MicroRNAs in Liver Regeneration

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Abstract
Liver maintains a unique tremendous regeneration capacity in response to partial hepatectomy (PH) or injury. Hepatocyte proliferation critically contributes to the process of liver regeneration (LR), which is regulated by various cytokines and growth factors. However, the molecular basis of LR remains unclear. Emerging evidence indicates that microRNAs (miRNAs, miRs) are involved in controlling hepatocyte proliferation during LR. In this review, an overview is provided to cover recent achievements in studies on the roles of miRNAs in LR. Studies on the regulatory effects of miRNAs and associated molecular mechanisms in LR will help enhance the understanding of the regenerative process and open up a new prospect for liver transplantation.

Introduction
Currently, the most classical model of liver regeneration (LR) is established by Higgins and Anderson through a two-thirds hepatectomy on rodents [1]. The liver restores its original weight within two weeks following partial hepatectomy (PH) [2-5]. LR is a continuous pathophysiological process with a sequence of distinct phases, including an initiation phase, a proliferation phase, and a termination phase [2, 6]. In the initiation phase, hepatocytes are in a state of reproduction capacity [2, 6]. In the proliferation phase, cell population is growing up [2, 6]. In the termination phase, regeneration is terminated through an apoptosis-proliferation balance [2, 6]. Most normal hepatocytes remain in a relatively dormant state.
in the baseline. After PH, numerous hepatocytes enter G<sub>1</sub> phase from G<sub>0</sub> phase of the cell division cycle, indicating a proliferative activity. In recent years, a well-orchestrated interplay of cytokines, growth factors and signaling pathways has emerged as important regulators in the process of priming and proliferation phases of LR, including tumor necrosis factor (TNF), interleukin-6 (IL-6), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and transforming growth factor–α (TGF–α) [7-14]. Once the liver restores to its original mass, LR enters the termination stage which is controlled by a group of growth inhibitors, such as activin A and TGF-β [15-21].

Recently, increasing evidence has indicated roles of microRNAs (miRNAs, miRs) in regulating proliferation of hepatocytes [22]. MiRNAs are a class of small non-coding RNAs post-transcriptionally regulating gene expressions through combining to the 3’ UTR of their target gene messenger RNAs (mRNAs), which leads to mRNA degradation and/or translation inhibition. MiRNAs participate in many important biological processes including cell growth, proliferation, differentiation, apoptosis and metabolism [23-25]. Dysregulated miRNAs have been reported to be involved in a spectrum of liver diseases [26-32]. Interestingly, circulating miRNAs have emerged as promising biomarkers for the diagnosis and prognosis of liver disease, cancers or many other diseases [22, 33, 34]. Growing evidence has indicated that miRNAs play roles in LR via either accelerating or inhibiting the proliferation of hepatocytes. This review covers recent advances in regulatory effects of miRNAs and potential mechanisms in LR.

**Dynamic expressions of miRNAs during liver regeneration**

In previous studies, miRNA profiles in livers of rodents receiving PH were analyzed. In a murine model of 50% PH, most miRNAs, including miR-16, miR-22, miR-23, miR-24, miR-29, miR-30, miR-122a, miR-126, and miR-145 were decreased during the process of LR, while some miRNAs, including miR-21, miR-26b, miR-192 and miR-194, were increased in the first day and then decreased in the following two days during the process of LR [35].

Besides that, 70% PH was the most used experimental murine model which helps elucidate the mechanisms underlying LR, where some miRNAs, including miR-26a, miR-33, miR-127, miR-150 and miR-378, were decreased [36-40], while some other miRNAs, including miR-34a, miR-122, miR-203 and miR-221, were increased during the process of LR [41-45] (Table 1). Interestingly, certain miRNAs, including miR-21, could be further altered during liver regeneration in response to chronic ethanol feeding; the latter inhibits and delays liver regeneration in experimental animal models [46, 47]. To confirm potential target genes of these abnormally expressed miRNAs, the upregulation of cyclins (A, D, E, F, and G), cyclin-dependent kinases (CDKs) and E2F transcription factors that function as regulators for cell cycle and proliferation was also identified [48-51]. However, further studies are highly needed to clarify the direct association between these miRNAs and LR.

