MiR-199a-5p regulates rat liver regeneration and hepatocyte proliferation by targeting TNF-α TNFR1/TRADD/CASPASE8/CASPASE3 signalling pathway

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ABSTRACT
Abnormally expressed miR-199a-5p (miR-199a) has been frequently reported in multiple types of malignancies. Nevertheless, its effect in liver regeneration (LR) is largely still unclear. Herein, we investigated the function of miR-199a in hepatocyte proliferation during LR. As a result, miR-199a expression was significantly increased 12–30 h, in rat hepatic tissue, after partial hepatectomy (PH). The down-regulated expression of miR-199a inhibited proliferation as well as promoted cell apoptosis of BRL-3A. Additionally, TNF-α was found as a target of miR-199a. The administration of TNF-α siRNA regulated the effects of miR-199a on hepatocyte proliferation as well as miR-199a-modulated TNF-α/TNFR1/TRADD/CASPASE8/CASPASE3 signalling pathways. Taken together, these present findings suggested that miR-199a promoted hepatocyte proliferation as well as LR via targeting TNF-α/TNFR1/TRADD/CASPASE8/CASPASE3.

INTRODUCTION

The liver is an important organ of the human body and plays an important role in maintaining the physiological activities of the body [1]. Specifically, when the liver is partially resected or damaged by external factors, compensatory proliferation can occur and the liver can quickly recover to its original liver weight, it is called liver regeneration (LR) [2]. LR could be generally categorized into three stages, including initial (2–6 h post PH), progress (12–72 h post PH), as well as termination (120–168 h post PH). A series of factors could modulate LR, including cytokines, growth factors, miRNAs, etc. [3–5], which is a hot field of modern biomedical research.

microRNAs (miRNAs), a kind of noncoding small RNA, are capable of modulating multiple biological activities via relevant target genes [6–8]. Emerging evidence demonstrates that miRNA plays an important role during LR [4,5]. Particularly, some recent studies have shown that miR-429 [9], miR-1907 [10], miR-27a/b [11], miR-221 [12], miR-21 [13,14] and miR-378 [14] regulates liver cell proliferation and liver regeneration through different target genes. But the mechanism is largely unclear. Nevertheless, it remains unknown of other types of miRNAs modulating hepatocyte proliferation during LR as well as the underlying mechanisms of these regulatory effects.

Herein, in the present research, we demonstrated that miR-199a expression was obviously enhanced in rat liver 12–30 h post PH, followed by an investigation of effects of miR-199a in hepatocytes. As a result, miR-199a could regulate hepatocyte proliferation, G1-to-S phase transition through targeting TNF-α, and TNF-α/TNFR1/TRADD/CASPASE8/CASPASE3 signalling pathways. Taken together, miR-199a was identified as a trigger of hepatocyte proliferation as well as LR by targeting TNF-α/TNFR1/TRADD/CASPASE8/CASPASE3 axis.

MATERIALS AND METHODS

Rat model of PH

Adult Sprague Dawley rats, weighed 230 ± 20 g, were purchased from animal centre of Henan Normal University. The animal experiment was approved by Animal Care and Use Committee of Henan Normal University (License No: SYXK2008-0105) and performed strictly in line with the present Animal Protection Law of China. Model preparation was described in our previous study [9]. In brief, 48 rats were randomly assigned to 8 groups (n = 6 each), including three PH groups and three sham-operated (SO) groups as well as two normal control group. To be specific, 70% hepatectomy was performed on rats in PH groups, with same treatment performed on rats in PH groups, with same...
conducted in PH group except hepatic resection in SO group. At sampling, rats were sacrificed, followed by hepatic resection and immediate conservation into liquid azote and subsequently at −80 °C until further experiment.

**Cell culture as well as administration**

BRL-3A, rat liver cell line, was purchased from cell bank of School of Basic Medicine of Peking Union Medical College (Beijing, China), which was maintained in DMEM (Life technologies, Waltham, MA) containing 10% FBS (Gibco) and 1% penicillin/streptomycin in a 5% CO2 incubator at 37 °C. MiR-199a inhibitor (Ribobio, China), miR-199a mimics (RiboBio) or negative controls (NCs) were transfected into BRL-3A for 48 h, respectively, to inhibit or overexpress miR-199a. The sequences of miR-199a mimic/inhibitor/NCs were showed in Table 1. Additionally, siRNA-TNF-α (sequence-01, 02 or 03, 50nM, Ribobio, Table 2) or siRNA-NC was transfected into BRL-3A for 48 h, respectively, for further investigation of the effects of TNF-α in miR-199a-related hepatocyte proliferation as well as cell growth.

