Life Sciences 232 (2019) 116656

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Dicer1 facilitates liver regeneration in a manner dependent on the inhibitory effect of miR-21 on Pten and Rhob expression

Tao Lv[a], Lingxiang Kong[a], Li Jiang[a], Hong Wu[a], Tianfu Wen[a], Yujun Shi[b,c], Jiayin Yang[a,*]

[a] Department of Hepato-Biliary-Pancreatic Surgery, West China Hospital of Sichuan University, Chengdu 610041, China
[b] Laboratory of Pathology, West China Hospital, Sichuan University, Chengdu 610041, China
[c] Key Laboratory of Transplant Engineering and Immunology, Ministry of Health, Chengdu 610041, China

ARTICLE INFO

Keywords:
Dicer1
Liver regeneration
miR-21
Pten
Rhob

ABSTRACT

Aims: Tamoxifen-induced liver-specific *Dicer1* deletion (*iDicer1*$^{-/-}$) in mature mice may provide clues demonstrating the genuine effects of acute loss of *Dicer1* and miRNAs in the liver regeneration process.
Main methods: In this study, mice with tamoxifen-induced *Dicer1* deletion through the Cre/LoxP system were constructed and then underwent classic 70% partial hepatectomy or CCl4-induced liver injury. To rescue the inhibitory effect of Dicer1 ablation on liver regeneration, miR-21 agomir was injected into the tail vein of *iDicer1*$^{-/-}$ mice.
Key findings: Unlike constitutive embryonic deletion of Dicer1, tamoxifen-induced Dicer1 deletion did not result in severe liver injury or lesions, providing an ideal model for investigating acute loss of Dicer1 and miRNAs in liver regeneration. Dicer1 deletion led to impaired liver regeneration through the inhibitory effect of miR-21 on PTEN and Rhob expression.
Significance: In our previous study, we found that embryonic loss of Dicer1 impairs hepatocyte survival and leads to chronic inflammation and progenitor cell activation, while the role of Dicer1 in liver regeneration remains largely unknown. We clearly identified the promotion effect of Dicer1 on liver regeneration by increasing miR-21 expression, which inhibits the expression of two negative cell proliferation regulators, Pten and Rhob.

1. Introduction

The liver, the largest digestive organ in the human body, plays critical roles in biological synthesis, metabolism and detoxication. As one of the most mysterious organs, the liver maintains a unique self-regeneration capacity in response to injury from trauma, drugs and viruses [1,2]. This peculiarity of the liver is the clinical basis of liver resection and liver transplantation, the only curative method for end-stage liver diseases, and it is the theoretical basis of the treatment of liver failure caused by various liver diseases. Although the liver possesses an enormous regenerative capacity, insufficient compensation for the loss of liver function plagues the treatment of liver failure [3,4]. Persistent injury and insufficient regeneration are the main causes of acute and chronic liver failure. Small-for-size syndrome caused by living-donor transplantation and liver resection is a common clinical example of insufficient regeneration [5]. Many HCC patients lose the chance for surgery because of the lack of residual liver volume. Therefore, studying the molecular mechanisms and regulatory factors of liver regeneration and developing new methods for the treatment of inadequate liver regeneration capacity are essential for clinical treatment.

Hepatocytes, characterized by their complex function and active metabolism, are the most efficient “stem cells” in liver regeneration, during which a large number of genes precisely regulate physiological activities [6]. MiRNAs are a class of small noncoding RNAs (18–24 nucleotides in length) that inhibit target genes' translation by binding to their 3′ untranslated region. The generation of functional miRNAs is precisely controlled. First, the pri-miRNA is cleaved by the Drosha-Dgcr8 complex into a pre-miRNA (60–70 nucleotides in length) in the nucleus, which is transported into the cytoplasm and then cleaved by the Dicer1-TRBP complex into a mature miRNA [7,8]. Most miRNAs are Dicer1-dependent and play critical roles in embryo development, proliferation, apoptosis and signaling pathways [9].

It has been reported that >300 miRNAs are involved in liver regeneration, of which >100 miRNAs are activated at the early stage of liver regeneration [10,11]. Recently, increasing evidence has indicated

* Corresponding author at: Department of Hepato-Biliary-Pancreatic Surgery, West China Hospital of Sichuan University, 37 Guoxue Road, Chengdu 610041, China.
E-mail address: yangjygyz@163.com (J. Yang).

https://doi.org/10.1016/j.lfs.2019.116656
Received 20 May 2019; Received in revised form 7 July 2019; Accepted 12 July 2019
Available online 12 July 2019

that miRNAs are involved in liver regeneration, but their roles remain unclear [12]. Embryonic deletion of Dicer1 causes a global loss of mature miRNAs, resulting in a lethality phenotype at E8 due to the loss of stem cell pluripotency and proliferation [13]. Thus, the conditional deletion of Dicer1 using the Cre system has been used to evaluate miRNA function in a range of organs, including the liver [14–16]. Liver-specific ablation of Dicer1 resulted in several consequences: Dicer1 depletion impaired hepatocyte survival and triggered long-lasting compensatory proliferation; the loss of Dicer1 induced robust ductular reaction and fibrosis; and the Dicer1-deficient liver finally developed HCC. Although these findings have revealed several functions of Dicer1 in the liver, its role in liver regeneration remains unknown, and the mouse model adopted has displayed obvious limitations for the pre-existing lesions caused by Dicer1 ablation after birth. In our study, we established tamoxifen-induced liver-specific Dicer1-deleted mice, which helped us to explore the effect of Dicer1 and the subsequent deficiency of miRNAs on liver regeneration and metabolism.

2. Material and methods

2.1. Animal studies

Tamoxifen-induced liver-specific Dicer1 knockout mice were generated by crossing Dicer1$^{flox/flox}$ mice with mice expressing Cre recombinase and mutated estrogen receptor (ER) under the control of the albumin promoter, as previously described [17]. Tamoxifen management followed the guidelines of Jackson Laboratory. Tamoxifen is dissolved in corn oil at a concentration of 20 mg/ml by shaking overnight at 37 °C. For adult mice, a standard dose of 100 μl tamoxifen/corn oil solution is effective for inducing recombination. Administer tamoxifen via intraperitoneal injection once every 24 h for a total of 5 consecutive days. Following the final injection, mice should be quarantined for 24 h before returning to their normal animal room. For avoiding the side effect of tamoxifen, there is a 7 day waiting period between the final injection and necropsy/histological analysis. The mice were maintained under an alternating 12-h light/dark cycle, fed regular chow, and provided water ad libitum. Animal procedures and care were conducted in accordance with the institutional guidelines and in compliance with national and international laws and policies. To assess the role of Dicer1 in liver regeneration, we performed 70% PH on eight- to twelve-week-old Dicer1 conditional knockout (*iDicer1*$^{-/-}$) mice and their sex-matched wild-type (WT) littermates one week after the last tamoxifen injection. Acute toxic hepatic injury was induced by intraperitoneal injection of 10 ml/kg body weight of a 10% CCl4 solution in olive oil. A single dose of 5-bromo-2′-deoxyuridine (BrdU; Sigma, St. Louis, MO, USA) was injected intraperitoneally at 50 mg/kg animal weight (10 mg/ml in phosphate-buffered saline) 1 h before sacrifice [18]. Serum was collected for the biochemistry measurement. Liver specimens were harvested at the indicated time points for histological analysis or nucleic acid and protein isolation.

