Large-Scale Quantitatively Detecting and Analyzing of ceRNAs reveal NOTCH3 mRNA, miR-369-3p and rno-Rmdn2_0006 Together Regulate the Hepatocytes in G0 Phase and in G1 Phase During the Initiation Stage of Rat Liver Regeneration

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Research Article

Keywords: Rat liver regeneration, Hepatocytes in G0 phase or in G1 phase, ceRNA comprehensive analysis, Neurogenic loci notch homologous protein 3 (NOTCH3), cicRNA-miRNA regulatory axis

Posted Date: August 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-794431/v1

Abstract

Aims:

Generally, key events of the liver regeneration initiation (LRI) are how the hepatocytes in G0 phase change to G1 phase. We use the data from large-scale quantitatively detecting and analyzing (LQDA) to reveal ceRNAs together regulate the hepatocytes in G0 phase or in G1 phase during LRI.

Main methods:

The hepatocytes of the rat liver right lobe were isolated at 0 hour, 6 hour and 24 hour after partial hepatectomy (PH), their ceRNA expression abundance was measured by LQDA, and the correlation of their expression, interaction and role were revealed by ceRNA comprehensive analysis.

Key findings:

It was found that expression of NOTCH3 mRNA is up-regulated at 0 h, but miR-369-3p and rno-Rmdn2_0006 of hepatocytes not change significantly at that, meanwhile, the G0 phase-related gene *CDKN1c* promoted by NOTCH3 up-regulation, and the G1 phase-related gene *PSEN2* inhibited by NOTCH3 down-regulation at that. On the contrary, NOTCH3 mRNA and rno-Rmdn2_0006 were up-regulated at 6 h, but miR-136-3p was down-regulated at that, the G1 phase-related genes *CHUK, DDX24, HES1, NET1* and *STAT3* promoted by NOTCH3 up-regulated, and the G0 phase-related gene *CDKN1a* inhibited by NOTCH3 down-regulated at that. These results suggested that the ceRNAs and the NOTCH3-regulated G0 phase- and G1 phase-related genes show a correlation in expression, interaction and role, they together regulate the hepatocytes in G0 phase at 0 h and in G1 phase at 6 h.

Significance:

These discovers are helpful to understand the mechanism which ceRNA together regulate the hepatocytes in G0 phase or G1 phase.

1. Introduction

Liver is an important organ of higher animals and contains hepatocytes, bile duct epithelial cells, oval cells, astrocytes, sinus endothelial cells, Kupffer cells, lacuna cells, dendritic cells, etc. Of them, the hepatocytes account for about 80% of the total number of liver cells and 75% of the dry weight of the liver (Amelia, 2015). Liver has functions of metabolism, detoxification, defense, etc. (Amelia F, 2015; Forbes et al., 2016),and a strong ability to regenerate (Michalopoulos et al., 1997). When liver is injured by some factors, for example, surgery, trauma, infection, necrosis etc., the number of hepatocytes are decreased sharply, and various feedback signals stimulate them to rapidly change from static state to grow state, so that the lost or damaged liver tissue is restored, including its structure and function (Hu et al., 2014; Riddiough et al., 2020), that is called as liver regeneration (Fausto, 2012).

The non-coding RNA (ncRNA) is a class of RNA found in etonymeal organisms in recent years with important physiological functions (Hombach et al., 2016). Of them, microRNA (miRNA) is a group of single-stranded RNA with a length of about 22 nt, which can bind to mRNA and inhibit its role (Chipman et al., 2019; Afonso-Grunz et al., 2015; Chen et al., 2016). For instance, Chen et al. discovered that miR-21 binds to PTEN mRNA, inhibits PTEN formation, promotes hepatocytes proliferation and LR (Chen et al., 2016). Tao et al. reported that miR-612 inhibits the proliferation and migration of hepatocellular carcinoma (HCC) by acting on AKT2 mRNA (Tao et al., 2013). Chen et al. found that miR-1 promotes cell proliferation by targeting histone deacetylase 4 (HDAC4), but miR-133 enhances cell proliferation by inhibiting serum reactive factor (SRF) (Chen et al., 2006).

Meanwhile, another class of ncRNA found in recent years was named as circular RNA (circRNA). They were formed by the reverse splicing of 5'and 3'ends of the linear RNA, was as miRNA sponge to bind with miRNA (Panda et al., 2018), and showed important physiological functions (Hansen et al., 2013). For instance, Guo et al. found that circ_03848, circ_08236, circ_13398 and circ_15013 regulate cell proliferation through binding with several miRNAs (Guo et al., 2019). Li et al. reported that circ137 and circ2270 regulate hepatocyte proliferation by interacting with miR-127 (Li et al., 2017). Shang et al. found that hsa_circ_0005075 and hsa-miR-23b-5p regulate proliferation, invasion and metastasis of liver cancer cells through the circRNA-miRNA-mRNA axis (Shang et al., 2016).