**Cell viability assay**

Cell viability was measured by CCK-8 assay. Briefly, BRL-3A cells were placed into 96-well plates at a density of 5 × 10³ cells/well, followed by incubation at 37 °C overnight. MiR-199a mimics (100nM), miR-199a inhibitor (100nM) or NC transfection was subsequently performed. After 48 h of transfection, we added 10 μL of CCK-8 solution, followed by incubation at 37 °C for 2 h. Finally, Biotek reader (ELx800, Winoski, VT) was used to determine absorbance at 450 nm. The assay was conducted in triplicate.

**EdU incorporation assay**

EdU assay based on our previous study [9]. Briefly, Before fixation in 4% PFA for half an hour cells, an EdU solution with a final concentration of 50μM/L to add in the medium at 37 °C for 2 h, then 0.5% Triton X-100 was utilized for treatment for 10 min, followed by washing with PBS 3 times. Afterwards, 100 μL of 1 × Apollo® reaction cocktail was used in cells for 30 min, followed by cell nuclei staining by 5 μg/mL of DAPI for 30 min and subsequent photography by fluorescent microscope.

**Cell cycle analysis**

For this assay, cells were collected 48 h after transfection, washed with cold PBS for 2 times, followed by fixation in 70% alcohol at −20 °C for over 12 h. Afterwards, cells were washed with cold PBS twice, followed by reaction with 20 μg of PI (Sigma, Ronkonkoma, NY) as well as 50 μg of RNase A (Sigma, Ronkonkoma, NY) in PBS solution (1 mL) at 37 °C for half an hour. Finally, FACSCan was employed to analyze DNA content.

**Cell apoptosis**

For apoptosis assay, cells were collected after 48 h of transfection, followed by centrifugation at 4 °C at 1000 rpm for 5 min to discard the supernatant. Afterwards, cold PBS was used to wash cells for 3 times, followed by centrifugation to discard supernatant. Annexin V-FITC apoptosis detection kit was purchased (BD Pharmingen, San Jose, CA), and cells were added with 3.5 μL Annexin-V-FITC as well as 3.5 μL PI in 100 μL binding buffer for each tube, followed by well-mixture and incubation in dark for 30 min. Afterwards, cells were added with another 400 μL binding buffer, followed by flow cytometry.

**Plasmid construction as well as luciferase reporter assay**

PCR was used to amplify the 3'UTR of TNF-α which contained miR-199a recognition sequence, with PCR product subsequently cloned into psiCHECK-2 Luciferase vector (Promega, Madison, WI). Additionally, primer-based overlapping PCR was used to synthesize a mutant TNF-α 3'UTR. Cells were placed into a 24-well plate (1 × 10³/well), followed by transfection of reporter plasmid, Renilla luciferase control vector, as well as miR-199a mimic/miR-199a inhibitor. After transfection for 48 h, protein samples were collected, followed by analysis by dual-luciferase reporter assay system (Promega) in line with standard instructions.

**RNA extraction as well as qRT-PCRs**

Total RNA, along with miRNA, was isolated with Trizol reagent (Invitrogen, Carlsbad, CA), followed by cDNA synthesis by Reverse Transcription System Kit (Promega, Madison, WI). Afterwards, SYBR® Green I was purchased for qRT-PCR on a Rotor-Gene 3000 real-time analyzer (Corbett Robotics, Brisbane, Australia), which was performed in triplicate. Primer sequences are summarized in Table 3. U6, as well as β-actin, were utilized as internal controls to normalize the expression of miRNA and total mRNA. Finally, the 2−ΔΔCt method was employed for calculation of the relative expression of target genes.

