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Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation

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ABSTRACT

Hepatic stellate cells (HSCs) activation is an initial event in liver fibrosis. MicroRNAs (miRNAs) have been found to play essential roles in cell differentiation, proliferation, and fat metabolism. In this study, we showed that down-regulation of two over-expressed miRNAs, miR-27a and 27b allowed culture-activated rat HSCs to switch to a more quiescent HSC phenotype, with restored cytoplasmic lipid droplets and decreased cell proliferation. Mechanistically, retinoid X receptor α was confirmed to be the target of miR-27a and 27b. These results indicated a new role and mechanism of miR-27a and 27b in regulating fat metabolism and cell proliferation during HSCs activation.

1. Introduction

Hepatic stellate cells (HSCs) normally reside in the space of Disse, containing bunches of vitamin A-riching lipid droplets, while activated HSCs lose cytoplasmic lipid droplets and trans-differentiate to proliferative, fibrogenic myofibroblasts, and play an essential role in the formation of liver fibrosis [1]. We hypothesized that microRNA (miRNA) could take part in such transdifferentiation.

MiRNAs are endogenous small (~22nt) non-coding RNAs that regulate gene expression by specifically interacting with 3′untranslated region (3′UTR) of target gene mRNAs to repress translation or enhance mRNA cleavage [2], and play essential roles in a variety of cellular processes, including cell differentiation, proliferation, and fat metabolism [3–6]. Recently, miRNAs have caused tremendous excitement in cancer research [7,8]. Their relationship with inflammation, immunity is just beginning to be explored [9,10]. Identification of abnormally expressed miRNAs in pathologic state will be helpful to further understand the mechanism of disease and modulation of their activity may be of therapeutic benefit [11]. However, the role of miRNAs in liver fibrosis has not been addressed.

In this study, we focused on two miRNAs (27a and 27b) that over-expressed in primary culture-activated rat HSCs. Down-regulation of miR-27a and 27b allowed culture-activated rat HSCs to switch to a more quiescent HSC phenotype, with restored cytoplasmic lipid droplets and decreased cell proliferation. Mechanistically, retinoid X receptor α (RXRα) was confirmed to be the target of miR-27a and 27b. These results indicated a new role and mechanism of miR-27a and 27b in regulating fat metabolism and cell proliferation during HSCs activation.

2. Materials and methods

2.1. Cell isolation and culture

Normal male Sprague–Dawley rats (body weight about 400 g) were cared for according to the principles of Guide for the Care and Use of Laboratory Animals formulated by Fudan University. HSCs were isolated from the livers through two steps of digestion as described previously [12]. By analysis through autofluorescence

Abbreviations: α-SMA, α-smooth muscle actin; BrdU, bromodeoxyuridine; HSCs, hepatic stellate cells; LI, labeling index; miRNA, microRNA; MRE, microRNA recognition element; RXRα, retinoid X receptor α; 3′UTR, 3′untranslated region

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of vitamin A and exclusive tests of trypan blue, the purity and vitality of the isolated HSCs were more than 90% and 95%, respectively. According to the changes of phenotype, HSCs maintained for 3 days were regarded as quiescent HSCs, for 10 days as activated HSCs. Passage two HSCs were used in transient transfection. Both HSCs and human embryonic kidney cell line 293T were maintained in DMEM (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen).

2.2. Immunofluorescence and Sudan III staining

HSCs grown on coverslips were fixed with 2% paraformaldehyde for 15 min on ice, followed by incubation with monoclonal antibodies against desmin (Chemicon Temecula, CA, USA) at 37 °C for 1 h, FITC labeled secondary antibody (Proteintech, Chicago, IL, USA) at room temperature for 45 min, and finally mounted with glycerol. Lipid droplets were stained with Sudan III solution for 2 min at room temperature.

2.3. RT-PCR and real-time RT-PCR

Total RNAs were extracted using TRIzol reagent (Invitrogen). cDNAs were synthesized with Thermoscript RT-PCR system for First-Strand cDNA Synthesis (Invitrogen) and NCode miRNA First-Strand cDNA Synthesis kits (Invitrogen). For quantitative detection of RXRα mRNAs and mature miRNAs, the templates and primer sets (Table 1) were mixed with SYBR Premix Ex Taq (Takara, Tokyo, Japan), and real-time PCR was performed using Rotor-Gene 3000 (Corbett Research, Sydney, Australia). For the detection of miRNA precursors, RT-PCR was performed as reported [13] and the term 'miRNA precursors' was used to embrace both pri-miRNA and pre-miRNA. The expression of mRNAs and miRNAs were normalized to β-actin and U6snRNA, respectively.