2.2. Reintroduction of miR-21 in the liver

The *iDicer1*$^{-/-}$ mice were given a tail vein injection of miR-21 agomir (5 nmol/mouse) (n = 18) or miRNA negative control (Ribo-bio, Guangzhou, China) six hours after 70% PH, and the liver samples were collected at the indicated time points [19,20]. Agomirs are chemically modified miRNAs that possess advantages such as increased serum stability, improved cellular uptake and increased stability in cells and are popular for in vivo applications.

2.3. Reverse transcription and quantitative real-time PCR

Total RNA was isolated from liver tissues using TRIzol reagent (Invitrogen, CA, USA), and cDNA was synthesized by iScript™ cDNA Synthesis Kits following the manufacturer's protocol (Bio-Rad, CA, USA). Quantitative real-time PCR (qRT-PCR) was performed using a standard SYBR green PCR kit (Bio-Rad, CA, USA), and PCR-specific amplification was done in the Bio-Rad CFX96 real-time PCR machine. For miRNA quantification, Bulge-loop miRNA qRT-PCR Primer Sets (one RT primer and a pair of qPCR primers for each set) specific for miR-122-5p, miR-21a-5p and let7a-5p were designed by RiboBio (Guangzhou, China). The relative expression of genes, including U6, miR-122-5p, miR-21a-5p and let7a-5p, was calculated with the $2^{-\Delta\Delta Ct}$ method.

2.4. Histology and immunohistochemistry

Hematoxylin and eosin (H&E) staining and immunohistochemical staining for Ki-67, BrdU, and CK19 were carried out on 5-μm-thick paraffin liver sections. The percentage of positive nuclei was counted in five consecutive high-power fields.

2.5. Western blotting

Liver samples were homogenized in RIPA lysis buffer with phenylmethanesulfonylfluoride fluoride (PMSF) (Beyotime, China), incubated on ice for 1 h and centrifuged for 15 min at 12,000 g at 4 °C. Supernatants were collected. The protein concentration was measured by BSA assay (Cowin Biotech, China). For western blotting, 60 μg total protein per lane was used to examine the expression levels of target proteins. Rabbit anti-Dicer1 (Novus, Colorado, USA); rabbit anti-Pten, anti-cyclin D1, anti-CDK1, anti-CDK2, anti-CDK4, anti-cyclin A, anti-cyclin B, and anti-AKT (Abcam, CA, USA); and rabbit anti-p-AKT and anti-Rhob (CST, MA, USA) antibodies were used. ECL reagent was used for chemiluminescence detection. Signal intensities were quantified and normalized to GAPDH intensity by ImageJ software.

2.6. Statistical analysis

All data are expressed as the mean ± standard deviation (mean ± SD). The SPSS statistical package was used (version 21.0, SPSS Inc., Chicago, IL, USA) for statistical analysis. Significance was determined with a two-tailed Student's t-test. A $P < 0.05$ was considered significant.

3. Results

3.1. The generation of tamoxifen-induced hepatocyte-specific Dicer1-deficient mice

To investigate the role of Dicer1 in liver regeneration and avoid the preexisting lesions caused by embryonic deletion of Dicer1, including robust ductular reaction, fibrosis and compensatory proliferation as previously described [16] (Fig. 1A), we first generated tamoxifen-induced hepatocyte-specific Dicer-deficient mice by crossing Dicer1$^{flox/flox}$ with Albumin-creERT transgenic mice as previously described [17], and the construction strategy is shown below (Fig. 1B). The knockout efficiency was measured by western blot (Fig. 1C). The serological detection results showed that there was no significant difference between WT and *iDicer1*$^{-/-}$ mice, indicating that the acute depletion of Dicer1 did not cause obvious liver injury, which was similar to the histological analysis of *iDicer1*$^{-/-}$ mouse liver (Fig. 1D, E), while the Dicer1-dependent miRNAs were significantly reduced in *iDicer1*$^{-/-}$ mouse liver (Fig. 2A).

3.2. Acute loss of Dicer1 impairs early liver regeneration in mice

To determine the role of Dicer1 in liver regeneration, we performed classic 70% partial hepatectomy (pH) on *iDicer1*$^{-/-}$ mice and WT littermates. Remnant livers were harvested at 24 h, 36 h, 48 h, 72 h, 5 days, and 7 days after surgery. Although all the mice were able to