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**Table 1.** Sequences of miR-199a mimics/inhibitors and related controls.

| miRNA       | Sequences (5’→3’)                        |
|-------------|------------------------------------------|
| MiR-199a mimic sense | CCCAGUGUUCAGACUUCUGUUUC |
| MiR-199a mimic antisense | GAACAGGUAGUCUGAACACUGGG |
| MiR-199a inhibitor | GAAACAGGUAGUCUGAACACUGGG |
| NC mimic sense | UUUGUACUACACAAAGACUAGCG |
| NC mimic antisense | CAGUACUUUUGUGAUAGCACA |
| NC inhibitor | CAGUACUUUUGUGAUAGCACAA |

**Table 2.** The sequence of TNF-α siRNAs.

| SiRNA          | Target sequence                      | Sequence                           |
|----------------|--------------------------------------|------------------------------------|
| TNF-α-01       | CCAAATGCGGATGATACCTCAA               | 5’-CCAUGGCAUGGAUCCUCAAGdTdT-3’     |
| TNF-α-02       | GCCGATGAGGTTGTGTACCTTAA             | 3’-dTdTGGUUACGCUACUAAGGU-5’        |
| TNF-α-03       | GCGCATTGCGCACCTTCAA                 | 5’-GCCAGUUGUGGCCAGUACUAAUdTdT-3’   |
|                |                                      | 3’-dTdTGGUAACCCGCAAGGAAU-5’        |
Western blot

RIPA lysis buffer complemented with proteinase inhibitors was utilized to homogenize cell samples, followed by incubation in ice for 30 min as well as centrifugation at 12,000 g at 4°C for 15 min. The protein sample was subjected to SDS-PAGE, then transferred to NC membrane (GE Healthcare). Afterwards, the membrane was reacted with rabbit anti-TNF-α (Bioss, 1:1000), rabbit anti-TNFR1 (Bioss, 1:1000), rabbit anti-TRADD (Bioss, 1:1000), rabbit anti-CASPASE8 (CST, 1:3000), rabbit anti-CASPASE3 (CST, 1:3000) rabbit anti-CCND1 (Bioss, 1:1000), rabbit anti-CCNA2 (Bioss, 1:1000), and rabbit anti-BCL2 (Boster, 1:1000) overnight at 4°C, followed by incubation with HRP-conjugated secondary antibodies goat anti-rabbit IgG (Sigma, 1:5000). Afterwards, Amersham ECL substrates were used for visualization of protein band, followed by measurement of band density using ImageQuant TL software. β-actin (CST, 1:3000) was used as loading control.

Statistical analysis

SPSS 18.0 software was employed for statistical analysis. Data were shown as mean ± SD. Independent t-test or ANOVA with post hoc test was employed for comparison between groups. A p-value < .05 indicated statistical significance.

Results

MiR-199a is related to the proliferation phase of rat LR

Our previous studies have identified several MiRNAs associated with the initiation stage of liver regeneration, miR-199a being one of them [15]. Here, we found that miR-199a was significantly up-regulated at 12–30 h after PH compared with the corresponding SO groups using qRT-PCR assay (Figure 1(A)), which was consistent with miRNA microarray data (Figure S1). Hepatocyte proliferation rate in this time period was observed by immunofluorescence and Western blot analysis and was represented by amount of PCNA positive cells and PCNA protein level (Figure S2). Additionally, miR-199a expression was detected in BRL-3A, showing the up-regulation of miR-199a in hepatocytes 3–6 days after culture (Figure 1(B)). This period coincided with the high expression of PCNA, indicating that this was the logarithmic growth stage of BRL-3A cells [9]. Therefore, the role of miR-199a in

![Figure 1](image-url)

Table 3. Primers used in reverse transcription and quantitative real-time PCR.

| MiRNA and genes | Primers sequences (5'→3') |
|-----------------|----------------------------|
| MiR-199a RT     | GTCGTATCCAGTGCCAGGGGTCCGAGGTATT |
| MiR-199a FP     | CGCAGCTTGATCGACGCAACAG |
| MiR-199a RP     | CCCAGTGTTCAGACTACCTG |
| U6 FP           | GTGCAGGGTCGAGGT |
| U6 RP           | CTCGCTTCGGCAGGACA |
| TNF-α FP        | AACCGTTACGAAATTGGCT |
| TNF-α RP        | TACCTCGAACTTCGCTCTCAAGG |
| CASPASE3 FP     | AGCTGGACCTGGTTGATTGAG |
| CASPASE3 RP     | ACCATGACCCCCCTGTTGA |
| CASPASE8 FP     | ATATCCAGATGAGGAGAC |
| CASPASE8 RP     | GGGGTGTCTTATGCTTACATCAT |
| TRADD FP        | AAAGAAGAAAGTGGCAATCTACAGAGG |
| TRADD RP        | TTCTGGTGGCTAGAATTTTCTCAAC |
| TNFR1 FP        | GGTACTGGCCGGTGTTTCG |
| TNFR1 RP        | GGTCTAGGCTGGATAGAGG |
| BCL2 FP         | CGACCTCTGGTTTGGATTTCCTCG |
| BCL2 RP         | TTTTATATTTTGTTTGGGCA |
| CCND1 FP        | AAAATGCGCGAGGGGAGG |
| CCND1 RP        | GAAGTCTGGTTTGGGTTAG |
| CCNA2 FP        | TTTTATATTTTGTTTGGGCA |
| CCNA2 RP        | GCTGATAGGCTGGAGGAGG |
| β-actin FP      | ACATCCTGGAAGACTCTTGCTGAGT |
| β-actin RP      | GTGCTAGGAGCCGGAGAATCT |
rat hepatocyte proliferation, as well as LR, was further examined.