In another study performed by Shu et al. [52], a biphasic expression pattern of miRNA in livers post PH has been detected. It was characterized by an upregulation of ~40% of the miRNAs tested at 3-18 hrs post PH, which was followed by a downregulation of ~70% of the miRNAs tested at 24 hrs post PH. Specially, biphasic expressions of let-7, miR-21, miR-29 and miR-30 were further confirmed using qRT-PCRs. These miRNAs were firstly increased (3 hrs post PH) and then decreased (24 hr post PH) in livers. Intriguingly, the overexpression of these miRNAs at the priming stage of LR triggers the downregulation of miRNA-processing genes, including Rnasen, Dgcr8, Dicer, Tarbp2 and Prkra, which in turn results in the downregulation of miRNAs in the subsequent stage of LR to allow the liver to regenerate via a negative feedback mechanisms [38, 52-54].

In addition, several studies have confirmed that miRNA profiles were associated with three discrete cellular polysome populations, including free and cytoskeleton- and membrane-bound ones in both quiescent and regenerating rat liver [55-57]. As LR progresses, several miRNAs change their polysome distribution pattern which is likely to
follow the changes of their target mRNAs. Therefore, the interplay between polysomes and miRNAs may be involved in the regulation of posttranscriptional gene expressions.

### MiRNAs accelerate liver regeneration

**MiR-21 accelerates hepatocyte proliferation**

In Song et al.’s study, an animal model was established by inactivating the DiGeorge syndrome critical region gene 8 (DGC8), which resulted in miRNA deficiency [38]. Intriguingly, a delay in cell cycle progression from G₁ to S phase was detected in this animal model, which proved the role of miRNAs in controlling cell growth and proliferation. The miRNA expression was analyzed during the early stage of LR post PH in wild-type mice. The results showed a strong induction of miR-21 at 18 hr post PH. Moreover, B-cell translocation gene 2 (Btg2), a cell cycle inhibitor, was confirmed as a direct target gene of miR-21 [58-60]. Btg2 could suppress the activation of fork-head boxM1 (FoxM1), an essential protein for DNA synthesis in hepatocytes post PH [38]. Therefore, hepatocyte proliferation could be accelerated through the inhibitory effect of miR-21 on Btg2.

To investigate how miR-21 stimulates LR, Ng et al. established a rodent model incapable of inducing miR-21 expression in hepatocytes using a miR-21 antisense oligonucleotide (miR-

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**Table 1. Aberrantly expressed microRNAs during liver regeneration**