On the other hand, neurogenic loci notch homologous protein 3 (NOTCH3), a transcrip factor with n-terminal extracellular domain (ECD), intermediate transmembrane domain and c-terminal intracellular domain (NICD) (Fleming., 1998; Hosseini-Alghaderi et al., 2020; Palermo et al., 2014), was one number of NOTCH family. One of its functions is to regulate cell proliferation. (Tang et al., 2019; Jing et al., 2019; Su et al., 2019; Hassan et al., 2016; Jung et al., 2011; Serafin et al., 2011). For example, Tang et al. found that inhibition of *NOT*CH3 reduce the rate of cell proliferation in osteosarcoma cells (Tang et al., 2019). Jing et al. found that increased *NOTCH3* expression leads to goblet cell proliferation, and decreased *NOTCH3* expression leads the proliferation of goblet cell low (Jing et al., 2019). Su et al. found that *NOTCH3* methylation reduce cell viability and tumor cell proliferation (Su et al., 2019). Hassan et al. found that non-small-cell type lung carcinoma cells (NSCLC) exhibit high *NOTCH3* expression and cell cycle arrest when *NOTCH3* expression is reduced (Hassan et al., 2016; Jung et al., 2011). Serafin et al. found that *NOTCH3* over-expression can promote the proliferation of colorectal cancer cells, but cell is in arrest when it is inhibited (Serafin et al., 2011).

To investigate the together regulatory role of ceRNAs to LR, this paper detected their expression changes by high-throughput biotechnology, analyzed their expression correlation by bioinformatics and systemic biological methods, constructed their interaction networks by Cytoscape 3.2 software, and revealed their role following the above detection and analysis.It was found that rno-Rmdn2_0006 and miR-369-3, influence expression of the G0 phase- and G1 phase-related genes which are regulated by NOTCH3, and the status of hepatocytes in G0 phase and in G1 phase. The results are reported as follows.

2. Materials And Methods

2.1. Preparation of the rat liver regeneration model induced by 2/3 hepatectomy

The 2/3 hepatectomy (partial hepatectomy, PH) was performed as described by Higgins (Higgins et al., 1931). The male Sprague-Dawley rats of 10 weeks old and 250 ± 10 g weight were used in the experiments. The liver right lobe of six rats were taken at 0 hour, 6 hour and 24 hour after PH, and were mixed at the corresponding time point. At the same time, the sham operation (SO) was set up as control. All experimental procedures in this study were carried out according to The guidelines for the protection and use of experimental animals published by the Ministry of Science and Technology of the People's Republic of China.

2.2. Isolation and identification of the rat hepatocytes

According to smedsrod et al (Xu et al., 2009), at 9:00–11:00 in the morning, the rats with PH and SO recovered to the experimental time were perfused under aseptic conditions, digested with collagenase, collected cells and placed in 60% saline solution methods, hepatocytes were centrifuged by Percoll (200g, 5min). Cell viability was evaluated by trypan blue staining. Cell purity was evaluated by fluorescence immunochemistry of Cy3 labeled ALB and G6P. G0 phase hepatocytes were identified by fluorescence immunochemistry of FITC labeled PCNA and Cy3 labeled G6P. G1 phase hepatocytes were identified by fluorescence immunochemistry of FITC labeled CCND1 and Cy3 labeled G6P. S-phase hepatocytes were identified by fluorescent immunochemistry with ccna2 and Cy3 labeled G6P.

2.3. A large-scale quantitative detection of mRNA

The total RNA was extracted, the ribosomal RNA was removed, the integrity of RNA was evaluated, and the mRNAs were detected. Rat genome 2302.0 chip was used for detection, and the detection was repeated 3 times. The ratio of gene expression was calculated with the mRNA signal value of regenerating liver cells 24 h after PH recovery as control (Cimica et al., 2007). The difference of mRNA expression between PH group and so group was calculated by *F* test (*P* value). The genes with *P* value ≤ 0.05 were regarded as liver regeneration related genes. T test was used to calculate the difference of mRNA expression at 0 h and 6 h after PH (*P* value) (Nishimoto et al., 2013). When *p* value ≤ 0.05, the difference is significant; when *p* value ≤ 0.01, the difference is extremely significant.

2.4. A large-scale quantitative detection of miRNA

The total RNA was extracted, the ribosomal RNA was removed, the integrity of RNA was evaluated, the agarose electrophoresis of samples was performed, the 25 b p RNA was recovered, the library was built and the single end sequencing was performed. Sequencing was performed according to truseq stranded total RNA with ribo zero gold (Illumina, USA). Then, quality control of Q20, removal of splice sequences and sequences less than 15 b p and more than 41 b p in length, filtering out reads containing N base, database analysis, sequence annotation, quantitative miRNA, etc. The ratio value of miRNA, correlation of liver regeneration (*P* value) and expression difference (*P* value) were calculated according to "materials and methods 3".

2.5. A large-scale quantitative detection of circRNA

The total RNA was extracted, the ribosomal RNA and linear RNA were removed, the integrity of RNA was evaluated, the library was built, the circRNA was sequenced, the 150 / 125 b p terminal pairs (reads) were obtained, the sequence was read, matched, quantified, annotated, the sequence reliability was verified, the chromosome was located, and the circRNA was quantified. Then, the ratio value, correlation of liver regeneration (*P* value) and expression difference (*P* value) were determined according to "materials and methods 3".