**Down-regulated expression of miR-199a inhibits hepatocyte proliferation**

MiR-199a mimics/inhibitors (50, 100, as well as 150 nM), or NCs were transfected into BRL-3A for investigation of the roles of miR-199a on proliferation as well as cell growth. Consequently, the expression of miR-199a was increased/decreased after transfection of miR-199a mimics/inhibitors (Figures 2(A,B)). Moreover, miR-199a levels were significantly raised/reduced in BRL-3A following transfection with mimics/inhibitors (100 as well as 150 nM; *p < .01* vs. control) after 48 h, respectively. Thus, 100 nM concentration was chosen for subsequent research. CCK-8 assay along with Edu cell proliferation assays showed that miR-199a inhibitors reduced BRL-3A proliferation (Figure 2(C–E)), miR-199a mimics had no obvious effect on BRL-3A proliferation (Figure 2(C–E)). In addition, flow cytometry indicated miR-199a had no significant effect on the cell cycle of BRL-3A (Figure 2(F)).

**Down-regulated expression of miR-199a promotes hepatocyte apoptosis**

MiR-199a inhibitors or NCs were transfected into BRL-3A to determine the role of miR-199a on hepatocytes apoptosis.

There was an increased apoptotic rate in cells transfected with miR-199a inhibitor (Figure 3), suggesting that miR-199a down-regulate expression promoted BRL-3A apoptosis.

**MiR-199a has a negative association with TNF-α expression both in vivo and in vitro**

Like previous studies, miRNAs should work with targeting genes, for effective functions. To this end, miRwalk was used for the prediction of the target gene of miR-199a to exploit the underlying mechanism of miR-199a in LR and hepatocyte proliferation. Intriguingly, targets belonging to cell cycle as well as apoptosis-associated gene ontology (GO) terms attracted our attention. We further concentrated on TNF-α, with progressively absent expression in proliferation phase of LR (Figure 4(A,B)). Meanwhile, the mRNA and protein expression of TNF-α in BRL-3A was decreased but increased following the treatment of miR-199a mimics or miR-199a inhibitor (Figure 4(C,D)). Therefore, TNF-α may be a potential target gene of miR-199a.

**TNF-α is a direct binding target of miR-199a**

To prove that TNF-α is a target of miR-199a. Two plasmids were constructed by cloning WT as well as MUT 3’UTR into psiCHECK2 vectors, followed by validation of the potential physical interaction of miR-199a with TNF-α mRNA using
luciferase reporter assay. BRL-3A was co-transfected with scramble or miR-199a mimics or inhibitors along with WT/MUT-TNF-α-UTR, followed by determination of luciferase activity at 48 h-post transfection. As a result, luciferase activity was significantly declined and enhanced in miR-199a mimics and miR-199a inhibitor levels, respectively (Figure 5(A,B)), while there was no obvious alteration in MUT 3’UTR vector and miR-199a mimic or inhibitor co-transfected cells with control. Collectively, TNF-α was a direct binding target of miR-199a.

siR-TNF-α reverses miR-199a suppression-inhibited cell growth in vitro

Three siRNAs were designed based on TNF-α sequence, followed by qRT-PCR as well as a Western blot for determination of siRNA knockdown efficiency. Consequently, both mRNA, as well as protein expression of TNF-α, was significantly decreased in BRL-3A following the administration of siRNA-TNF-α (siR-TNF-α-2) (Figure 6(A,B)), which were selected for further assays. MiR-199a inhibitor, as well as siR-TNF-α, were co-transfected into BRL-3A for further validation of the interaction of TNF-α with miR-199a on proliferation and growth of BRL-3A. As a result, the inhibitory influence of miR-199a inhibitor on hepatocyte proliferation and growth was abolished following co-transfection with miR-199a inhibitor and siR-TNF-α (Figure 6(C–E)). Implicating the tight association of TNF-α suppression with miR-199a-derived effects on hepatocyte proliferation as well as cell growth.