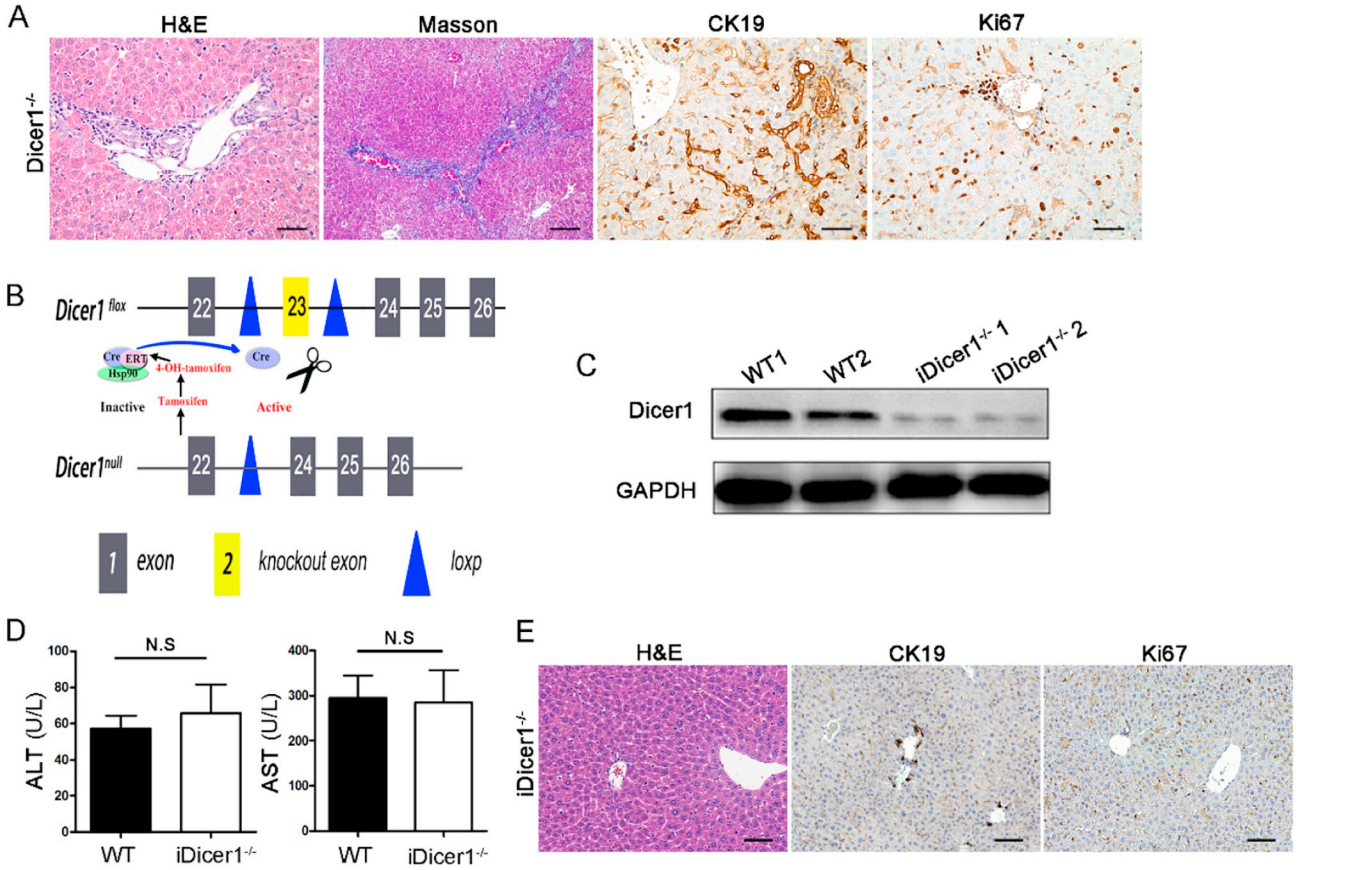

Fig. 1. The construction of *iDicer1*$^{-/-}$ mice. (A) H&E, Masson, CK19 and Ki-67 staining reveal pre-existing lesions in Dicer1 embryonic-deleted mouse liver. Scar bars, 50 μm, 200 μm, 50 μm and 50 μm, respectively. (B) Schematic diagram of the construction of *iDicer1*$^{-/-}$ mice. (C) Detection of Dicer1 protein in mouse liver. (D) Results of biochemical tests of WT and *iDicer1*$^{-/-}$ mice (n = 5). N.S., not significant. (E) H&E, CK19 and Ki-67 staining results indicate that no obvious liver injury occurred in *iDicer1*$^{-/-}$ mouse liver. Scar bar, 100 μm.

approximately complete the liver regeneration process within 7 days after surgery, in the early stage of regeneration, i.e., 36 h, 48 h, and 72 h, the liver/body weight ratio of *iDicer1*$^{-/-}$ mice was lower than that of WT mice (Fig. 2B), suggesting that the acute deletion of Dicer1 delayed the liver regeneration process.

To further evaluate the effect caused by Dicer1 deletion in liver regeneration, immunohistochemical staining of proliferating cell markers Ki-67 and BrdU was carried out. We quantified the number of Ki-67-positive cells in WT and *iDicer1*$^{-/-}$ mice at each time point (Fig. 2C, D). At 36 h and 48 h after PH, the proportion of Ki-67-positive cells in *iDicer1*$^{-/-}$ mice was significantly lower than that in WT mice (20.05% and 27.96%, respectively, for *iDicer1*$^{-/-}$ mice, 66.63% and 66.36% for WT mice). These percentages were 3.32 and 2.37 times higher in the WT mice than the *iDicer1*$^{-/-}$ mice at 36 h and 48 h, respectively. Conversely, at 72 h and 5 days after PH, the percentages of Ki-67-positive cells in *iDicer1*$^{-/-}$ mice were obviously higher than in WT mice (Fig. 2D). Meanwhile, the results of the BrdU incorporation assay were basically the same as those of Ki-67 staining. (Fig. 2E, F). These results showed that the acute deletion of Dicer1 inhibited liver regeneration in mice, and the peak of hepatocyte proliferation was present at 72 h after PH, compared to 36 h in WT mice.

We next assessed the liver repair rate in mice in response to the acute toxic injury induced by CCl4 injection. The necrosis area in the *iDicer1*$^{-/-}$ liver was larger than in WT 72 h and 5 days after CCl4 management, which was in accordance with the results obtained from the surgery model (Fig. 2G).

Cell proliferation suppressor genes Pten and Rhob lead to PI3K/AKT signaling pathway inactivation and cell cycle inhibition.

In quiescent liver, mature hepatocytes are maintained in G0 phase, and once the liver is damaged or injured, hepatocytes self-proliferate and enter into the cell cycle. In response to classical 70% PH, mature hepatocytes in the remnant liver will react immediately, and at the early stage of regeneration, the elevated inflammatory cytokines, growth factors and portal pressure will trigger the synthesis of key pro-proliferation proteins, such as cyclins and cyclin-dependent kinases (CDKs) [21]. The expression levels of cyclins and CDKs can reflect the status of cell proliferation [22]. We examined and analyzed the expression of several cyclins and CDKs in both *iDicer1*$^{-/-}$ and WT mouse liver, and the results are presented in Fig. 3A-C. According to the histological results, Cyclin D1, Cyclin A, Cyclin B, Cyclin E, CDK1, CDK2 and CDK4 were all lower in *iDicer1*$^{-/-}$ liver before 72 h after PH, while the trend was reversed thereafter.

Among the seven cyclins and CDKs we examined, Cyclin D1 is most critical to the cell cycle, and as the earliest cyclin expressed in the liver regeneration process [23,24], the mRNA level of Cyclin D1 in the liver rose to a peak 12 h after hepatectomy, several times higher than that in the physiological state. Then, the Cyclin D1 protein steadily increased. Cyclin D1 forms complexes with CDK4 and CDK6, whose activity begins to rise in the middle of G1 phase, peaks in the late of G1 phase and acts on the check point, facilitating the liver cells' entrance into S phase from G1 phase [22,24]. In our study, Cyclin D1 was completely inhibited in Dicer1-deficient mice until 36 h after surgery (Fig. 3A, B), and it has been well documented that the silencing of Cyclin D1 can result in liver regeneration abnormity. This finding seems to explain the delay of early liver regeneration in *iDicer1*$^{-/-}$ mice.