2.6. Prediction of G0 phase- and G1 phase-related genes of liver regeneration relation

The NCBI website (https://www.ncbi.nlm.nih.gov/) and IPA software (Ingenuity Pathway Analysis) were used to predict G0 phase- and G1 phase-related genes. Then, the two predicted genes were integrated to get the type and quantity list of G0 and G1 related genes. Then, they were compared with the meaningful expression, differential expression and liver regeneration related genes (tables) of "materials and methods 3" in this paper, and the genes in both tables were obtained. They were regarded as G0/G1 related genes of liver regeneration.

2.7. Screening of the transcription factors of the liver regeneration relation, which regulate the cellular G0 phase and G1 phase

The list of transcription factors was obtained by screening NCBI website, then, their mRNAs were compared with the mRNA list of “materials and methods 6” of this paper to get their significant expression, and difference significantly or extremely at 0 hour and at 6 hour during LRI. This paper selectively analyze NOTCH3 mRNA content and how it is regulated by miRNA and circRNA.

2.8. Prediction of G0 phase- and G1 phase-related genes regulated by NOTCH3

The cistrome data browser website and IPA software were used to predict the G0 phase- and G1 phase-related genes of NOTCH3 regulation. Then, the genes obtained from the two were integrated to obtain the list of types and numbers of genes related to G0 and G1 regulated by NOTCH3. Then, they were compared with the meaningful expression, differential expression and liver regeneration related genes (tables) of "materials and methods 3" in this paper, and the genes in both tables were obtained. These genes were regarded as the G0/G1 phase related genes regulated by NOTCH3 and related to liver regeneration.

2.9. Prediction of miRNAs binding with NOTCH3 mRNA

The IPA software and the miRwalk website (http://mirwalk.umm.uni-heidelberg. de/.) were used to predict miRNAs binding with NOTCH3 mRNA. The total number and name of miRNAs, named as “theoretical miRNAs”, were listed by integrating the above two prediction lists. Finally, the "theoretical miRNA" was compared with the detection results of " materials and methods 4" in this paper, and the miRNA with significant or extremely significant expression difference at 0 h and/or 6 h was found, which was called "detection miRNA". The distribution of the "detection miRNA" in the NOTCH family was obtained by comparing with the miRNA binding to the mRNA of each member of the NOTCH family. The miRNA that only binds to NOTCH3 mRNA was selected as "target miRNA" for analysis.

2.10. Interaction network construction of ceRNAs

In this paper, miRNA-bound circRNA was predicted by miRanda software. Generally speaking, the destination miRNA sequence is input into miRNA (s) box of miRanda software, click on Species, Rat and Go in order to get the matching degree and binding energy between the destination miRNA and circRNA. miRNA/circRNA pairs with matching degree (Max Score) ≥ 150 and binding energy (Max Energy)≤-30 are considered as Mirna/circrna pairs. Then, compared with the results of "materials and methods 5", circRNA with significant or very significant expression difference at 0H and/or 6H was found, which is called "detection circRNA". Comparing the distribution of "detection circRNA" in "destination miRNA", we select the circRNA only combined with the latter, called "destination circRNA ", for follow-up analysis.

2.11. Interaction network construction of ceRNAs

The ceRNA interaction networks were constructed with Cytoscape 3.2 software. To summarize, the matchs of NOTCH3 mRNA and its bound miRNAs, miRNAs and their sponge melecular circRNAs were listed respectively, and the documents were saved. Then, click File–Import–Network–File, and load the above documents into the analysis bar respectively. At the same time, the column1 is set as the analysis condition of Source Node, and the column2 as Target Node, and then clicked Apply box to obtain their interaction network diagram. Then, click style-Shape to adjust the Shape of the network graph, and click Fill Color to adjust the Color of the network graph, and click File and Import to get the network graph of interaction.

2.12. Statistical analysis

In this paper, the ratio values of ceRNAs were culculated by which the gene expression signal values of control divide that of experimental group, the relative ceRNAs of liver regeneration were calculated by ratio values of ceRNAs and *F*-test of SPSS 17.0 software, the expression difference of ceRNAs at 0 hour and at 6 hour after PH were culculated by ratio values of ceRNAs and *t*-test of SPSS 17.0 software (Nishimoto et al., 2013).

3. Results

3.1. The rat liver regeneration and its hepatocytes

In this paper, a model of rat 2/3 hepatectomy(partial hepatectomy, PH)was prepared according to the method of Higgins et al (Higgins et al., 1931), the liver right lobe of rat was taken at 0 hour, 6 hour and 24 hour after PH (Fig. 1A), its hepatocytes were separated, and their mRNAs, miRNAs and circRNAs were detected quantitively by high-throughput biotechnology (Fig. 1B). The results showed that the growth index of the regenerated liver was consistent with the report, the activity and purity of the isolated liver cells were ≥ 95%, and the cytochemical characteristics of the G0, G1 and S liver cells were consistent with the report (Fig. 1).