Discussion

In recent decades, more and more studies have shown that miRNA is involved in liver regeneration [13,16–18], however, with unknown mechanisms. Herein, we reported that miR-199a showed an obvious up-regulation trend in rat liver 12–30 h post 2/3 PH. MiR-199a down-regulated expression inhibited hepatocyte proliferation in BRL-3A, which also negatively modulated TNF-α in vitro and in vivo. TNF-α suppression was confirmed as a requirement for
miR-199a down-expression-inhibited hepatocyte proliferation. We also showed that miR-199a regulated BRL-3A proliferation through TNF-α/TNFαR1/TRADD/CASPASE8/CASPASE3 signalling pathway.

MiR-199a is a class of small RNA that is evolutionally conserved and was first discovered during the development of the inner ear [19,20]. A large number of studies have shown that it plays an important role in a variety of tumour types [21–23]. MiR-199a has become an important tumour marker. Recently, some studies have shown that miR-199a is down-regulated in bladder urothelial carcinoma [24], hepatocellular carcinoma [25], and cutaneous squamous cell carcinoma [26], but up-regulated in gastric cancer [27], Marek’s disease virus-induced T cell lymphoma [28], and uveal melanoma [29].

Figure 4. MiR-199a negatively correlates with TNF-α expression at protein level both in vitro and in vivo. (A) qRT-PCR analysis for TNF-α level in liver tissue at 12–30 h after PH (compared with those at SO group). (B) Western blot analysis for TNF-α level in liver tissue at 12–30 h after PH (compared with those at SO group). (C) qRT-PCR analysis for TNF-α expression in BRL-3A after transfection with miR-199a inhibitor (miR-199a-i) or NC-I. (D) Western blot analysis for TNF-α expression in BRL-3A transfected with miR-199a inhibitor (miR-199a-i) or NC-I. β-actin/U6 were used as loading control. All data are represented as the mean ± SD, *p < .05, **p < .01.

Figure 5. TNF-α is a direct target of miR-199a. (A) The luciferase report analysis for the relative fluorescence level in BRL-3A cells after co-transfection of TNF-α wild-type and mutant 3’UTR with miR-199a mimic/inhibitor. (B) The miR-199a binding site in the 3’UTR of TNF-α and the corresponding mutation site. All data are represented as the mean ± SD, *p < .05.
Figure 6. siRNA- TNF-α reverses miR-199a inhibition-inhibited cell growth in vitro. (A,B) TNF-α level were measured in BRL-3A after treatment with siRNA- TNF-α (sequence-1,-2 or -3) by qRT-PCR and Western blot analysis. (C) Cell viability assay. (D) Edu (red) cell proliferation assay. (E) The percentage of Edu-positive cells was quantified. The results demonstrated that siRNA- TNF-α reversed the inhibitive effect of miR-199a-i on hepatocyte proliferation. All data are represented as the mean ± SD, *p < .05, **p < .01.

Figure 7. MiR-199a regulates proliferation in rat hepatocyte via TNF-α/TNFFR1/TRADD/CASPASE8/CAPASE3 signalling pathway. (A) qRT-PCR analysis for TNF-α, TNFR1, TRADD, CASPASE8, and CAPASE3, BCL2, CCND1 and CCNA2 in rat hepatocyte BRL-3A after co-transfection with siR- TNF-α, miR-199a inhibitor (miR-199a-i) or its negative control (NC-i). (B) Western blot analysis for TNF-α, TNFR1, TRADD, CASPASE8, and CAPASE3, BCL2, CCND1, and CCNA2 in rat hepatocyte BRL-3A after co-transfection with siR-TNF-α, miR-199a inhibitor (miR-199a-i) or its negative control (NC-i). All data are represented as the mean ± SD, *p < .05, **p < .01.
These results suggest that miR-199a has different functions in different cancers. Recent studies have shown that miR-199a can be used to regulate HBV replication [30]. Also, miR-199a is associated with the progression of liver fibrosis [31]. In addition, miR-199a is considered to be the assumed target for HCC diagnosis and treatment [32,33]. These above-described findings suggest that miR-199a plays an important role in the progression from chronic hepatitis to cirrhosis and finally to HCC. For further illustration of the mechanism of miR-199a in LR, qRT-PCR was used to confirm the significant up-regulation of miR-199a 12–30 h after liver resection, implicating the possible key role of miR-199a in hepatocyte proliferation during LR. Moreover, miR-199a expression was artificially decreased in BRL-3A, which revealed that miR-199a down-regulation inhibited proliferation but promoted apoptosis in BRL-3. The present outcomes form the basis to further investigate the mechanism of miR-199a-modulating hepatocyte proliferation. In addition, up-regulated expression of miR-199a in proliferative phase of LR requires further investigation.

