting the cytohubba plug-in.

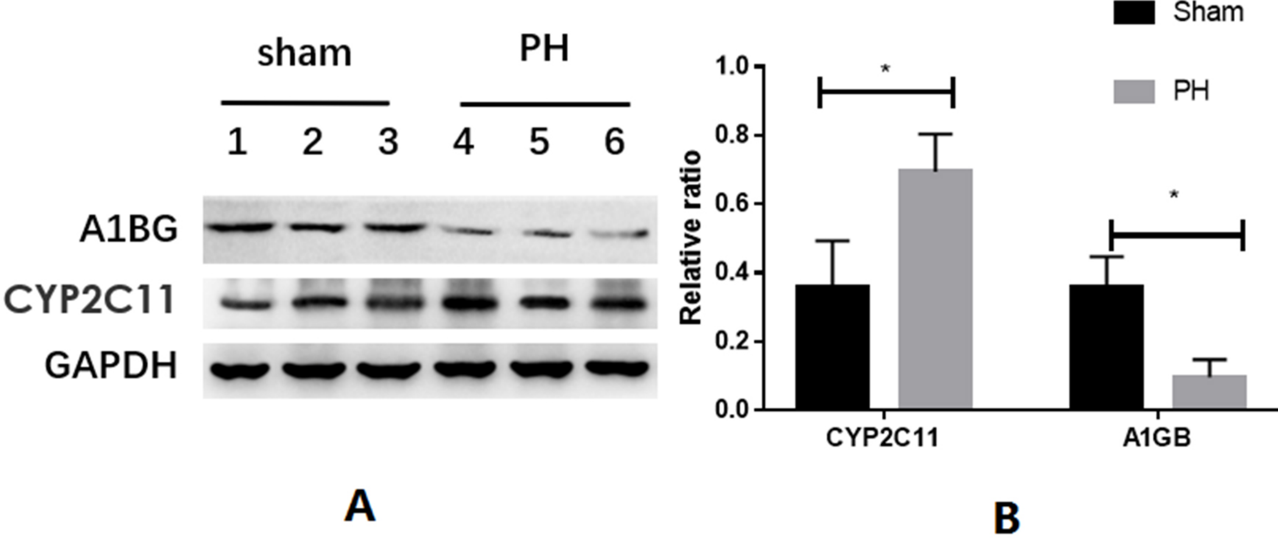

Fig. 5. Western blotting Verification results. (A) Representative Western blotting analysis on the expression of A1GB, Cyp2c11 and GAPDH (loading control) in the liver of male rats at 6 days after PH. (B) Relative staining intensity ratios of A1GB and Cyp2c11 compared between PH group and SHAM group. Data represent mean ± SD. for PH group. *$p < 0.05$, versus SHAM group.

Cyp2c11 was upregulated and A1bg was downregulated in the PH group compared with the SHAM groups, (*$p < 0.05$), which is consistent with the results of our microarray analysis (Fig. 5).

5. Discussion

Before extracting data, it is of necessity to verify the chip quality at first and then find and reject the problematic chips to ensure the reliability of subsequent analysis. RLE and NUES can reflect the consistency of parallel experiments. An RLE plot exhibits the difference between a gene's expression level and the median for that gene, assuming that most genes are not differentially expressed. The boxes should be close to 0. A NUSE plot however is scaled so that the median standard error across arrays is 1 for each gene and thus boxes should be close to 1 [13]. If there is a large deviation in the box plot of a chip, it indicates that there is a problem with the chip [14]. RNA degradation

is another important factor which can affect chip quality. Since RNA degradation starts from the 5' end, the fluorescence intensity at the 5' end is much lower than that at the 3' end [15]. To assess the viability of the microarray using the RNA degradation plot, we look at the parallelism and similarities between samples slopes in which an extreme difference in one or more of the samples slopes means that the sample is not viable for use. The detection results of the above three indicators show that the chips included in this analysis are reliable in quality, and the batch difference of detection results is small, thus providing credible original data for performing subsequent analysis.