3.2. Interactions of NOTCH3 mRNA with miRNAs, circRNAs and other mRNA of hepatocytes

The combine of ceRNAs was analyzed by miRwalk website and miRanda software. The results showed that 131 kinds of miRNA, which are bound with NOTCH3 mRNA, are significantly expressed at 0 h and at 6 h after PH. Of them, 55 types inhibited each other at 0 h and 59 types at 6 h, 33 kinds promoted each other at 0 h and 57 types at 6 h. On the other hand, 527 kinds of circRNA, which are bound with miRNA, are significantly expressed at 0 h and at 6 h after PH. Of them, 189 kinds of circRNA were inhibited each other at 0 h and 195 types at 6h, 200 kinds promoted each other at 0 h and 288 types at 6 h, respectively. However, the 21 kinds of genes, were regulated by NOTCH3 and G0 phase- or G1 phase-related, which come from the above-mentioned title in result “the expression changes of mRNA, miRNA and circRNA of hepatocytes”. Of them, 8 kinds were inhibited by NOTCH3 at 0 h, and 10 types at 6 h, but 4 kinds were promoted by NOTCH3 at 0 h, and 7 types at 6 h (Table 1).

Table 1

Combine of NOTCH3 mRNA with miRNAs, circRNAs and other mRNA of hepatocytes.

time poit	correlation	NOTCH3 mRNA	miRNAs	circRNAs	other mRNAs*
0 h	promotion	1	33	200	4
	inhibition	0	55	189	8
6 h	promotion	1	57	288	7
	inhibition	0	59	195	10
	total	1	131	527	21
* refers to the mRNAs of genes regulated by NOTCH3					

3.3. The interaction of NOTCH3 mRNA with miRNA, circRNA and other mRNAs of rats hepatocytes

The interaction of the above-mentioned ceRNAs was analyzed by Cytoscape 3.2 software. The results showed that 59 types of miRNAs interacted with NOTCH3 mRNA at 0 h (Fig. 2A), 38 types at 6 h (Fig. 2D), 59 types of miRNAs and 144 kinds of circRNAs interact to form 819 interaction pairs at 0 h (Fig. 2B), and 38 types of miRNAs and 99 kinds of circRNAs interact to form 303 interaction pairs at 6 h (Fig. 2E). Continually, NOTCH3 mRNA, 59 types of miRNAs and 144 kinds of circRNAs interact to form 819 interaction pairs at 0 h (Fig. 2C), but NOTCH3 mRNA, 38 types of miRNAs and 99 kinds of circRNAs to form 303 interaction pairs at 6 h (Fig. 2F).

3.4. Expression correlation of NOTCH3 mRNA, miRNAs and circRNAs of hepatocytes in G0 and G1 phase

The expression correlation of miRNAs, circRNAs and NOTCH3 mRNA of hepatocytes in G0 phase and G1 phase was analyzed by system biology methods, it was found that NOTCH3 mRNA, miR-369-3p, which combine NOTCH3 mRNA, and rno-Rmdn2_0006, which combine miR-369-3p do not show significant expression change at 0 h, but rno-Rmdn2_0006 was up-regulated, and miR-369-3p down-regulated at 6 h (Table 3).

The base sequence of miR-369-3p was analyzed by the miRbase software and found that its sequence is aauaauacaugguugaucuuu (Table 2). Meanwhile, the mother source gene (MSG) of rno-Rmdn2_0006 was analyzed by its site on chromosome, and found it is *RMDN2.*

Table 2

Expression correlation of ceRNAs of hepatocytes at 0 h and 6 h after PH.

ceRNA	base sequence	ceRNA of hepatocytes			
		Contol	0 h	6 h	Significance
NOTCH3mRNA		1 ± 0	1.53 ± 1.14	2.53 ± 2.60	0.00**
miR-369-3p	aauaauacaugguugaucuuu	1 ± 0	0.70 ± 0.22	0.28 ± 0.03	0.03*
rno-Rmdn2_0006	rmdn2	1 ± 0	1.26 ± 0.34	2.62 ± 0.70	0.01**
* significant; ** extremely significant					

3.5. Expression correlation of *NOTCH3* and NOTCH3-regulated G0 phase- and G1 phase-related genes of hepatocytes

The expression correlation of *NOTCH3* and the NOTCH3-regulated G0 phase- and G1 phase-related genes of hepatocytes were analyzed by the system biology methods. It was found that *NOTCH3* of hepatocytes

does not show significant expression change, the G0 phase-inhibited gene *CDKN1c*, which are regulated by NOTCH3, was up-regulated at 0 h after PH. However, *NOTCH3* of hepatocytes was up-regulated, the G1 phase-promoted genes *CHUK, DDX24, HES1, NET1* and *STAT3*, which are regulated by NOTCH3, were up-regulated at 6 h after PH (Table 3).