2.4. Western blot analysis

Whole cell lysates were obtained by on ice scraping cells into RIPA buffer. RXRα, collagen type I and alpha-smooth muscle actin (α-SMA) proteins were analyzed by Western blot using RXRα monoclonal antibody (Chemicon), collagen type I polyclonal antibody (Calbiochem, San Diego, CA, USA) and α-SMA monoclonal antibody (Sigma, St. Louis, MO, USA). Signals were detected by Super signal Western Pico chemiluminescent substrate (Pierce, Rockford, IL). Control for loading was checked by re-probing the membrane with β-actin antibody (Sigma).

2.5. Transient transfection of anti-miR miRNA Inhibitors

The passage one HSCs were re-seeded at a density of 2.5×10^4 cells per well in 24-well plates or 2.5×10^5 per 6-cm dishes. After 24 h, anti-miR miRNA specific Inhibitors, anti-miR-27a, anti-miR-27b or negative control anti-miR-neg (Ambion, Austin, TX, USA) were transiently transfected by Lipofectamine 2000 (Invitrogen) into these passage two HSCs for 42 h, at a final concentration of 50 nM, 75 nM or 100 nM.

2.6. Bromodeoxyuridine (BrdU) incorporation assay

For evaluation of cell proliferation, cells were incubated with 50 μg/ml BrdU (Sigma) to incorporate BrdU into DNA in cells engaged in DNA synthesis (cells in S phase of the cell cycle) at the last 8 h of anti-miR miRNA Inhibitors transfection. BrdU incorporation was determined by monoclonal antibody to BrdU (Sigma) and Texas red-labeled secondary antibody (Proteintech). The nuclei were counterstained with hoechst33258 (Sigma). For each well, five areas were randomly counted at a magnification of 200. The results were expressed as the labeling index (LI) according to the following formula: number of BrdU-positive nuclei ×100/number of total nuclei.

2.7. Plasmid construction

The putative miR-27 anchor element from 2962 to 2990 of RXRα mRNA 3′UTR (5′-GGCCTTGCTGCAGCTCGTCTGACTGTGAA-3′) was termed as miR-27 recognition element (MRE27) [14]. The synthesized RXRα 3′UTR fragment containing three copies of MRE27 with flanking Xho1 and Not1 restriction enzyme cohesive end were annealed and ligated into the psiCHECK-2 Vector (Promega, Madison, WI, USA) between the XhoI and NotI sites located 3′ to the renilla luciferase translational stop codon, resulting in single insertion as psiCHECK-2/RXRαMRE27x3. In this vector, the post-transcriptional

Table 1
Sequence of primers used for PCR analyses.

Gene		Primers	Length of production
Rat RXRα	Sense	5′-caacgggtcgaggctccatg-3′	727 bp
	Anti-sense	5′-gagctggggttcagccccat-3′	
Rat β-actin	Sense	5′-aggatgcagaaggagattactgc-3′	219 bp
	Anti-sense	5′-aaaacgcagctcagtaacagtgc-3′	
miR-9	Sense[a]	5′-tctttggttatctagctgtatga-3′	About
miR-19a	Sense[a]	5′-tgtgcaaatctatgcaaaactga-3′	70 bp
miR-19b	Sense[a]	5′-gcaaatccatgcaaaactga-3′	
miR-27a	Sense[a]	5′-ttcacagtggctaagttccgc-3′	
miR-27b	Sense[a]	5′-ttcacagtggctaagttctgc-3′	
miR-30a	Sense[a]	5′-tgtaaacatcctcgactggaag-3′	
miR-30c	Sense[a]	5′-tgtaaacatcctacactctcagc-3′	
miR-30d	Sense[a]	5′-tgtaaacatccccgactggaag-3′	
miR-122	Sense[a]	5′-tggagtgtgacaatggtgtttgt-3′	
miR-124	Sense[a]	5′-gcacgcggtgaatgcc-3′	
miR-128a	Sense[a]	5′-tcacagtgaaccggtctctttt-3′	
miR-128b	Sense[a]	5′-tcacagtgaaccggtctctttc-3′	
miR-130a	Sense[a]	5′-cagtgcaatgttaaaagggcat-3′	
miR-130b	Sense[a]	5′-cagtgcaatgatgaaagggcat-3′	
miR-148b	Sense[a]	5′-tcagtgcatcacagaactttgt-3′	
miR-152	Sense[a]	5′-tcagtgcatgacagaacttgg-3′	
miR-294	Sense[a]	5′-aaagtgcttccctttgtgtgt-3′	
miR-301	Sense[a]	5′-cagtgcaatagtattgtcaaagcat-3′	
miR-302	Sense[a]	5′-taagtgcttccatgttttggtga-3′	
miR-373	Sense[a]	5′-gaagtgcttcgatttggggtgt-3′	
miR-450	Sense[a]	5′-tttttgcgatgtgttcctaatg-3′	
miR-454	Sense[a]	5′-tagtgcaatattgcttatagggttt-3′	
miR-455	Sense[a]	5′-atgcagtccacgggcatatacact-3′	
miR-493	Sense[a]	5′-ttgtacatggtaggctttcatt-3′	
miR-520a	Sense[a]	5′-aaagtgcttcccttggactgt-3′	
miR-520b	Sense[a]	5′-aaagtgcttcctttagaggg-3′	
miR-520c	Sense[a]	5′-aaagtgcttcctttagagggtt-3′	
miR-520d	Sense[a]	5′-aaagtgcttctctttggtgggtt-3′	
miR-520e	Sense[a]	5′-aaagtgcttcctttttgaggg-3′	
miR-520f	Sense[a]	5′-aagtgcttcctttagagggtt-3′	
miR-616	Sense[a]	5′-actcaaaacccttcagtgactt-3′	
miR-721	Sense[a]	5′-cagtgcaattaaaaggggaa-3′	
pre-miR-27	Sense	5′-gcagggcttagctgcttg-3′	66 bp
	Anti-sense	5′-ggcggaacttagccactgt-3′	
pre-miR-128	Sense	5′-tggattcggggccgtag-3′	62 bp
	Anti-sense	5′-aaagagaccggttcactgtgag-3′	
pre-miR-301	Sense	5′-gctctgactttattgcactactg-3′	60 bp
	Anti-sense	5′-gctttgacaatactattgcactg-3′	
U6snRNA	Sense	5′-ctcgcttcggcagcaca-3′	94 bp
	Anti-sense	5′-aacgcttcacgaatttgcgt-3′	