MiRNA exerts its biological function through its target genes. Studies have shown that the effects of miRNA in the proliferation phase of LR depend on its specific target genes [9,34,35]. For further investigation of the action mechanism of miR-199a in modulating hepatocyte proliferation, targets belonging to cell cycle- as well as apoptosis-related GO terms attracted our attention, and we further concentrated on TNF-α, with progressively absent expression in proliferation phase of LR. MiR-199a mimics could decrease, whereas miR-199a inhibitor could enhance both mRNA and protein expression of TNF-α in BRL-3A. TNF-α was further validated as a direct target of miR-199a by dual-luciferase reporter assay.

TNF-α is a kind of pleiotropic cytokine, which was discovered by Carswell in 1975 and secreted by a variety of cells in various inflammatory and immune responses [36]. There are two types of tumour necrosis factor receptor (TNFR) families, TNFR1 and TNFR2, but most of the biological activity of TNF-alpha is through binding to TNFR1 [37]. TNFR1 is universally expressed in all cell types, playing an important role in NF-κB pathway activation. TNFR1 ligation could trigger receptor trimerization, which subsequently recruits TRADD, the latter activates caspase-8 and induce cell apoptosis by binding with a specific death domain (DD) in cytoplasmic domain of TNFR1 [38]. In turn, activated caspase-8 could activate effector caspses, including caspase-3, to trigger cell apoptosis via digesting upwards of hundreds of proteins [39]. The molecular context of TNFR1 activation is thought to determine whether it promotes regeneration or contributes to hepatocyte death [40]. Studies have shown that cell populations, involved in normal liver physiology, including hepatocytes, endothelial cells as well as neutrophils, undergo apoptosis through TNF/TNFR1-induced death signals [41]. Our outcomes implicated that decreased expression of TNF-α reversed the inhibitory effect of miR-199a inhibitor on hepatocytes.

The binding of TNF-α to TNFR1 triggers DISC formation, consisting of TRADD, FADD, caspase-8, as well as hepatic DISCs assembly, thereby initiating a caspase cascade causing hepatocyte apoptosis [42]. Studies have shown that DISC is related to TRADD adaptor protein to initiate procaspase-8 cleavage into caspase-8 as well as subsequent activation of downstream effector caspses (including caspase-3) [43]. The correlation of FADD, TRADD, as well as procaspase-8 with TNFR1, causes apoptosis by protein cleavage and activation of downstream apoptotic effector caspses in hepatocytes [44], which implicates the recruitment of TRADD adaptor proteins as a critical step during the induction of TNF/TNFR1-mediated apoptotic DISC association. However, it is unclear whether TNF-α-mediated apoptotic is modulated by miR-199a in hepatocytes. TNF-α was significantly decreased in rat liver 12–30 h post PH. Nevertheless, down-regulation of miR-199a prompted TNF-α, TNFR1, TRADD, CASPASE8, and CASPASE3 expression in BRL-3A. SiR- TNF-α reverses the role of down-regulated miR-199a expression in promoting TNF-α, TNFR1, TRADD, CASPASE8 and CASPASE3 expression. Therefore, it is speculated that miR-199a could modulate rat hepatocyte proliferation via the activation of TNF-α/TNFR1/TRADD/CASPASE8/CASPASE3 pathway.

Conclusions

MiR-199a expression is up-regulated in the proliferative phase of LR. Additionally, miR-199a regulates hepatocyte proliferation via targeting TNF-α/TNFR1/TRADD/CASPASE8/CASPASE3 signalling pathways. Therefore, miR-199a might be used as a promising target to modulate LR as well as hepatic malignancy progression.

Disclosure statement

No potential conflict of interest was reported by the authors.

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