Table 3

Expression correlation of *NOTCH3* and the NOTCH3-regulated G0 phase- and G1 phase-related genes of hepatocytes.

mRNA	**Expression of *NOTCH3* and the NOTCH3-regulated genes**			
	Control	**0 h**	**6 h**	**Significance**
NOTCH3 mRNA	1 ± 0	1.53 ± 1.14	2.53 ± 2.60	0.00**
G0 phase-related genes				
NOTCH3 promoted				
CDKN1c	1.00	3.05 ± 1.27	1.08 ± 0.47	0.00**
NOTCH3 inhibited				
CDKN1a	1.00	0.39 ± 0.07	0.93 ± 0.15	0.00**
G1 phase-related genes				
NOTCH3 promoted				
CHUK	1.00	1.00 ± 0.17	1.52 ± 0.14	0.00**
DDX24	1.00	0.78 ± 0.11	1.63 ± 0.11	0.00**
HES1	1.00	1.07 ± 0.11	1.80 ± 0.16	0.00**
NET1	1.00	0.87 ± 0.14	2.03 ± 0.06	0.00**
STAT3	1.00	0.74 ± 0.15	2.88 ± 0.24	0.00**
NOTCH3 inhibited				
PSEN2	1.00	1.07 ± 0.21	0.45 ± 0.06	0.00**
** extremely significant				

3.6. Association correlation with the role of ceRNA and NOTCH3-regulated G0 phase- and G1 phase-related genes

of hepatocytes

This paper showed that NOTCH3 mRNA, miR-369-3p and rno-Rmdn2_0006 do not change meaningfully at 0 h after PH, but the G0 phase-related gene *CDKN1a* down-regulated, which is inhibited by NOTCH3, and the G1 phase-related genes *CHUK*, *DDX24*, *HES1*, *NET1* and *STAT3* promoted by NOTCH3 were up-regulated at that, suggesting that all of them are fit with the physiological status of hepatocytes in G0 phase (at 0 h in Fig. 3). On the other hand, the expression of miR-369-3p, which inhibits NOTCH3 mRNA, was down-regulated at 6 h after PH, but the NOTCH3 mRNA and rno-Rmdn2_0006 were up-regulated, the G0 phase-related gene *CDKN1c* was up-regulated, which is promoted by NOTCH3, and expression of the G1 phase-related gene *PSEN2* inhibited by NOTCH3 was down-regulated at that, suggesting that all of them are fit with the physiological status of hepatocytes in G1 phase (at 6 h in Fig. 3).

4. Discussion

In the present,it is well known that a large number of genes, RNAs and proteins exist in cells, in the same time, most physiological activities including liver regeneration are controlled by content changes of the above-mentioned elements and their complex interaction and regulation. Although the data obtained by biological high-throughput technology make understand the mechanism of a biological process probable, there are still many difficulties in technology and method. In this paper, the above-mentioned data were used to reveal the mechanism regulating hepatocytes in G0 phase or in G1 phase during the rat liver regeneration initiation (LRI), and inspiring results were gained.

It was reported that the hepatocytes are the main cells of liver structure and function, accounting for about 80% of the total number of hepatic cells and 75% of the total weight of liver, and can reflect and represent most functions of liver tissue (Amelia, 2015). In general, most hepatocytes of adult rat liver are in rest, named as G0 phase. After partial hepatectomy (PH) is performed in rats, the hepatocytes of the remnant liver were rapidly activated, and to 6 hour after PH, the hepatocytes changed to the G1 phase synchronously, to 24 hour after PH, DNA synthesis of hepatocytes went on synchronously, named as S phase (Fausto et al., 2006). In this paper, to ensure the experimental reliability and avoid contamination interference, the 2/3 hepatectomy (PH), hepatocytes isolation, and biological high-throughput detection of ceRNAs were performed in the sterile room. To eliminate the blood interference to the experimental results, the blood in the liver tissue were removed by perfusing PBS buffer. To analyze whether the ceRNA expression changes were meaningful in rat liver regeneration, the ceRNA expression abundance of hepatocytes in S phase (at 24 h after PH) were used as control of the G0 phase (at 0 h) and G1 phase (at 6 h). To exclude the operation influence on liver regeneration, the sham operation (SO) was used as control to PH (Xu et al., 2009; Taub, 2004).

In the research aspect of NOTCH3 role, Alquda et al. found that highly expressed NOTCH3 promotes cell proliferation through cell cycle proteins (Alquda et al., 2013). Kurakazu et al. found that down-regulation of *CDKN1a* leads to cell cycle arrest (Kurakazu et al., 2019; Zhang et al., 2018). Mademtzoglou et al. found that *CDKN1c* can be used as a cell cycle repressor, the cell proliferation stops when *CDKN1c* is

highly expressed, but its proliferation enhances when *CDKN1c* is low (Mademtzoglou et al., 2018; Chen et al., 2018). Liu et al. found that *CHUK* can promote the G1 phase of the cells (Liu et al., 2016). Shi et al. found that *DDX24* was over-expressed in proliferating cells and under-expressed in arrest cells (Shi et al., 2016). Rani et al. found that *HES1* can induce cell proliferation (Rani et al., 2016; Hirata et al., 2002; Harris et al., 2019). Ahmad et al. found that over-expression *NET* leads to cell proliferation, but under-expressed inhibits cell proliferation (Ahmad et al., 2014; Zong et al., 2020). Bai et al. found that the over-expression *STAT3* promotes cell proliferation, but cell is in arrest when it is inhibited (Bai et al., 2019; Lu et al., 2018). Janicki et al. found that over-expression *PSEN2* leads to cell arrest, but under-expression promotes cell proliferation (Janicki et al., 2000; Azimi et al., 2018; Janicki et al., 1999). The above-mentioned results showed that the *NOTCH3* expression and NOTCH3 content influences cell phase.