[a] Sense primers for mature miRNAs were provided here, anti-sense primer was provided by Invitrogen as Universal q-PCR Primer, so the length of production was not clear, according to our gel analysis, it was about 70 bp.

regulation of renilla luciferase was potentially regulated by RXRα MRE27 fragment. The activity of renilla luciferase was normalized by the internal firefly luciferase activity. MiR-27a and 27b expression plasmids, pcDNA6.2-GW/EmGFP-mir-27a and pcDNA6.2-GW/EmGFP-mir-27b, were constructed according to manufacture's instructions (Invitrogen). pcDNA6.2-GW/EmGFP-mir-neg vector was provided by Invitrogen as control. The nucleotide sequences of constructed plasmids were confirmed by DNA sequencing (Invitrogen).

2.8. Luciferase assay

The 293T cells were seeded into 24-well plates 24 hs before transfection and transiently cotransfected with 0.1 μg psiCHECK-2/RXRαMRE27x3 reporter plasmid and 0.4 μg pcDNA6.2-GW/EmGFP-mir-27a, mir-27b or mir-neg by Lipofectamine 2000 (Invitrogen). Luciferase assays were performed 42 h later using the Dual-Luciferase reporter system (Promega). The renilla and firefly luciferase signal were detected using the Veritas Microplate Luminometer (Turner Biosystems, Sunnyvale, CA).

2.9. Statistics assay

Values were expressed as means ± S.D. Comparison between means was made by ANOVA. When appropriate, ANOVA was followed by Tukey's posthoc test to do pair-wise comparisons between all groups. Differences were considered significant if $P < 0.05$. All assays were performed in triplicate and experiments repeated at least three times with independently isolated cells.

3. Results and discussion

3.1. MiR-27a and 27b were up-regulated in activated HSCs

Rat HSCs in primary culture were used to determine the miRNAs differently expressed during HSCs activation. The positive identification of the cells was determined by autofluerecence of vitamin A and immunofluerecent staining with intermediate filament-desmin, a rat HSCs specific marker (Fig. 1A and B). With continued culture on plastic, cells appeared "activated" (i.e., increased spreading and diminished vitamin A-riching lipid droplets) (Fig. 1C and D) and began proliferating.

Mature miRNAs that differently expressed in quiescent and activated HSCs were evaluated by real-time PCR. In activated HSCs, some mature miRNAs were over-expressed with fold changes of >1.5 (including miR-27a, 27b and miR-30a, 30c, 30d), while the expression of a number of miRNAs were depressed (Fig. 2B and Table 2). To investigate the potential function and mechanism of these miRNAs during HSCs activation, we focused on miR-27a and 27b which are two paralogous sequences of miR-27 that differ at only one position and both over-expressed in activated HSCs. The precursors for miR-27a and 27b were amplified using the same pair of primers. In accordance with mature miR-27a and 27b, the expression of their precursors also increased in activated HSCs (Fig. 2C). MiR-27a and 27b has been reported to be involved in the regulation of cell proliferation and differentiation in breast cancer and liver cancer [15–17]. We were interested to know if miR-27 also played a role in HSCs activation.

3.2. Down-regulation of miR-27a and 27b allows HSCs to restore their ability to accumulate cytoplasmic lipid droplets and decreases HSCs proliferation

To further determine the biological function of miR-27 during HSCs activation, miR-27a and 27b were inactivated by anti-miR-27a and anti-miR-27b in fully activated passage two HSCs. The effects of anti-miR-27a and anti-miR-27b on the expression of miR-27a and 27b were monitored and validated by quantitative real-time PCR (Fig. 3A).