We next investigated the reason why Dicer1 deletion led to Cyclin D1 inhibition. The PI3K/AKT/mTOR signaling pathway is widely reported in various cells and is a key regulator of cell proliferation,

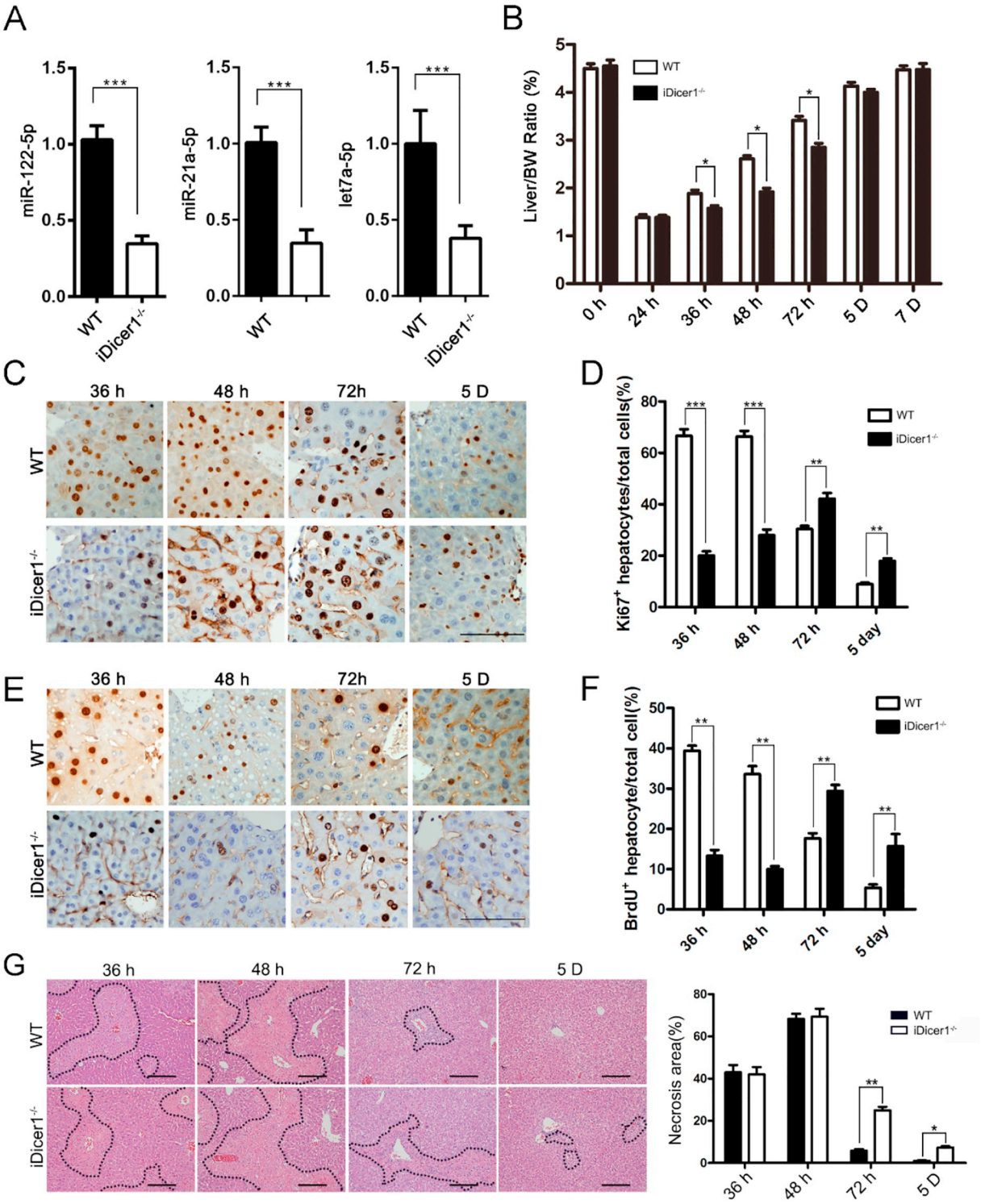

Fig. 2. Liver regeneration is impaired in *iDicer1*$^{-/-}$ mice. (A) miR-122-5p, miR-21-5p and let7a-5p, representative microRNAs in liver, are dramatically decreased in *iDicer1*$^{-/-}$ mouse liver. (B) Liver/body weight ratio at the indicated time points after 70% PH. (C-D) Ki-67 staining of liver sections and the statistical analysis of Ki-67-positive cell ratios. Scar bar, 100 μm. (*E*-F) BrdU staining of liver sections and the statistical analysis of BrdU-positive cell ratios. Scar bar, 100 μm. (G) Liver necrosis assessed by H&E staining of liver sections after CCl4 administration and the statistical analysis of necrosis area. Scar bar, 100 μm. The data are presented as the means ± s.e.m.; the *P* value was determined by the two-tailed Student's *t*-test, **P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.

survival and apoptosis. AKT, phosphorylated by PI3K, can activate the mTOR signaling pathway, which is pivotal for the expression of Cyclin D1 [25,26]. The tumor suppressors Pten and Rhob can inactivate this PI3K/AKT/mTOR/Cyclin D1 axis [27].

According to our western blot assay, we found that Pten and Rhob were highly expressed in *iDicer1*$^{-/-}$ mouse liver, while p-AKT was lower, at 24 h and 36 h after 70% PH (Fig. 3D, E). Therefore, we speculated that deletion of Dicer1 caused miRNA-mediated inhibition of Pten and Rhob to diminish or disappear, elevating the expression of Pten and Rhob, which finally resulted in PI3K/AKT signaling pathway and Cyclin D1 inhibition at the early stage of liver regeneration. However, which miRNA is the primary cause of this phenomenon remains unknown.