In the research aspect of miR-369-3p role, Youn et al. found that low expression of miR-369-3p can reduce the proliferation of chondrocytes, but high expression of miR-369-3p would can help the proliferation of chondrocytes (Youn et al., 2018). Wei et al. found that miR-369-3p can act on *CXCR4*, and its down-regulated expression can increase the number of migratory bone marrow mesenchymal stem cells (BMSC) (Wei et al.,2016). Meanwhile, the prediction by IPA(Ingenuity Pathway Analysis)software,miRanda software and miRwalk website showed miR-369-3p can bind with rno-Rmdn2_0006, however, it was not found the report about role of rno-Rmdn2_0006.

The above-mentioned results suggested that NOTCH3 mRNA is released at 6 h after PH, which is inhibited by miR-369-3p, the released NOTCH3 mRNA forms NOTCH3 probably, the latter promotes the expression of G1 phase-related genes, and inhibit the expression of G0 phase-related genes, making the hepatocytes in G1 phase. On the contrary, the NOTCH3 mRNA was combined by miR-369-3p at 0 h after PH, leading to NOTCH3 difficult to be formed, G1 phase-related genes promoted by NOTCH3 difficult to express, and G0 phase-related genes inhibited by NOTCH3 to express, making the hepatocytes in G0 phase. The results of this study are helpful to understand the mechanism of circRNA, miRNA and mRNA regulating the G0 or G1 phase of liver cells.

Declarations

Author contributions

ZXY and XCS conceived the project. ZXY and WZH designed the research. LYF, GH, GJL, JW, CCF, LJT, ZKC and XCS performed the research. ZXY and WZH analyzed the data, ZXY, WZH and LYF wrote the manuscript.

Acknowledgements

This project is supported by National Natural Science Foundation of China (31572270, 81771226) and National Youth Natural Science Foundation (U1804186, U1404312, 31601038, 31201093, 81200317).