We first examined whether suppression of miR-27a and 27b affected the morphological features of activated HSCs. One of the most distinct morphological features of activated HSCs was diminished cytoplasmic lipid droplets. In some activated HSCs

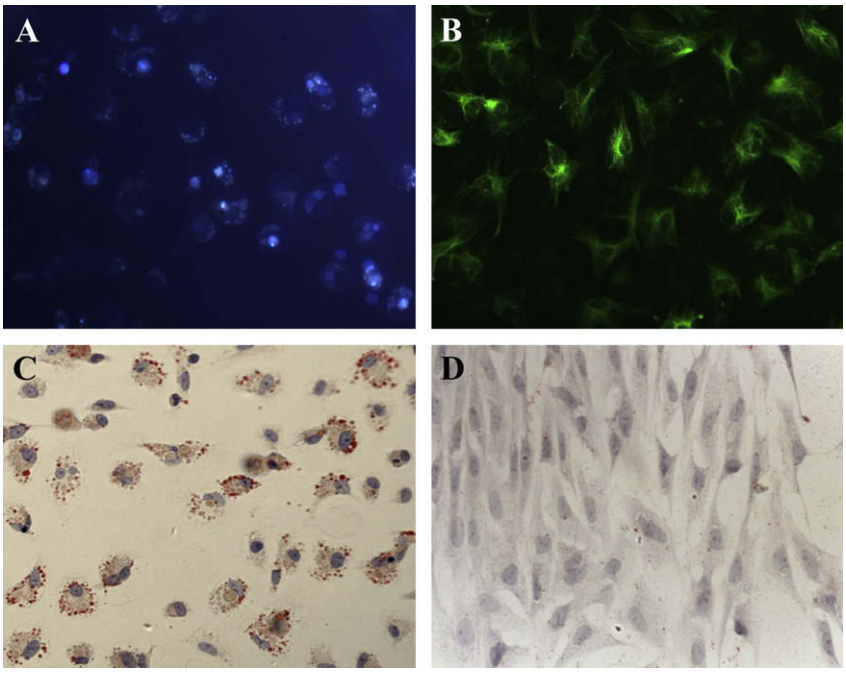

Fig. 1. Identification and culture-activation of primary rat HSCs: (A) Autofluorescence of cytoplasmic vitamin A lipid droplets in D3 HSCs, excitated at 330 nm. (B) Immunofluorescent staining of desmin in D10 HSCs, FITC. (C) Bunches of cytoplasmic lipid droplets in D3 HSCs, Sudan III staining. (D) Depletion of cytoplasmic lipid droplets in D10 HSCs, Sudan III staining. Original magnification ×200. D3 and D10 HSCs represent day3 quiescent and day10 activated HSCs.