In the proliferation phase, both the regenerated liver and liver tumors show the strong proliferation ability of liver cells. However, because tumor cells lack the negative regulation mechanism for termination, their cells can divide indefinitely. Liver regeneration is a complex and delicate process regulated by multiple factors. When the regeneration level can satisfy the body's metabolic needs, it will trigger a termination signal to stop cell proliferation. In the present study, altogether 74 DEGs were selected from the rat liver microarray at the termination phase of liver regeneration, further validated by Western blotting assay, and analyzed by KEGG and GO enrichment analyses, so as to reveal the roles of these genes at the termination phase of liver regeneration. Among the seven pathways screened by KEGG analysis, MAPK and TGF-β signal pathways were the most noteworthy. MAPK family is a group of serine-threonine protein kinases which can be activated by various extracellular stimuli, and is mainly involved in a lot of important physiological/pathological processes such as cell growth, differentiation, stress and inflammatory reaction. However, it has been reported that p38, as a MAPK subfamily, exert an important role in proliferation, which is closely related to the pathophysiological environment in which hepatocytes live. Studies conducted by Campbell *et al*. [16] showed that p38α is a negative regulator of hepatocyte proliferation. In the liver regeneration model of mice after PH operation with specific liver cell knockout, the early phase of hepatocyte regeneration exhibits enhanced proliferation activity, and its mechanism may be related to the antagonism of p38α against JNK-c-Jun pathway activity. In the model of chronic biliary cirrhosis, the specific knocking out of liver cells p38α will lead to mitotic retardation and cytokinesis failure, thus reducing the proliferation of liver cells and ultimately lowering the life span of mice [17].

Fortier *et al*. [18] confirmed through the CCl4-induced acute liver injury model that specific knockout of p38α of hepatocytes altered the immune microenvironment after injury, enhanced the infiltration of inflammatory cells, and mediated the formation of immune microenvironment conducive to liver tissue repair through the chemotaxis of CCL2/CCL5 cytokines. Studies have proved that TGF-β signaling pathway is an important signal to inhibit the proliferation of hepatocytes for the reason that it is closely related to the activation of hepatic stellate cells (HSCs). As the most important non parenchymal cells in the liver microenvironment, HSCs are involved in the pathophysiological processes of liver fibrosis, hepatocellular carcinoma and liver regeneration after transdifferentiate into myofibroblasts (MFS). Inhibition of TGF-β pathway interferes with epithelial mesenchymal transition of HSCs during liver regeneration and reduces the nuclear aggregation of β-Catenin and the expression of cytochrome P450, resulting in delayed proliferation. The activation of this pathway can directly promote liver fibrosis [19,20]. A transcriptome analysis of HCC indicated that 40% of samples have TGF-β signaling pathway gene mutation, and up-regulation of this pathway will promote inflammation and fibrosis, while down-regulation will reduce tumor inhibitory activity and significantly shorten the survival time of patients [21]. In addition, recent research results also confirm our GO enrichment analysis results. Notch signaling pathway can promote the proliferation of hepatocytes. Overexpression of Notch-1 can up-regulate the expression of cyclin A1, D1, E and other cyclins [22]. The activation of Notch pathway in hepatocytes is the key condition of liver homeostasis, metabolism, regeneration, vascular physiology and biliary tract morphogenesis [23]. Notch pathway in liver is significantly activated after PH operation, and inhibition of this pathway can lead to the imbalance of cell cycle and its related proteins, and the mechanism involved may be in association with NICD/Akt/HIF-1α pathway [24]. Exosomes derived from hepatocytes can promote intercellular communication during regeneration, and exosomes derived from bone marrow mesenchymal stem cells can inhibit HSCs activation through Wnt/β-catenin pathway, consequently alleviating CCl4-induced liver fibrosis [25,26]. At the end of liver regeneration, the molecular function of DEGs is enriched in the activities of various metabolic related enzymes including aromatase and steroid hydroxylase, also indicating that the most important metabolic function of liver has been restored.

In comparison with the control group, the top 10 hub genes related to the termination phase of liver regeneration screened from PPI network were all up-regulated. Ste2 gene regulates the expression of estrogen sulfotransferase 2, maintaining the steady state of estrogen by sulfonating and inactivating estrogen. It has been reported that this enzyme is the direct transcription target of Nrf2. The latest research shows that Nrf2 is activated in the early phase of tumorigenesis in rat model of fatty hepatitis with fibrosis, and knockout of Nrf2 gene can inhibit the amplification of initial cell clone, which can thus prevent the occurrence of HCC [27,28]. This may be the reason for the increased expression of the gene in the late phase of liver regeneration. A study conducted by Zhao *et al*. [29] showed that tumor-like growth in the early phase of liver development is positively and negatively coordinated by α-2u globulin (extra-

cellular domain)/ppp2r2a-pik3c3 (MAPK signaling pathway)/Hsd3b5 (metabolic pathway), evidently showing that Hsd3b5 gene plays an important role in the biosynthesis of all steroid hormones. In addition, another article by Xiu *et al*. [30] also showed a significant expression decrease in Hsd3b5 after lowering serum testosterone and estrogen. It is of note that the expression level of Myc, as a oncogene, is still up-regulated at the termination phase of liver regeneration. The sustained overexpression of c-Myc caused by the deletion of hepatocyte nuclear factor 4α (HNF4 α) will eventually lead to the non-functional continuous proliferation of liver after PH operation [31]. Moreover, it also indirectly confirmed that there must be a series of negative modulation signals against its proliferation-promoting effect at this phase. In liver cells, Btg2 as a cell cycle inhibitor can prevent FoxM1 activation and inhibit DNA synthesis [32]. MiR-6875-3p can directly inhibit Btg2 expression and up-regulate FAK/Akt pathway activity, which can thus promote HCC invasion and metastasis [33]. PRMT5 can promote tumor proliferation by down-regulating Btg2 expression through ERK pathway [34]. The above results confirm the positive and negative effects of some hub genes in the termination phase of liver regeneration. However, there are no related reports concerning the roles of Mup5, Rup2 and UST4r genes in liver regeneration and HCC, which need to be further explored.