<table>
<thead>
<tr>
<th>MiRNAs</th>
<th>Variation tendency in initiation and proliferation phase</th>
<th>Variation tendency in termination phase</th>
<th>Direct Target(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiR-16</td>
<td>down-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
</tr>
<tr>
<td>MiR-22</td>
<td>down-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
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<tr>
<td>MiR-23</td>
<td>down-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
</tr>
<tr>
<td>MiR-23b</td>
<td>-</td>
<td>down-regulated</td>
<td>Smad3</td>
<td>[57-60]</td>
</tr>
<tr>
<td>MiR-24</td>
<td>down-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
</tr>
<tr>
<td>MiR-26a</td>
<td>down-regulated</td>
<td>down-regulated</td>
<td>CCND2, CCNE2</td>
<td>[39]</td>
</tr>
<tr>
<td>MiR-29</td>
<td>down-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
</tr>
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<td>MiR-30</td>
<td>down-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
</tr>
<tr>
<td>MiR-31</td>
<td>down-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
</tr>
<tr>
<td>MiR-33</td>
<td>down-regulated</td>
<td>-</td>
<td>CDK6, CCND1</td>
<td>[40]</td>
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<tr>
<td>MiR-122a</td>
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<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
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<td>MiR-126</td>
<td>down-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
</tr>
<tr>
<td>MiR-127</td>
<td>down-regulated</td>
<td>-</td>
<td>Bcl6, Setd8</td>
<td>[36]</td>
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<td>down-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
</tr>
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<td>MiR-150</td>
<td>down-regulated</td>
<td>-</td>
<td>VEGF-A</td>
<td>[37]</td>
</tr>
<tr>
<td>MiR-378</td>
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<td>down-regulated</td>
<td>Odc1</td>
<td>[38]</td>
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<td>MiR-21</td>
<td>up-regulated</td>
<td>down-regulated</td>
<td>Btg2, Rhob</td>
<td>[35, 38, 53-57]</td>
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<tr>
<td>MiR-26b</td>
<td>up-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
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<tr>
<td>MiR-192</td>
<td>up-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
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<td>MiR-194</td>
<td>up-regulated</td>
<td>down-regulated</td>
<td>-</td>
<td>[35]</td>
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<tr>
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<td>up-regulated</td>
<td>INHBB, Met</td>
<td>[41, 87-88, 93]</td>
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<td>-</td>
<td>HO-1</td>
<td>[42, 67-69]</td>
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<tr>
<td>MiR-203</td>
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<td>-</td>
<td>SOCS3</td>
<td>[43-44, 74-76]</td>
</tr>
<tr>
<td>MiR-221</td>
<td>up-regulated</td>
<td>-</td>
<td>Arnt</td>
<td>[45]</td>
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21-ASO), which led to complete elimination of miR-21 surge induced by PH in hepatocytes [61]. Immunostaining and Western blotting analysis demonstrated that cyclin D1 was induced at 18 hr after PH in livers of control mice while mice injected by miR-21-ASO 6 hr after PH displayed attenuated induction of cyclin D1. These data indicate that miR-21 is responsible for cyclin D1 induction and subsequent G1 phase transition of hepatocytes following PH. In addition, gene ontology analysis was carried out to determine the mechanisms how miR-21 induces cyclin D1 expression. Among the candidate genes, the mRNA and protein levels of Rhob, a well-known GTPase as a suppressor of cancer cell growth, transformation, and metastasis, were found to be negatively correlated with the expression of miR-21 induced by PH. Moreover, Rhob mRNA and protein levels were increased in livers of mice treated with miR-21-ASO injection. Based on the results from bioinformatics and luciferase assays, the inverse correlation between miR-21 and Rhob was further clarified. Viewed together, Rhob is directly inhibited by the miR-21 surge induced by PH during the early phase of LR. More importantly, the activation of Akt1 and mammalian target of rapamycin complex-1 (mTORC1) following Rhob inhibition is confirmed, which is responsible for mediating the positive effect of miR-21 on cyclin D1 translation and enhanced liver regenerative capacity post PH.

A recent study from Yan et al. demonstrated that the expression of miR-21 began to decrease immediately after the transition of most hepatocytes from G1 to S phase, suggesting that miR-21 may promote cell cycle progression before the entry into S phase [62]. Flow cytometry analysis showed that the proportion of S-stage hepatocytes was significantly increased or decreased by miR-21 mimics or inhibitors, respectively, indicating that miR-21 promotes cell cycle progression via accelerating the transition of hepatocytes to S phase. Furthermore, it has been reported that miR-21 negatively regulated PTEN at post-transcriptional level in cultured hepatocytes. As the PI3K/Akt signaling pathway is negatively regulated by PTEN, they further confirmed that the activation of PI3K/Akt signaling pathway due to PTEN inhibition is responsible for the promoting effect of miR-21 on LR.

Interestingly, it has also been reported that ursodeoxycholic acid (UDCA), a strong inducer of miR-21, could further upregulate miR-21 expression in LR post PH, which might provide novel approaches to improve the regenerative capacity of liver [63].

**MiR-23b suppresses TGF-β1-induced apoptosis in hepatocytes by Smad3 inhibition**

The TGF-β is an essential regulator involved in the cessation of liver growth once liver is regenerated to normal mass [20, 21]. A previous study has demonstrated that miR-23b could downregulate the TGF-β/bone morphogenetic protein (BMP) signaling by inhibiting Smads [64-66]. In fact, it has been shown that miR-23b was notably reduced at the termination stage of LR (120 hr after PH) [67]. Moreover, miR-23b mimics promoted while miR-23b inhibitor prevented the proliferation and cell cycle progression of BRL-3A cells *in vitro*. Smad3, one downstream protein of TGF-β1 signaling, was markedly down regulated in miR-23b overexpressing cells. Luciferase assays further confirmed Smad3 as a direct target gene of miR-23b. In addition, flow cytometry showed that up-regulation of miR-23b could suppress TGF-β1-induced apoptosis in BRL-3A cells via inhibition of Smad3 expression. Intriguingly, TGF-β1 has been shown to inhibit miR-23b expression at the transcriptional level. Thus, the TGF-β1/miR-23b/smad3 signaling is supposed to be a novel mechanism in the termination stage of LR.