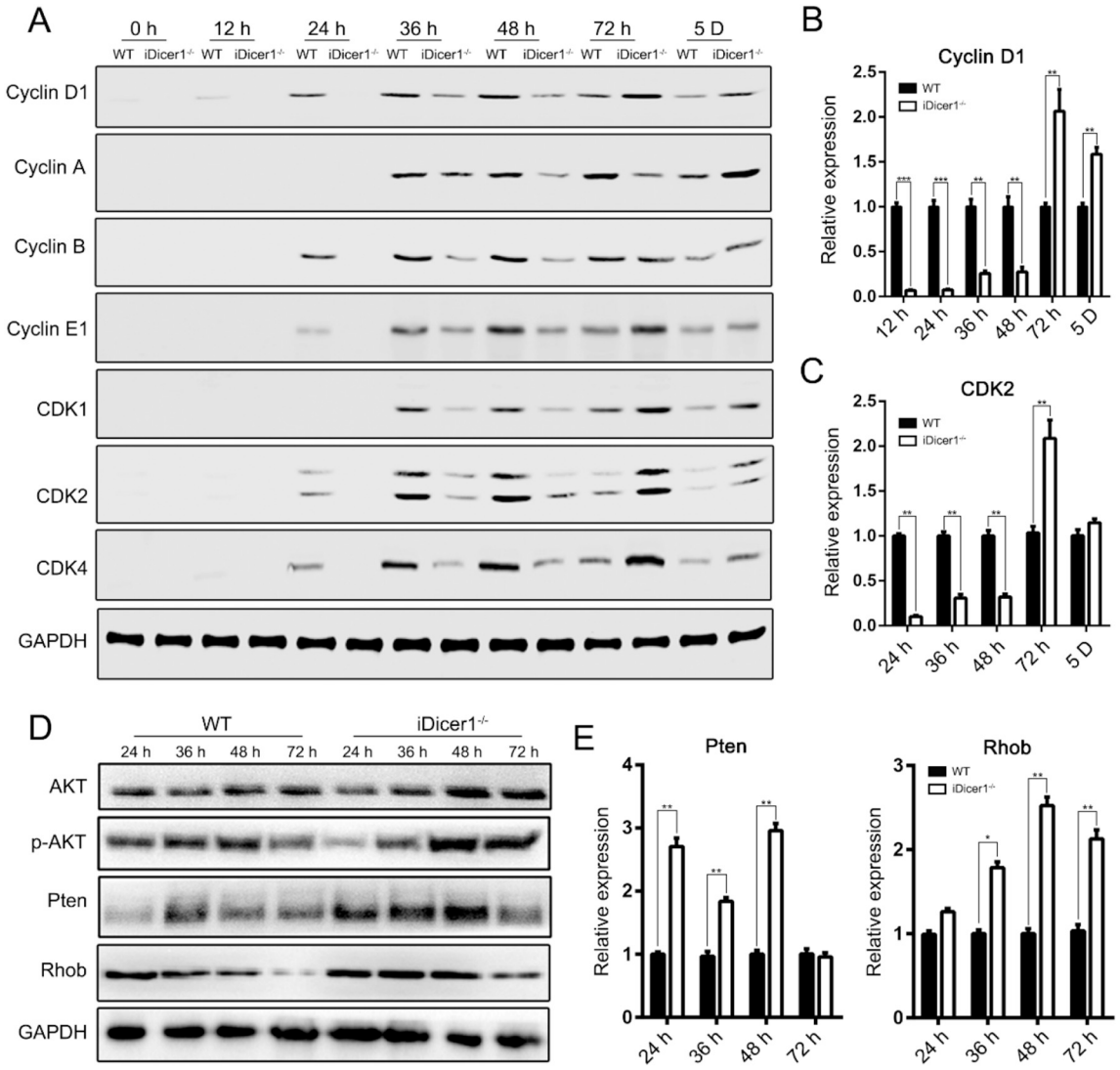

Fig. 3. Detection and quantification of Cyclins and key regulator proteins in liver regeneration. (A) Western blot assays for Cyclin D1, Cyclin A, Cyclin B, Cyclin E1, CDK1, CDK2 and CDK4. GAPDH served as a loading control. (B–C) The relative expression of Cyclin D1 and CDK2 at the indicated time points after 70% PH. (D) The detection of key proteins (AKT, p-AKT, Pten and Rhob) in regenerating livers; GAPDH served as a loading control. (E) Statistical analysis of the relative expression of Pten and Rhob at the indicated time points after 70% PH. The data are presented as the means ± s.e.m.; the *P* value was determined by the two-tailed Student's t-test, $^{*}P<0.05$, $^{**}P<0.01$.

3.3. miR-21 rescues the liver regeneration delay caused by Dicer1 deletion

Considering that the biological function of Dicer1 is mainly involved in miRNA maturation, we wondered whether the transfusion of one miRNA into Dicer1-deficient mice could restore the liver regeneration ability. According to a literature review and website prediction (TargetScan and miRBase), we found that both Pten and Rhob are target genes of miRNA-21 [21,28,29], which plays a pivotal role in liver regeneration. Then, we investigated the changes in liver regeneration by transfusing its analog (miRNA-21 agomir).

The *iDicer1*$^{-/-}$ mice were given a tail vein injection of miR-21 agomir at 6 h after 70% PH, and livers were harvested at the indicated time points (Fig. 4A). To our surprise, there was no significant difference in liver/body weight ratio between *iDicer1*$^{-/-}$ and WT littermates (Fig. 4B). Although the proliferation index of *iDicer1*$^{-/-}$ mice given miR-21 agomir was still slightly lower than that in WT mice, no obvious disparity was found according to the Ki-67 and BrdU immunohistochemical staining results (Fig. 4C, D).

We also analyzed the expression levels of cyclins, CDKs and the key proteins identified above (Pten, Rhob and p-AKT) (Fig. 5A, B). The results showed that the expression disparity of those proteins, especially Pten, Rhob and p-AKT, was almost eliminated after miR-21 agomir transfusion (Fig. 5A, B). According to the histological results, no overt difference was found between the two groups, which further confirmed that the effect of Dicer1 on liver regeneration was miR-21 dependent (Fig. 5C).

4. Discussion

Increasing evidence has shown that miRNAs play pivotal roles in liver physiological and pathological conditions, including liver regeneration [30]. Because of the complex regulatory relationship between miRNAs and target genes and the co-regulatory effect of different miRNAs regarding one target gene, the role of a single miRNA in liver regeneration cannot be accurately reflected in liver regeneration. The general effect of all miRNAs in liver regeneration has not been reported. In this study, by deleting the Dicer1 gene in mature mouse liver, we ablated almost all miRNAs in the liver and observed alterations in liver regeneration. Although the role of Dicer1 in liver has been reported previously [14–16], the preexisting lesions caused by embryonic