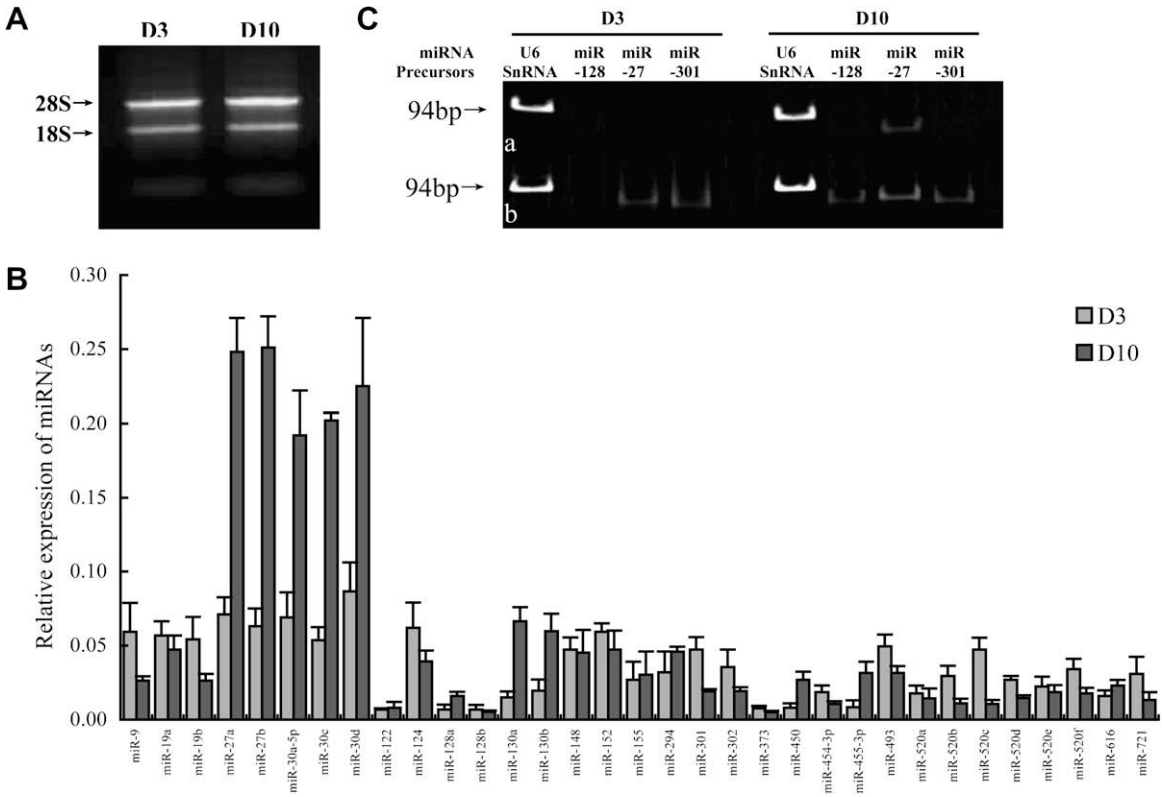

Fig. 2. Expression of miRNAs in D3 and D10 rat HSCs: (A) Five microgram total RNAs were loaded into each lane, the 1% agarose gel was stained with 0.1 μg/ml ethidium bromide; (B) Expression of mature miRNAs in D3 and D10 rat HSCs were evaluated by real-time PCR; normalized to U6snRNA. (C) Expression of miRNA precursors in D3 and D10 HSCs were analyzed by RT-PCR with 25 cycles (a) or 28 cycles (b). D3 and D10 HSCs represent day3 quiescent and day10 activated HSCs.

transfected with both anti-miR-27a and anti-miR-27b, large and small lipid droplets reappeared in perinuclear cytoplasm, as demonstrated by Sudan III staining (Fig. 3B), and the number of Sudan III positive-staining cells increased in a dose dependent manner (Fig. 3C). These observations indicated a negative role of miR-27 in lipid anabolism regulation; down-regulation of miR-27a and 27b allows activated HSCs to restore their ability to accumulate cytoplasmic lipid droplets. However, no significant cytoplasmic lipid droplets could be found in HSCs transfected with either anti-miR-27a or anti-miR-27b, which suggested that the blockage of one paralog of over-expressed miR-27a or 27b is not sufficient to induce above phenotypic switch in activated HSCs. MiR-27a and 27b share the same miRNA binding sites in the 3′UTR of predicted target mRNAs, increased expression of either of them would down-regulate their target genes. Both miR-27a and 27b were up-regulated in activated HSCs, so cotransfection of anti-miR27a and anti-miR27b were required to assess the role of these miRNAs in HSCs. According to this preliminary finding, in the studies afterward, only anti-miR-27a and anti-miR-27b cotransfection groups were set.

Table 2
Differently expressed miRNAs in quiescent (D3) and activated (D10) HSCs.

MiRNA	D3HSC average	S.D. ±	D10HSC average	S.D. ±	Folds increased
miR-130a	0.015	0.004	0.066	0.009	3.427
miR-27b	0.063	0.012	0.251	0.021	2.984*
miR-455	0.008	0.005	0.032	0.007	2.762
miR-30c	0.054	0.009	0.202	0.005	2.762*
miR-27a	0.071	0.012	0.248	0.023	2.494*
miR-450	0.008	0.003	0.027	0.005	2.392
miR-130b	0.020	0.008	0.060	0.012	2.046
miR-30a	0.069	0.017	0.192	0.030	1.783*
miR-30d	0.087	0.020	0.225	0.046	1.598*
miR-19b	0.054	0.015	0.026	0.005	−0.517
miR-9	0.059	0.020	0.026	0.003	−0.558
miR-721	0.031	0.012	0.013	0.005	−0.571
miR-301	0.047	0.009	0.019	0.002	−0.596
miR-520b	0.030	0.007	0.011	0.003	−0.627
miR-520c	0.047	0.008	0.011	0.003	−0.778

The expression of miRNAs were detected by real-time PCR, data normalized to U6snRNA, *$P < 0.05$.

Another obvious and important parameters of HSCs activation is increased cell proliferation. We next examined whether inactivation of miR-27a and 27b inhibits this parameter. By BrdU incorporation assay, we found that cotransfection of anti-miR-27a and anti-miR-27b decreased the percentage of activated HSCs in S phase of the cell cycle (Fig. 3D). In breast cancer cell lines, both miR-27a and 27b were abundantly expressed and have been reported to regulate several genes involved in cell proliferation and differentiation [15,16]. Down-regulation of miR-27a by antisense miR-27a decreased the percentage of MDA-MB-231 cells in S phase of cell cycle [16]. In human hepatocellular carcinoma cells, miR-27a has also been proved to function as proliferation-promoting factor [17]. So increased expression of miR-27 may facilitate cell proliferation, thus exhibit oncogenic activity. Our findings proved that increased expression of both miR-27a and 27b also played a role in promoting cell proliferation during HSCs activation.

Hoechst33258 staining showed that cell apoptosis was not affected by cotransfection of anti-miR-27a and anti-miR-27b, and other characteristics of activated HSCs such as enhanced matrix protein collagen type I expression and expression of α-smooth muscle actin (α-SMA) were almost not affected (Fig. 3E).