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Figures

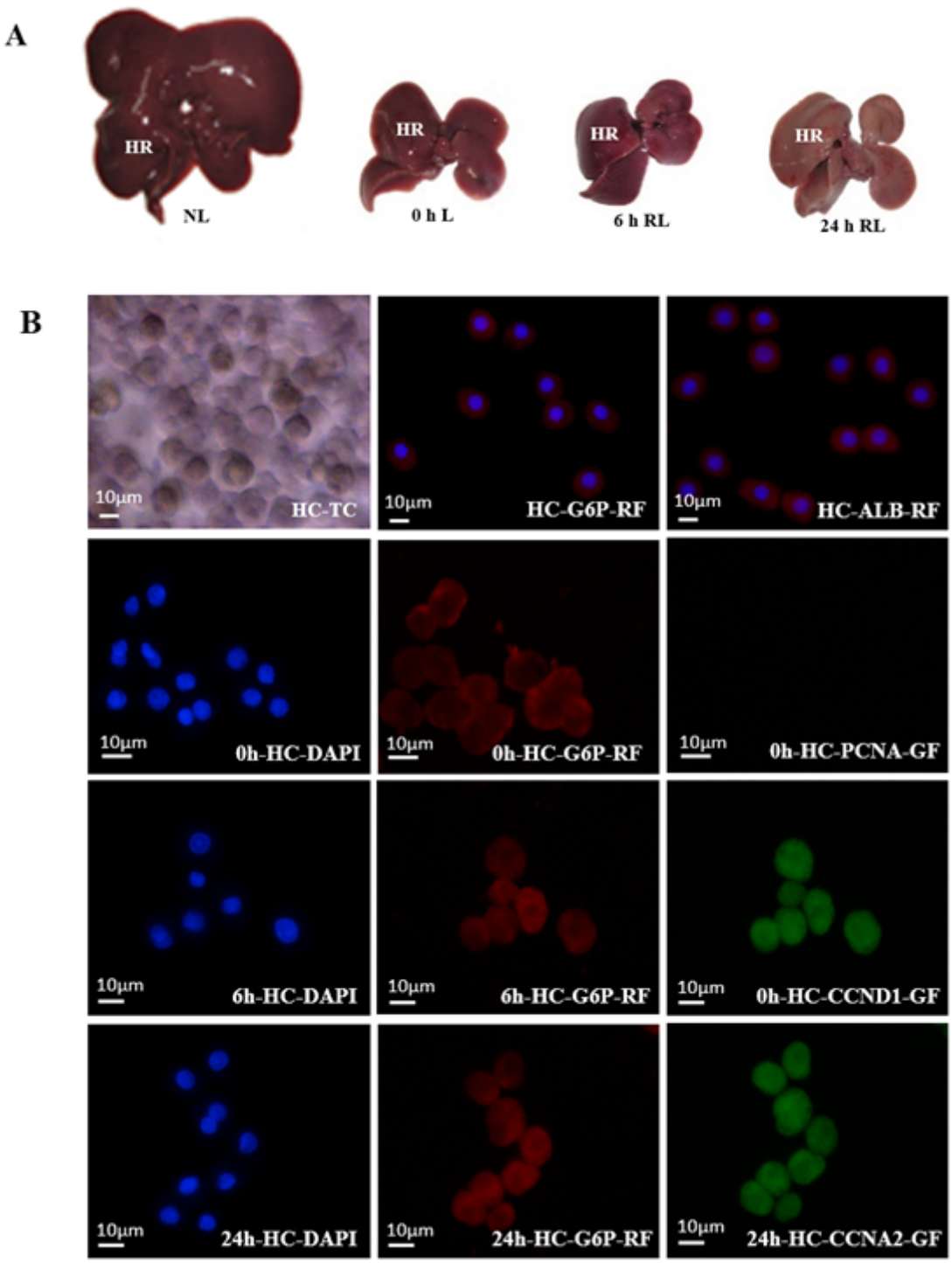

Figure 1

(A) Preparation of rat 2/3 hepatectomy (partial hepatectomy, PH) model; (B) Isolation and identification of the hepatocytes NL. Normal liver of adult rats; L. Liver; HR. Right lobe of liver; RL. Regenerated liver; HC. Hepatocytes; TC. Trypan blue dyeing; G6P. Glucose-6-phosphatase; ALB. Albumin; PCNA. Proliferating cell nuclear antigen; CCND1. Cyclin D1; CCNA2. Cyclin A2; RF. Red fluorescence; DAPI. Blue fluorescence; GF. Green fluorescence 3.2. Interactions of NOTCH3 mRNA with miRNAs, circRNAs and other mRNA of hepatocytes

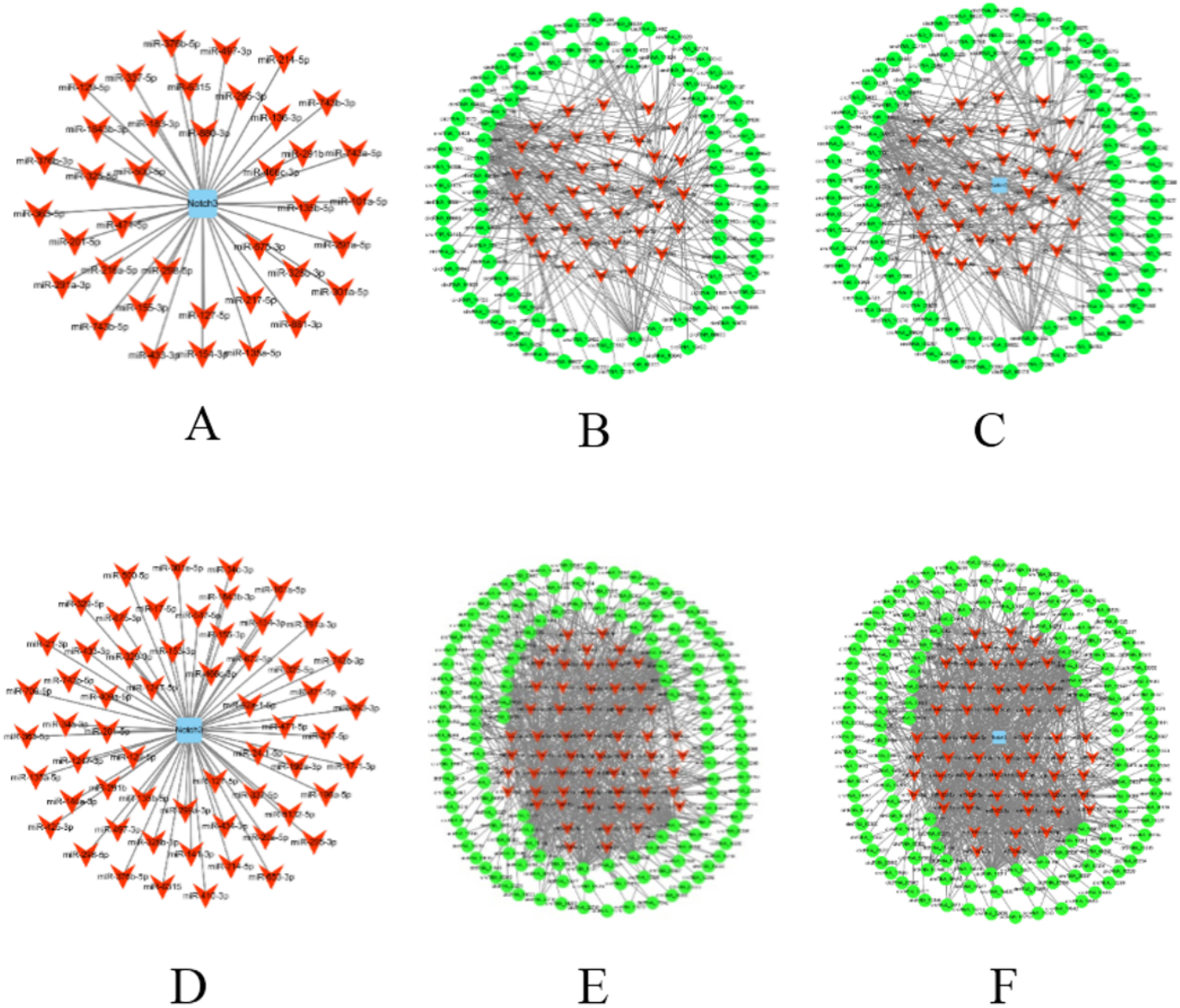

Figure 2

Interaction of mRNAs, miRNAs and circRNAs of hepatocytes in G0 phase and G1 phase in LR A-C: At 0 h after PH; D-F: At 6 h after PH; Blue/red: mRNAs-miRNAs interaction; Red/green: miRNAs-circRNAs interaction; Blue/red/green: mRNAs-miRNAs- circRNAs interaction.