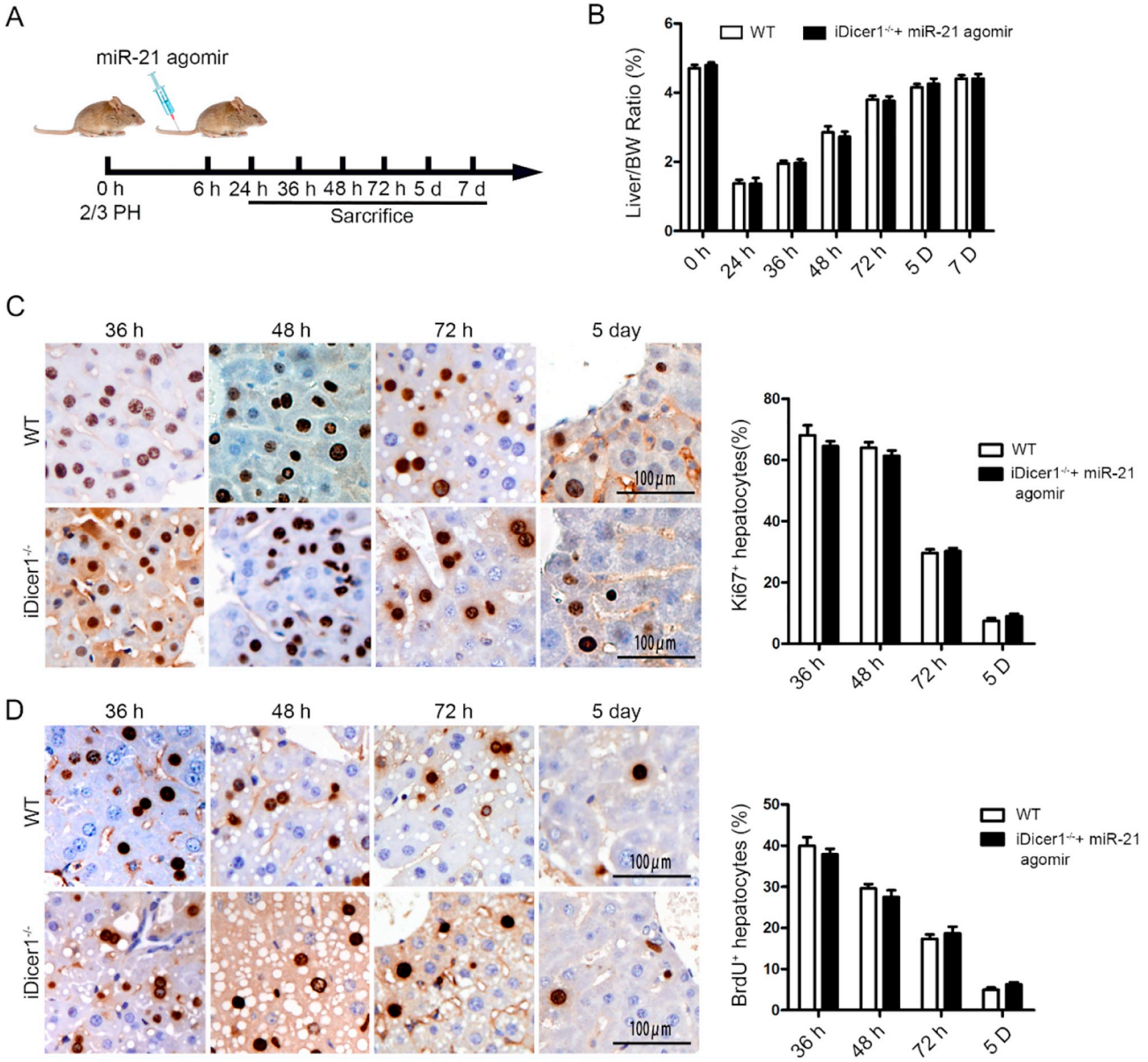

Fig. 4. MiRNA-21 analog rescues the inhibition effect caused by Dicer1 deletion. (A) Schematic diagram for transfusing miRNA-21 agomir. (B) No obvious disparity in liver/body weight ratios was found between *Dicer1*$^{-/-}$ and WT mice after transfusing miR-21 agomir. (C-D) Ki-67 immunohistochemical staining and statistical analysis after 70% PH. (E-F) BrdU incorporation assay and statistical analysis. Scale bar, 100 μm.

deletion of Dicer1 make it unsuitable for evaluating the role of Dicer1 in liver regeneration. In our mouse model, acute loss of Dicer1 caused by tamoxifen administration successfully knocked down Dicer1 protein and related miRNAs without obvious liver injury, providing an ideal model for investigating the role of Dicer1 and miRNAs as a whole in liver regeneration.

MiRNA-21 is generally regarded as an oncogene because of its role in the occurrence, development and metastasis of multiple tumors [31,32]. Increasing evidence has proven that miRNA-21 plays a very important role in liver pathology. MiRNA-21 was elevated in the sera of HBV and HCV patients, regardless of whether it was associated with liver cancer or cirrhosis [33]. In addition, miRNA-21 can inhibit the expression of the negative regulatory protein SMAD7, an important factor in hepatic fibrosis (TGF-β). However, if overexpressed, miRNA-21 can lead to the activation of the TGF-β signaling pathway and promote the occurrence of hepatic fibrosis [34]. MiRNA-21 is highly expressed in hepatocellular carcinoma, and it inhibits the expression of PTEN and activates the PI3K/AKT signaling pathway, promoting the occurrence of hepatocellular carcinoma [29]. In liver regeneration, miR-21 is elevated at early stage of liver regeneration, which may be partly due to the activation of IL/Stat3 signaling pathway with 6 h after hepatectomy [35]. The role of miRNA-21 is controversial. Song et al. found that miRNA-21 and miRNA-378 play an important role in liver regeneration by specifically downregulating the Dgcr8 gene in liver cells, and they had a reinforcing effect on each other [30]. Ng et al. found that CylinD1 levels declined after miRNA-21 was knocked out after hepatectomy, which made it difficult for cells to enter the S phase [4,31]. As a result, liver regeneration is inhibited, and miRNA-21 increases cyclin D1 levels by activating Akt1/mTOR. In ethanol-fed rats, inhibition of miRNA-21 facilitates liver regeneration, revealing the inhibiting effect of miRNA-21 in liver regeneration [36]. In our study, we observed that the effect of Dicer1 on liver regeneration was mainly dependent on miR-21 (Fig. 5C). Although the inhibitory effect of Dicer1 deletion on liver regeneration was not completely rescued by miRNA-21 agomir transfusion, which was also consistent with the theoretical notion of multiple miRNAs regulating liver regeneration, the situation

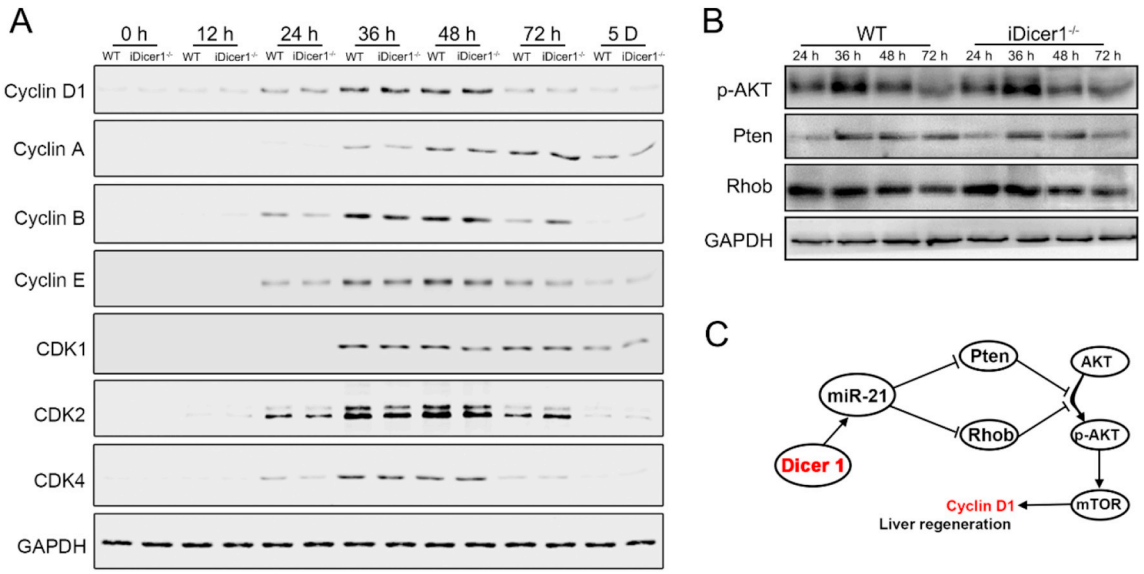

Fig. 5. Western blot detection of cyclins, CDKs (A) and key regulator proteins (B) in regenerating livers. (C) Proposed mechanism of Dicer1-mediated liver regeneration is dependent on the inhibition effect of miR-21 on Pten and Rhob, which facilitates the expression of Cyclin D1.

was greatly improved in *iDicer1*$^{-/-}$ mice.