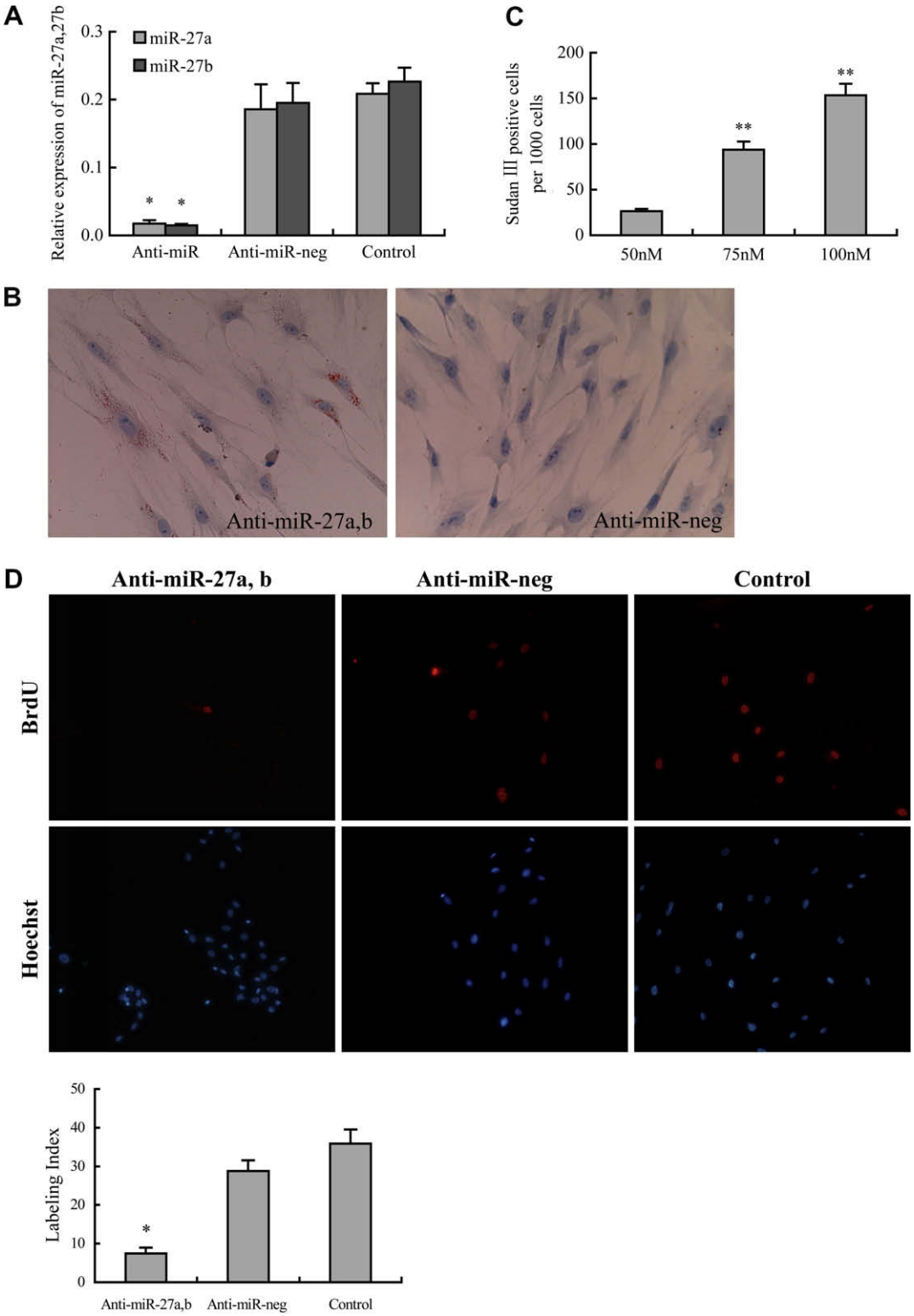

Fig. 3. Down-regulation of miR-27a and 27b allows HSCs to restore their ability to accumulate cytoplasmic lipid droplets and decreases HSCs proliferation: (A) 75 nM anti-miR-27a or anti-miR-27b transfection caused down-regulation of miR-27a or 27b in passage two HSCs, cells treated with transfection medium served as control, normalized to U6snRNA. $^{*}P < 0.05$ compared with anti-miR-neg. (B) Accumulation of cytoplasmic lipid droplets in passage two HSCs cotransfected with 75 nM anti-miR-27a, b, Sudan III staining. Original magnification ×400. (C) The number of Sudan III staining cells increased in a dose-dependent manner after cotransfected with anti-miR-27a, b, $^{**}P < 0.01$ compared with the other two. (D) BrdU incorporation assay in passage two HSCs cotransfected with 75 nM anti-miR-27a, b, nuclei were counterstained with hoechst33258, cells treated with transfection medium served as control, Original magnification ×200. $^{*}P < 0.05$ compared with anti-miR-neg. (E) D10 activated HSCs synthesized more collagen type I and expressed α-SMA, $^{**}P < 0.01$ compared with D3 HSCs. The expression of collagen type I and α-SMA were not affected in passage two HSCs cotransfected with 75 nM anti-miR-27a, 27b for 42 h. The expression of collagen type I and α-SMA protein was determined by Western blot analyses using whole cell lysates, β-actin served as loading control. $^{\#}P > 0.05$ compared with those transfected with anti-miR-neg. D3 and D10 HSCs represent day3 quiescent and day10 activated HSCs; anti-miR-27a, b represents anti-miR-27a and anti-miR-27b.