6. Conclusions

To conclude, liver regeneration is a complex biological process regulated by multiple factors. The main feature of liver regeneration is that it can stop proliferation and produce functional hepatocytes in accordance with the needs of the body at the end phase. In this study, we successfully screened and analyzed the DEGs in rat liver tissue at the end phase of liver regeneration, and preliminarily understood its related functions and signal pathways. In the meanwhile, we also analyzed the hub genes with negative regulatory effect, such as Ste2, Btg2, and Hsd3b5, thus providing a new idea for the screening of liver tumor research targets and the exploration of pathological mechanism.

Abbreviations

DEGs, Differentially expressed genes; GEO, Gene Expression Omnibus; HCC, Hepatocellular carcinoma; HNF4 α, Hepatocyte nuclear factor 4α; HSCs, Hepatic stellate cells; LR, Liver regeneration; MAPK, Mitogen-activated protein kinase; MFS, Myofibroblasts; NUSE, Normalized unscaled standard errors; PH, Partial hepatectomy; PPI, Protein-protein interaction; RLE, Relative log expression; STRING, Search Tool for the Retrieval of Interacting proteins; TGF-β, transforming growth factor-β.

Author contributions

JG designed and supervised the study. HS. and MW. contributed to the implementation of the research, analysis of the results and writing of the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

All animals' care and experimental protocols were complied with the Animal Management Rules of the Ministry of Health of the People's Republic of China and all experimental designs were approved by the Animal Care and Use Committee of Second affiliated Hospital of Chongqing Medical university.

Acknowledgment

We thank the Experimental Animal Center of Chongqing Medical university (Chongqing, China) for providing us with animals required and we would also like to thank all the peer reviewers for their opinions and suggestions.

Funding

This study was supported by grants from nature science foundation of Chongqing (no: cstc2019jcyj-zdxmX0027).

Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/JOMH/18/1/10.31083/jomh.2021.051.

References

[1] Higgins GM, Anderson RM. Experimental pathology of liver: restoration of liver of white rat following partial surgical removal. Archives of Pathology & Laboratory Medicine. 1931; 12: 186–202.

[2] Forbes SJ, Newsome PN. Liver regeneration-mechanisms and models to clinical application. Nature Reviews Gastroenterology & Hepatology. 2016; 13: 473–485.

[3] Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology. 2006; 43: S45–S53.

[4] Mao SA, Glorioso JM, Nyberg SL. Liver regeneration. Translational Research. 2014; 163: 352–362.

[5] Brettschneider J, Collin F, Bolstad BM, Speed TP. Quality assessment for short oligonucleotide microarray data. Technometrics. 2008; 50: 241–264.

[6] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, *et al*. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research. 2015; 43: e47.

[7] Gautier L, Cope L, Bolstad BM, Irizarry RA. Affy-analysis of Affymetrix GeneChip data at the probe level. Bioinformatics. 2004; 20: 307–315.

[8] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols. 2009; 4: 44–57.

[9] Kanehisa M. Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Research. 2000; 28: 27–30.

[10] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, *et al*. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nature Genetics. 2000; 25: 25–29.

[11] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Research. 2003; 13: 2498–2504.

[12] Vella D, Zoppis I, Mauri G, Mauri P, Di Silvestre D. From protein-protein interactions to protein co-expression networks: a new perspective to evaluate large-scale proteomic data. EURASIP Journal on Bioinformatics & Systems Biology. 2020; 2017: 6.

[13] Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010; 11: R106.

[14] Gandolfo LC, Speed TP. RLE plots: visualizing unwanted variation in high dimensional data. PLoS ONE. 2018; 13: e0191629.

[15] Fasold M, Binder H. Estimating RNA-quality using GeneChip microarrays. BMC Genomics. 2013; 13: 186.

[16] Campbell JS, Argast GM, Yuen SY, Hayes B, Fausto N. Inactivation of p38 MAPK during liver regeneration. The International Journal of Biochemistry & Cell Biology. 2011; 43: 180–188.