Cyclins and CDKs are key regulators of the cell cycle and proliferation. Cyclin D1, CDK4, and CDK6 form a complex whose activity begins to increase in the middle of G1, reaches a peak in late G1, and plays an important role in cell cycle selective differentiation by acting on the restriction point [22]. The Cyclin D1-CDK4 complex can promote the phosphorylation of Rb1 such that it dissociates from the Rb1/E2F1 complex, promoting the expression of cell cycle genes downstream of E2F1 and achieving the G1/S transition [37]. Cyclin D1 is a critical cycle protein that promotes the G1/S transition and enables cells to enter the cell cycle and complete cell division. CCND1, the gene encoding cyclin D1, is located on chromosome 11q13 and is regulated by miRNAs such as miRNA-33 and miRNA-489. The downregulation or deletion of Cyclin D1 leads to severe liver regeneration inhibition.

Liver regeneration was significantly improved in Dicer1 knockout mice by transfusing miRNA-21 analogs, reaching a nearly wild-type liver regeneration status. Most miRNAs suffer from maturation disorder after Dicer1 knockout, and the liver regeneration process is affected after hepatectomy, together with the decrease in cyclin D1. The overall result can be summarized as follows: Liver regeneration was inhibited by Dicer1 knockout, and the peak of regeneration was delayed. microRNA-21 analogs both compensated for all missing miRNA functions and increased the expression of Cyclin D1 via the activation of the PI3K/AKT pathway. The possible reasons involved are as follows: There are many changes in miRNAs in normal mice after liver resection, and the changes can be divided into two types, up- and downregulation, and interconversion occurs in the process of liver regeneration. In the case of Cyclin D1, in the early stage of liver regeneration, the positive regulators miRNA-21 and miRNA-489, are upregulated, while negative regulator miRNA-33 is downregulated; at the end of liver regeneration, miRNA-33 is upregulated to lower the level of Cyclin D1, while miRNA-21 is downregulated. The levels of both miRNA-21 and miRNA-33 are decreased after Dicer1 deletion, and the major discrepancy with the control group is the positive regulator miRNA-21. This disparity was compensated by transfusing microRNA-21 analogs, which can compensate for Cyclin D1 reduction after Dicer1 knockout, such that hepatocytes could enter the cell cycle smoothly, thereby rescuing the inhibition of liver regeneration caused by Dicer1 deletion.

Gene modification and analog transfusion not only provide new ideas for study but also provide clues for clinical diagnosis and treatment. Because miRNAs have great stability in plasma and serum, the miRNAs in the blood have a very high potential value for the diagnosis and treatment of clinical diseases [38]. Chronic hepatitis C infection causes elevation of miRNA-155 in serum and peripheral blood mononuclear cells [39], and liver injury can lead to elevated miRNA-122 in plasma, which is even more sensitive than enzyme tests [40,41]. In the treatment regimen, there has been clinical success in reducing the level of HCV by inhibiting miRNA-122, and anti-miRNA-122 LNA inhibitors have been used in clinical trials. The initial results show that HCV resistance does not develop. With the advantages of specific amplification effects, long half-lives and low-toxicity side effects, miRNA-targeted drugs have wide application prospects. It has been demonstrated that miRNA-21 plays an important role in liver regeneration [20]. For cases of hepatic failure caused by clinical regeneration disorder, the introduction of miRNA-21 analogs is undoubtedly a bold therapeutic strategy and innovation, but its actual therapeutic effect and clinical value need to be verified by further clinical trials.

5. Conclusion

In summary, we demonstrate that acute loss of Dicer1 leads to liver regeneration inhibition compared with that in WT littermates. Although the Dicer1 deletion reduces the levels of almost all Dicer1-dependent miRNAs, the introduction of miRNA-21 restores the expression of Cyclin D1 by inhibiting Pten and Rhob, which facilitates the activation of the PI3K/AKT/mTOR axis and then rescues the inhibition effect of liver regeneration caused by Dicer1 deletion. Our study has clarified the role of miRNA-21 in the promotion of liver regeneration, providing a theoretical basis for the use of miRNAs in the treatment of clinical liver regeneration disorders.

Author contributions

T. L., Y. J. S. and J. Y. Y. conceived and designed the experiments; T. L., L. X. K. and L. J. performed the experiments; T. L., L. X. K., and Y. J. S. analyzed the data; H. W., T. F. W., and J. Y. Y supervised the whole study. T. L. and J. Y. Y. wrote the paper.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

We thank Zhenru Wu and Yongjie Zhou for their assistance with pathology techniques and animal models. This study was supported by grants from the National Natural Science Foundation of China (NM. 81470037 and 817700653), the 1.3.5 Project for Disciplines of Excellence, West China Hospital, Sichuan University (ZY2017308), and Projects of the Ministry of Science and Technology (2017ZX10203205-005-002).