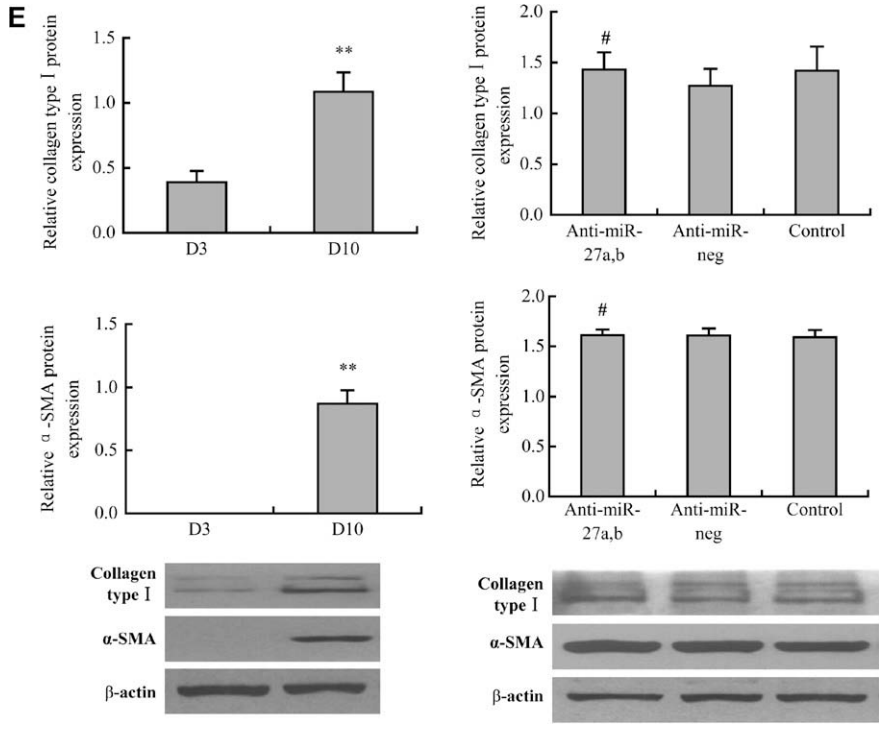

Fig. 3 *(continued)*

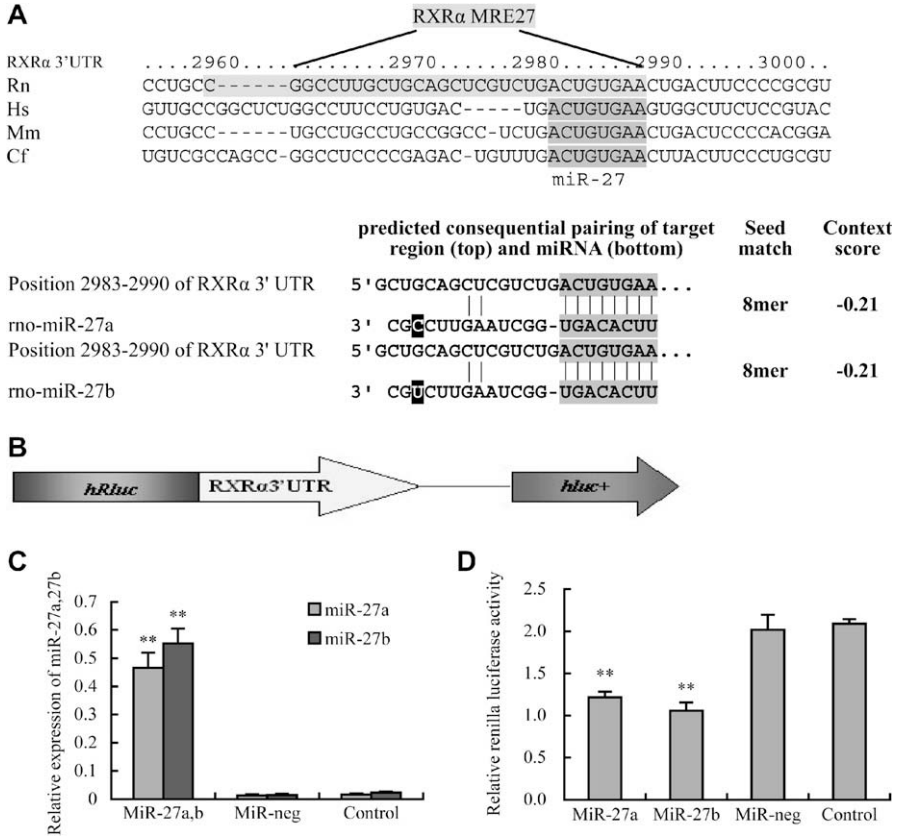

Fig. 4. Interaction of miR-27a and 27b with the 3′UTR of RXRα mRNA: (A) Conserved site for miR-27 and miR-27 recognition element (MRE27) in rat RXRα 3′UTR; predicted pairing of miR-27a and 27b with target region in rat RXRα 3′UTR. (B) Construct map of psiCHECK-2/RXRα MRE27x3 used for the luciferase assay. The 3′UTR of RXRα was cloned into the vector after the renilla luciferase gene (hRluc) to form a fusion transcript. Firefly luciferase gene (hluc+) was coexpressed in the vector as an internal control. (C) Transient transfection of miR-27a or 27b expression vectors significantly increased miR-27a or 27b expression in 293Tcells. (D) Relative renilla luciferase activity of psiCHECK-2/RXRα MRE27x3 in the presence of miR-27a, miR-27b or miR-neg expression vectors in 293T cells, the 293T cells simply transfected with psiCHECK-2/RXRα MRE27x3 served as control, normalized to firefly luciferase activity. $^{**}P < 0.01$ compared with those transfected with miR-neg expression vectors.

3.3. Interaction of miR-27a and 27b with the 3′UTR of retinoid X receptor α (RXRα) mRNA

The results presented so far demonstrate that inactivation of miR-27a and 27b in culture-activated HSC induces partial reversal of the cell phenotype to that of quiescent HSC. In order to explore the mechanisms involved, we performed computer-based sequence analysis by using Targetscan software [18]. The first eight nucleotide (seed sequence) from the 5′end of both miR-27a and 27b miRNA are complementary to the 2983–2990 nucleotides of the rat RXRα 3′UTR which are conserved across various species (Fig. 4A). Moreover, according to Targetscan, the context scores [19] of miR-27a and 27b are the lowest among all the conserved miRNAs predicted to target conserved sites in RXRα 3′UTR, which indicates that miR-27a and 27b are most likely to interact with RXRα 3′UTR and consequently down-regulate its expression at post transcriptional level.