**MiR-122, a miRNA biomarker of acute liver failure patients with spontaneous recovery**

LR is involved in the spontaneous recovery process of a portion of patients suffered from acute liver failure (ALF) [68, 69]. A study was conducted by John et al. to clarify the association between miRNA and spontaneous LR in ALF patients [42]. A notable increase in miR-122 level was detected in both serum and liver tissues of spontaneously recovered patients as compared to nonrecovered patients. Previously, heme oxygenase-1 (HO-1) was confirmed as a target gene of miR-122 with inhibitory effects on cell proliferation by upregulating p21 and downregulating TNF-α and IL-6 [70-72]. The authors demonstrated...
a significant reduction of p21 expression in liver tissues of ALF patients with spontaneous recovery and an induction of TNF-α and IL-6 in serum as compared to nonrecovered patients. These results suggested that miR-122 may accelerate LR through HO-1 inhibition in spontaneously recovered ALF patients. In addition, the authors further identified miR-122 as a biomarker in ALF patients with spontaneous recovery by an ROC curve analysis.

**A20 promotes hepatocyte proliferation through a miR-203-dependent manner**

A20, a zinc finger protein encoded by TNF-induced reaction genes, has been confirmed as a nuclear factor kappa B (NF-κB) inhibitor and a pro-proliferative factor in hepatocytes [73, 74]. In Silva et al.’s study, IL-6-induced signal transducer and activator of transcription 3 (STAT3) signaling was enhanced in A20 overexpressing hepatocytes [43, 44]. To further investigate the mechanism of A20 in accelerating LR, an animal model of A20 KO mice was established. The suppressor of cytokine signaling 3 (SOCS3) was found to be significantly increased in A20 KO livers, leading to subsequent inhibition of IL-6/STAT3 signaling. More importantly, SOCS3 was epigenetically regulated by miR-203 in hepatocytes [75-77]. As the upstream of SOCS3, miR-203 was significantly decreased in A20 KO mice. Taken together, these data suggest that A20 activates IL-6/STAT3 signaling and promotes hepatocyte proliferation via downregulating SOCS3 in a miR-203-dependent manner.

**MiR-221 promotes hepatocyte proliferation via inhibiting Arnt**

MiR-221 has been found to be upregulated in hepatocellular carcinoma (HCC) [78-80]. Meanwhile, it could delay fulminant liver failure and inhibit apoptosis of hepatocytes in mice, which implicates the potential promotive role of miR-221 in LR [81]. A recent study by Yuan et al. demonstrated higher numbers of BrdU- and Ki67-positive nuclei in cultured primary hepatocytes transfected with miR-221 mimics compared with control cells, indicative of the promotive effect of miR-221 in hepatocyte proliferation *in vitro* [45]. To further determine if miR-221 could enhance hepatocyte proliferation *in vivo*, adeno-associated virus type 8 (AAV8-Ttr-miR-221) was used to overexpress miR-221 in the mouse liver followed by 70% PH to induce hepatocyte proliferation *in vivo*. Immunohistochemical analysis showed higher numbers of Ki67, BrdU, and PCNA-positive nuclei in miR-221 overexpressing mice than the controls at the peak of DNA synthesis (36 hr after PH), indicating a rapid entry into S phase of the cell division cycle in miR-221 overexpressing hepatocytes. Furthermore, a class of important cyclins, including cyclin D1, E1, A2, and B1, were found to be upregulated at 36 and 72 hrs after PH in livers of mice overexpressing miR-221. In addition, *in silico* analyses and luciferase reporter assay identified aryl hydrocarbon receptor nuclear translocator (Arnt) as a novel target gene of miR-221. Yuan et al. demonstrated that miR-221 mimics could decrease the expression of Arnt both at the mRNA and protein levels in hepatocytes. Luciferase assays further confirmed Arnt as a target gene of miR-221. Taken together, loss of Arnt contributes to the miR-221-induced hepatocyte proliferation [45]. However, it is worth mentioning that in John et al.’s study, ALF patients with spontaneous recovery (SR) showed higher serum levels of miR-21, miR-122 and miR-221, but significantly lower levels of miR-21 and miR-221 in liver biopsies, compared to nonrecovered patients [42]. Unlike this reciprocal expression pattern of miR-21 and miR-221, miR-122 was elevated in serum and liver tissue of SR patients. This suggests that the pro-proliferation effects of a given microRNA may not completely overlap the function of that miR in the regenerated tissue.