References

[1] G.K. Michalopoulos, Liver regeneration, J. Cell. Physiol. 213 (2) (2007) 286–300.
[2] N. Fausto, J.S. Campbell, K.J. Riehle, Liver regeneration, J. Hepatol. 57 (3) (2012) 692–694.
[3] A. Rutherford, R.T. Chung, Acute liver failure: mechanisms of hepatocyte injury and regeneration, Semin. Liver Dis. 28 (02) (2008) 167–174.
[4] H. Bantel, Mechanisms of cell death in acute liver failure, Front. Physiol. 3 (2012) 79.
[5] S. Yagi, S. Uemoto, Small-for-size syndrome in living donor liver transplantation, Hepatobiliary Pancreat. Dis. Int. 11 (6) (2012) 570–576.
[6] H. Ji, Y. Lu, Y. Shi, Seeds in the liver, Acta Histochem. 119 (4) (2017) 349–356.
[7] D. Bartel, MicroRNAs: target recognition and regulatory functions, cell 136 (2) (2009) 215–233.
[8] V. Ambros, The functions of animal microRNAs, Nature 431 (7006) (2004) 350.
[9] N.C. Hoffend, W.J. Magner, T.B. Tomasi, The epigenetic regulation of dicer and microRNA biogenesis by Panobinostat, Epigenetics 12 (2) (2017) 105–112.
[10] I. Chaveles, A. Zaravinos, I.G. Habeos, et al., MicroRNA profiling in murine liver after partial hepatectomy, Int. J. Mol. Med. 29 (5) (2012) 747–755.
[11] R.E. Castro, D.M.S. Ferreira, X. Zhang, et al., Identification of microRNAs during rat liver regeneration after partial hepatectomy and modulation by ursodeoxycholic acid, Am. J. Physiol. Gastrointest. Liver Physiol. 299 (4) (2010) G887–G897.
[12] X. Chen, Y. Zhao, F. Wang, et al., MicroRNAs in liver regeneration, Cell. Physiol. Biochem. 37 (2) (2015) 615–628.
[13] E. Bernstein, S.Y. Kim, M.A. Carmell, et al., Dicer is essential for mouse development, Nat. Genet. 35 (3) (2003) 215.
[14] S. Sekine, R. Ogawa, R. Ito, et al., Disruption of Dicer1 induces dysregulated fetal gene expression and promotes hepatocarcinogenesis, Gastroenterology 136 (7) (2009) 2304–2315 (e4).
[15] S. Sekine, R. Ogawa, M.T. Mcmanus, et al., Dicer is required for proper liver zonation, J. Pathol. 219 (3) (2009) 365–372.
[16] X.F. Lu, Y.J. Zhou, L. Zhang, et al., Loss of Dicer1 impairs hepatocyte survival and leads to chronic inflammation and progenitor cell activation, World J Gastroenterol: WJG 21 (21) (2015) 6591.
[17] X.F. Lu, X.Y. Cao, Y.J. Zhu, et al., Histone deacetylase 3 promotes liver regeneration and liver cancer cells proliferation through signal transducer and activator of transcription 3 signaling pathway, Cell Death Dis. 9 (3) (2018) 398.
[18] J. Xia, Y. Zhou, H. Ji, et al., Loss of histone deacetylases 1 and 2 in hepatocytes impairs murine liver regeneration through Ki67 depletion, Hepatology 58 (6) (2013) 2089–2098.
[19] R. Yin, D. Guo, S. Zhang, et al., miR-706 inhibits the oxidative stress-induced activation of PKCα/TAOK1 in liver fibrogenesis, Sci. Rep. 6 (2016) 37509.
[20] R. Ng, G. Song, G.R. Roll, et al., A microRNA-21 surge facilitates rapid cyclin D1 translation and cell cycle progression in mouse liver regeneration, J. Clin. Invest. 122 (3) (2012) 1097–1108.
[21] R. Taub, Liver regeneration: from myth to mechanism, Nat. Rev. Mol. Cell Biol. 5 (10) (2004) 836.
[22] M. Malumbres, M. Barbacid, Cell cycle, CDKs and cancer: a changing paradigm, Nat. Rev. Cancer 9 (3) (2009) 153.
[23] J.H. Albrecht, B.M. Rieland, C.J. Nelsen, et al., Regulation of G1 cyclin-dependent kinases in the liver: role of nuclear localization and p27 sequestration, Am. J. Physiol. Gastrointest. Liver Physiol. 277 (6) (1999) G1207–G1216.
[24] M. Jaumot, J.M. Estanyol, J. Serratosa, et al., Activation of cdk4 and cdk2 during rat liver regeneration is associated with intranuclear rearrangements of cyclin-cdk complexes, Hepatology 29 (2) (1999) 385–395.
[25] J. Chen, M. Bai, C. Ning, et al., Gankyrin facilitates follicle-stimulating hormone-driven ovarian cancer cell proliferation through the PI3K/AKT/HIF-1α/cyclin D1 pathway, Oncogene 35 (19) (2016) 2506.
[26] C. Qiu, Q. Xie, D. Zhang, et al., GM-CSF induces cyclin D1 expression and proliferation of endothelial progenitor cells via PI3K and MAPK signaling, Cell. Physiol. Biochem. 33 (3) (2014) 784–795.
[27] B.D. Manning, L.C. Cantley, AKT/PKB signaling: navigating downstream, Cell 129 (7) (2007) 1261–1274.
[28] Y. Zhou, M. Xiong, L. Fang, et al., miR-21–containing microvesicles from injured tubular epithelial cells promote tubular phenotype transition by targeting PTEN protein, Am. J. Pathol. 183 (4) (2013) 1183–1196.
[29] F. Meng, R. Henson, H. Wehbe–Janek, et al., MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer, Gastroenterology 133 (2) (2007) 647–658.
[30] G. Song, A.D. Sharma, G.R. Roll, et al., MicroRNAs control hepatocyte proliferation during liver regeneration, Hepatology 51 (5) (2010) 1735–1743.
[31] S. Zhu, H. Wu, F. Wu, et al., MicroRNA-21 targets tumor suppressor genes in invasion and metastasis, Cell Res. 18 (3) (2008) 350.
[32] Y. Huang, Y.B. Yang, X.H. Zhang, et al., MicroRNA-21 gene and cancer, Med. Oncol. 30 (1) (2013) 376.
[33] P. Damania, B. Sen, S.B. Dar, et al., Hepatitis B virus induces cell proliferation via HBx-induced microRNA-21 in hepatocellular carcinoma by targeting programmed cell death protein4 (PDCD4) and phosphatase and tensin homologue (PTEN), PLoS One 9 (3) (2014) e91745.
[34] H. Zhu, H. Luo, Y. Li, et al., MicroRNA-21 in scleroderma fibrosis and its function in TGF-β-regulated fibrosis-related genes expression, J. Clin. Immunol. 33 (6) (2013) 1100–1109.
[35] A. Tscherner, A.C. Brown, L. Stalker, et al., STAT3 signaling stimulates miR-21 expression in bovine cumulus cells during in vitro oocyte maturation, Sci. Rep. 8 (1) (2018) 11527.
[36] E. Juskeviciute, R.P. Dippold, A.N. Antony, et al., Inhibition of miR-21 rescues liver regeneration after partial hepatectomy in ethanol-fed rats, Am. J. Physiol. Gastrointest. Liver Physiol. 311 (5) (2016) G794–G806.
[37] S.N. Khleif, J. DeGregori, C.L. Yee, et al., Inhibition of cyclin D-CDK4/CDK6 activity is associated with an E2F-mediated induction of cyclin kinase inhibitor activity, Proc. Natl. Acad. Sci. 93 (9) (1996) 4350–4354.
[38] G.A. Calin, C.M. Croce, MicroRNA signatures in human cancers, Nat. Rev. Cancer 6 (11) (2006) 857.
[39] C. Su, Z. Hou, C. Zhang, et al., Ectopic expression of microRNA-155 enhances innate antiviral immunity against HBV infection in human hepatoma cells, Virol. J. 8 (1) (2011) 354.
[40] M. Girard, E. Jacquemin, A. Munnich, et al., miR-122, a paradigm for the role of microRNAs in the liver, J. Hepatol. 48 (4) (2008) 648–656.
[41] W.C. Tsai, S.D. Hsu, C.S. Hsu, et al., MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis, J. Clin. Invest. 122 (8) (2012) 2884–2897.