[17] Tormos AM, Arduini A, Talens-Visconti R, del Barco Barrantes I, Nebreda AR, Sastre J. Liver-specific p38α deficiency causes reduced cell growth and cytokinesis failure during chronic biliary cirrhosis in mice. Hepatology. 2013; 57: 1950–1961.

[18] Fortier M, Cadoux M, Boussetta N, Pham S, Donné R, Couty JP, *et al*. Hepatospecific ablation of p38alpha MAPK governs liver regeneration through modulation of inflammatory response to CCl4-induced acute injury. Scientific Reports. 2019; 9: 14614.

[19] Chen Z, Wan L, Jin X, Wang W, Li D. Transforming growth factor-β signaling confers hepatic stellate cells progenitor features after partial hepatectomy. Journal of Cellular Physiology. 2019; 235: 2655–2667.

[20] Mehta KJ, Coombes JD, Briones-Orta M, Manka PP, Williams R, Patel VB, *et al*. Iron Enhances hepatic fibrogenesis and activates transforming growth factor-beta signaling in murine hepatic stellate cells. The American Journal of the Medical Sciences. 2018; 355: 183–190.

[21] Chen J, Zaidi S, Rao S, Chen JS, Phan L, Farci P, *et al*. Analysis of genomes and transcriptomes of hepatocellular carcinomas identifies mutations and gene expression changes in the transforming growth factor-beta pathway. Gastroenterology. 2018; 154: 195–210.

[22] Wang C, Qi R, Li N, Wang Z, An H, Zhang Q, *et al*. Notch1 signaling sensitizes tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in human hepatocellular carcinoma cells by inhibiting Akt/Hdm2-mediated p53 degradation and up-regulating p53-dependent DR5 expression. The Journal of Biological Chemistry. 2009; 284: 16183–16190.

[23] Morell CM, Strazzabosco M. Notch signaling and new therapeutic options in liver disease. Journal of Hepatology. 2014; 60: 885–890.

[24] Zhang F, Zhang J, Li X, Li B, Tao K, Yue S. Notch signaling pathway regulates cell cycle in proliferating hepatocytes involved in liver regeneration. Journal of Gastroenterology and Hepatology. 2018; 33: 1538–1547.

[25] Balaphas A, Meyer J, Sadoul R, Morel P, Gonelle-Gispert C, Bühler LH. Extracellular vesicles: future diagnostic and therapeutic tools for liver disease and regeneration. Liver International. 2019; 39: 1801–1817.

[26] Rong X, Liu J, Yao X, Jiang T, Wang Y, Xie F. Human bone marrow mesenchymal stem cells-derived exosomes alleviate liver fibrosis through the Wnt/β-catenin pathway. Stem Cell Research & Therapy. 2019; 10: 98.

[27] Guo Y, Hu B, Huang H, Tsung A, Gaikwad NW, Xu M, *et al*. Estrogen sulfotransferase is an oxidative stress-responsive gene that gender-specifically affects liver ischemia/reperfusion injury. Journal of Biological Chemistry. 2015; 290: 14754–14764.

[28] Orrù C, Szydlowska M, Taguchi K, Zavattari P, Perra A, Yamamoto M, *et al*. Genetic inactivation of Nrf2 prevents clonal expansion of initiated cells in a nutritional model of rat hepatocarcinogenesis. Journal of Hepatology. 2018; 69: 635–643.

[29] Zhao X, Zhuo H. ECR-MAPK regulation in liver early development. BioMed Research International. 2015; 2014: 850802.

[30] Wang XJ, Chamberlain M, Vassieva O, Henderson CJ, Wolf CR. Relationship between hepatic phenotype and changes in gene expression in cytochrome P450 reductase (POR) null mice. The Biochemical Journal. 2005; 388: 857–867.

[31] Huck I, Gunewardena S, Espanol-Suner R, Willenbring H, Apte U. Hepatocyte nuclear factor 4 alpha activation is essential for termination of liver regeneration in mice. Hepatology. 2019; 70: 666–681.

[32] Song G, Sharma AD, Roll GR, Ng R, Lee AY, Blelloch RH, *et al*. MicroRNAs control hepatocyte proliferation during liver regeneration. Hepatology. 2010; 51: 1735–1743.

[33] Xie Y, Du J, Liu Z, Zhang D, Yao X, Yang Y. MiR-6875-3p promotes the proliferation, invasion and metastasis of hepatocellular carcinoma via BTG2/FAK/Akt pathway. Journal of Experimental & Clinical Cancer Research. 2019; 38: 7.

[34] Jiang H, Zhu Y, Zhou Z, Xu J, Jin S, Xu K, *et al*. PRMT5 promotes cell proliferation by inhibiting BTG2 expression via the ERK signaling pathway in hepatocellular carcinoma. Cancer Medicine. 2018; 7: 869–882.