**MicroRNAs inhibit liver regeneration**

**MiR-26a inhibits hepatocyte proliferation by repressing cyclin expression**

Several studies have shown that miR-26a family members could suppress tumorigenesis in liver cancer cells and B lymphoma cells, and expression of miR-26a was also found to be significantly declined after 70% PH [39]. Adenovirus 5 (Ad5)-anti-miR-26a-LUC was utilized to downregulate miR-26a in the mouse liver which resulted in enhanced...
proliferation of hepatocytes and improved liver function characterized by reduced serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin (T-Bil) during PH. Overexpression of miR-26a via Ad5-miR26a-LUC had inverse effects. These data showed an inhibitory effect of miR-26a on hepatocyte proliferation during LR. Cyclin D2 and cyclin E2 were identified as putative target genes of miR-26a using qRT-PCRs and Western blotting analysis. Thus, miR-26a is supposed to inhibit hepatocyte proliferation during LR via decreasing cyclin D2 and cyclin E2.

**MiR-33 inhibits cell proliferation during LR**

MiR-33 is considered as a key transcriptional factor in cholesterol biosynthesis and transport [82, 83]. Meanwhile, as an intronic miRNA located within the gene encoding sterol-regulatory element-binding protein-2 (Srebp-2), miR-33 has been implicated in the regulation of cell cycle [84-86]. In Iwakiri et al.’s study, they found that miR-33 overexpression could inhibit CDK6 and cyclin D1 at mRNA level in A549 and Huh7 cell lines, which would lead to G1 cell cycle arrest and reduce cell proliferation [40]. Moreover, hepatic miR-33 level was found to be downregulated in the mouse liver at 24 hr after PH. Mice with anti-miR-33 treatment displayed enhanced hepatocyte proliferation and LR, paralleling with increased expressions of CDK6 and cyclin D1. Moreover, luciferase reporter assays confirmed that miR-33 directly targets the 3’-UTR of CDK6 and cyclin D1. Thus miR-33 reduces cell proliferation and cell cycle progression during LR via suppressing CDK6 and cyclin D1.

**MiR-34a suppresses hepatocyte proliferation in the termination stage of LR**

In Chen et al.’s study, a highly induction of miR-34a has been detected in livers of rats at 5 days after PH [41]. MTT assays and cell cycle analysis showed that the cell growth was significantly suppressed by overexpression of miR-34a in normal rat liver BRL-3A cells. As the correlation between LR termination and activin-A has been confirmed, the authors sought to verify the association between miR-34a and activin-A in termination regenerating livers. Activin-A, homodimer of two inhibin βB (INHBB) proteins, has been confirmed as an essential regulator to decelerate hepatocyte proliferation during the termination stage of LR [87, 88]. Using luciferase reporter assay, INHBB has been identified as a direct target of miR-34a. BRL-3A cell proliferation was strongly repressed in INHBB siRNA group, indicating that INHBB downregulation represses hepatocyte proliferation. Moreover, as Met is a previously validated target gene of miR-34a [89-92] and has been proved to be essential for promoting hepatocyte proliferation during LR [93], the authors further demonstrated that miR-34a could downregulate Met at mRNA and protein levels in both BRL-3A cells and regenerating liver tissues following PH [93]. In summary, miR-34a was strongly induced during the termination stage of LR. Up-regulation of miR-34a could suppress hepatocyte proliferation through its inhibitory effects on INHBB and Met. Besides Met, several other regulators for cell cycle, including NMYC, cyclin D1, cyclin E2, CDK4, and CDK6, have previously been validated as potential target genes of miR-34a [94, 95], though the downstream mechanisms mediating the inhibitory effect of miR-34a on hepatocyte proliferation during LR remain to be further elucidated.