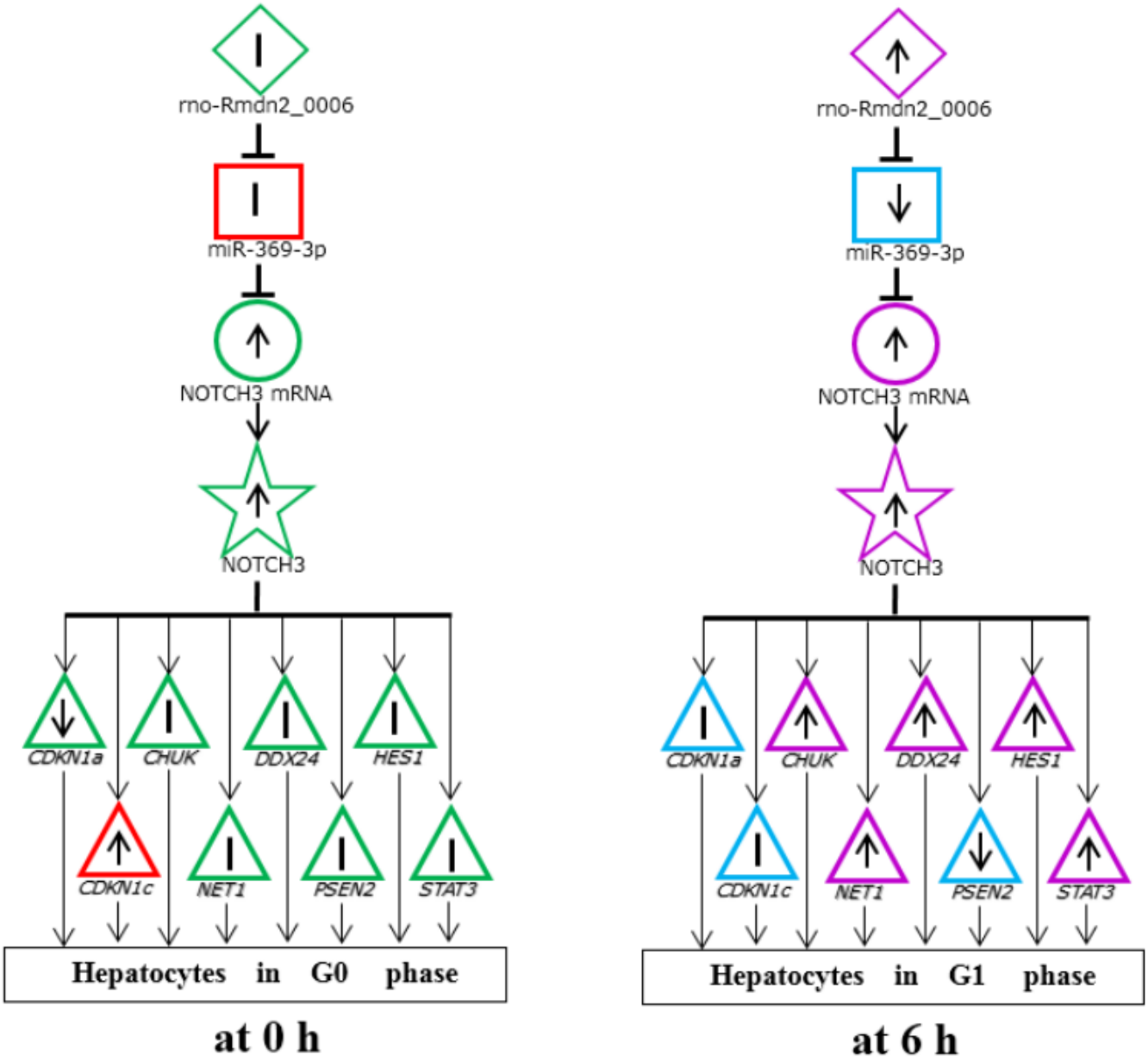

Figure 3

The role correlation of ceRNAs and the G0 phase- and G1 phase-related genes regulated by NOTCH3 during the rat liver regeneration 0 h□0 hour after PH□6 h□6 hour after PH□□□circRNA□□□miRNA□●□ NOTCH3 mRNA□□□NOTCH3□△□the genes regulated by NOTCH3□↑□the gene expression up-regulated□↓□ the gene expression down-regulated□|□the gene expression change not significant□⊥□inhibition□Red edge: promoting G0 stage; Green edge: inhibiting G0 stage; Purple edge: promoting G1 phase; Blue edge: inhibiting G1 phase