**MiR-127 suppresses hepatocyte proliferation through Bcl6 and Setd8 inhibition**

MiR-127 is a small non-coding RNA characterized by its overlapping gene structure and functions of regulating gene expressions involved in cell apoptosis [96]. Aberrant expressions of miR-127 have been implicated in suppressing the growth of several types of cancers [97-99]. A recent study by Pan et al. demonstrated that the miR-127 level was downregulated in the rat liver at 24 hr after PH [36]. Overexpression of miR-127 in BRL-3A rat immortalized hepatocytes reduced cell growth and caused cell cycle arrest in the G2/M phase. By using bioinformatics analysis including TargetScan, miRanda and Pictar algorithms, B-cell lymphoma 6 protein (Bcl6) and SET domain-containing protein 8 (Setd8) were identified as two candidate target genes of miR-127. Bcl6 contributes to cell activation, differentiation and proliferation and has been verified as a transcriptional repressor for p53, ATR, and CDKN1A.
Setd8, a member of the SET domain-containing methyltransferase family, plays a role in cell cycle regulation via targeting histone H4 lysine K20 (H4K20) for monomethylation [100]. Using qRT-PCR and Western blotting analysis, they found that the mRNA and protein levels of Bcl6 and Setd8 were remarkably increased in the rat liver at 24 hr after PH. Meanwhile, Bcl6 and Setd8 were downregulated at both mRNA and protein levels in rat hepatocytes treated with miR-127 mimics. Furthermore, Bcl6 si-RNA markedly reduced BRL-3A cell proliferation in vitro. Taken together, these results suggest that downregulation of miR-127 could promote hepatocyte proliferation through increasing Bcl6 and Setd8 during LR.

Inhibition of miR-150 induces hepatocyte proliferation

Vascular endothelial growth factor (VEGF), an angiogenesis-inducing factor, has been confirmed as a promotor for the proliferation of sinusoidal endothelial cells and hepatocytes during LR [101-103]. Thus, miRNAs targeting VEGF have the possibility of regulating hepatocyte proliferation. To elucidate the potential association between VEGF and certain miRNAs during LR, Yu et al. carried out in silico analysis to determine the putative miRNAs targeting VEGF-A [37]. Seven of the putative miRNAs were selected by bioinformatics algorithms for further screening. Based on the results from qRT-PCRs, miR-150 was found to be significantly downregulated at the early stage of LR and was considered to possess the closest correlation to the VEGF-A gene. As VEGF-A was remarkably increased in liver tissues during LR, this alteration may be due to the inverse correlation between miR-150 and VEGF-A. To further confirm this hypothesis, cultivated primary hepatocytes were transfected with miR-150 inhibitors and both mRNA and protein levels of VEGF-A in hepatocytes were increased. In addition, an increased protein level of hypoxia-inducible factor-1α (HIF-1α) was detected in liver tissues at 24 hr and 48 hr post PH. Hypoxia-induced hepatocytes displayed decreased expression of miR-150 and upregulation of VEGF-A, suggesting that the low-level expression of miR-150 in the early phase of LR is associated with induction of HIF-1α.

Downregulation of miR-150, miR-663 and miR-503 were also reported in liver biopsies of patients who had undergone auxiliary liver transplantation [104]. Moreover, inhibition of miRNA-150, miRNA-503 and miRNA-663 promoted hepatocyte proliferation in vitro.

MiR-378 suppresses LR by inhibiting ornithine decarboxylase (Odc1)

In Song et al.’s study, miR-378 expression was decreased in liver tissues following 70% PH, indicating the inverse correlation with LR [38]. Ornithine decarboxylase (Odc1), which encodes a polyamine-synthesizing enzyme needed for DNA synthesis [105], has been reported to have a pro-proliferation effect. Using luciferase reporter assays, Odc1 was confirmed as a direct target gene of miR-378. These data revealed that downregulation of miR-378 contributes to LR via activating Odc1.

Discussion

In this review, we summarized the current knowledge about differentially expressed miRNAs and their implications in the process of LR. The dynamic changes of miRNAs during liver regeneration include three types. MiR-16, miR-22, miR-23, miR-24, miR-26a, miR-29, miR-30, miR-31, miR-33, miR-122a, miR-126, miR-127, miR-145, miR-150 and miR-378 were downregulated during the process of LR. MiR-21, miR-26b, miR-192 and miR-194, were upregulated in the first day and then downregulated in the following two days. MiR-34a, miR-122, miR-203 and miR-221, were upregulated during the process of LR. Among the abnormally expressed miRNAs, miR-21, miR-23b, miR-122, miR-203 and miR-221 are verified as promoters for LR, while miR-26a, miR-33, miR-34a, miR-127, miR-150 and miR-378 have inverse effect (Fig. 1, 2). MiR-21, the most widely studied mRNA in LR, plays a positive role on hepatocyte proliferation through three different target genes, including suppressing Btg2, Rhob and PTEN. MiR-23b, which is notably reduced at the termination stage of LR, promotes hepatocyte proliferation through the TGF-β1/miR-23b/smard3 signaling pathway. MiR-122 is
identified as a biomarker with a high sensitivity of ALF patients with spontaneous recovery, and no direct evidence has been shown in its roles in LR. MiR-203, induced by A20, may lead to hepatocyte proliferation by activating IL-6/STAT3 signaling. MiR-221 has a promotive effect on LR by activating cyclin D1, E1, A2 and B1, and inhibiting Arnt. MiR-26a is supposed to inhibit hepatocyte proliferation during LR via suppressing cyclin D2 and cyclin E2. MiR-33 reduces cell proliferation and cell cycle progression during LR via inhibiting CDK6 and cyclin

**Fig. 1.** The role and molecular mechanisms of microRNAs in hepatocyte proliferation during liver regeneration.

**Fig. 2.** The role of microRNAs in the cell cycle of hepatocytes during liver regeneration.
D1. MiR-34a suppresses hepatocyte proliferation in the termination stage of LR through an inhibitory effect on INHBB and Met. MiR-127 reduces hepatocytes growth and causes cell cycle arrest in the G2/M phase through Bcl6 and Setd8 inhibition. Downregulation of miRNA-150, miRNA-503 and miRNA-663 promote hepatocyte proliferation in vitro. MiR-378 suppresses LR by inhibiting Odc1. Despite of a growing number of studies on miRNAs in the regulation of hepatocyte proliferation and LR, molecular and functional mechanisms of how miRNA dysregulation impacts LR are not completely understood.

This review focused on hepatocyte proliferation during the process of LR, under the hypothesis of the unimpaired hepatocyte proliferation. However, it's worth mentioning that several cell types can contribute to regeneration following toxin-mediated damage, such as oval/progenitor cells though the role of stem cells in LR is under debate [106]. The oval cells, named for their appearance, are associated with ductular reaction, a regenerative process occurs in LR [107, 108]. In addition, they express biliary epithelial cells (BECs)-markers, hepatocyte-specific markers and the embryonic liver marker α-fetoprotein. Kordes et al. identified the mesenchymal hepatic stellate cells (HSCs) as potential sources of oval cells for the regenerating liver [109]. They transplanted HSCs into rats with liver injury and determined these cells as a liver stem cell pool in the regenerating liver. However, the source of oval/progenitor cells remains unknown.

Two strategies could be proposed for enhancing LR by targeting miRNA. For those miRNAs enhancing LR, miRNA mimics could be used to enhance LR. A case in point is the first phase I study of miR-24 mimics used in liver cancer. The nature of miRNA mimics are double-stranded RNAs used to mimic endogenous miRNAs. For those miRNAs attenuating LR, miRNA antagonirs could be used to enhance LR. A case in point is the phase II trial of miR-122 antagonist used in hepatitis. Antagomirs are chemically engineered oligonucleotides which used to silence endogenous miRNAs [110].

In conclusion, better understanding of the roles of miRNAs in LR will definitely help enhance the understanding of the regenerative process and open up a new prospect for liver transplantation.

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Disclosure Statement

The authors declare there are no conflicts of interest.
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