RXRα is involved in multiple signaling pathways related to cell proliferation and differentiation, mainly as heterodimeric partner of several nuclear receptors. It has been documented that RXRα plays a central role in adipogenesis [20], probably as a heterodimeric partner for peroxisome proliferator-activated receptor γ. RXRα also suppresses DNA synthesis and causes cell growth arrest in a variety of cell types including HSCs [21–24]. The expression of RXRα significantly decreases in activated HSCs [25] and transfection of RXRα gene can inhibit HSCs proliferation and partly reverse the phenotype of activated HSCs [26]. However, the regulation of RXRα expression remains to be established. It has been proposed that reduced expression of RXRα relate to decreased mRNA stability [22]. Potentially, in activated HSCs, down-regulation of RXRα could occur through the binding of miR-27a or 27b to the 3′UTR of RXRα mRNA. And the phenotypic switch caused by inactivation of miR-27a and 27b in activated HSCs might due, in part, to up-regulated RXRα.

To investigate the potential interaction experimentally, the 3′UTR of rat RXRα mRNA was subcloned after the renilla luciferase coding sequence (Fig. 4B) and cotransfected with miR-27a or 27b expression vectors into 293T cells. Indeed, renilla luciferase activity decreased by 40-50% in 293T cells cotransfected with either miR-27a or miR-27b expression vectors compared with those cotransfected with miR-neg expression vectors (Fig. 4D). These findings show that both miR-27a and 27b can interact with the 3′UTR of RXRα and efficiently inhibit the translation from the chimeric transcript.

To learn whether miR-27a and 27b can affect endogenous RXRα expression, we next examined the effect of inactivation of miR-27a and 27b on RXRα in culture-activated HSCs. Real-time PCR and Western blots revealed significantly increased expression of both RXRα mRNA and protein in activated HSCs cotransfected by anti-miR-27a and anti-miR-27b (Fig. 5). The results showed above affirmed a negative role of miR-27a and 27b in the regulation of RXRα expression.

In conclusion, down-regulation of over-expressed miR-27a and 27b allowed activated HSCs to restore their ability to accumulate cytoplasmic lipid droplets and decreased HSCs proliferation. Moreover, the fatty acid metabolism and cell proliferation regulating properties of miR-27a and 27b maybe, at least partly mediated by affecting RXRα expression. Silencing of miR-27a and 27b in vivo may present a promising approach to the resolution of hepatic fibrosis.

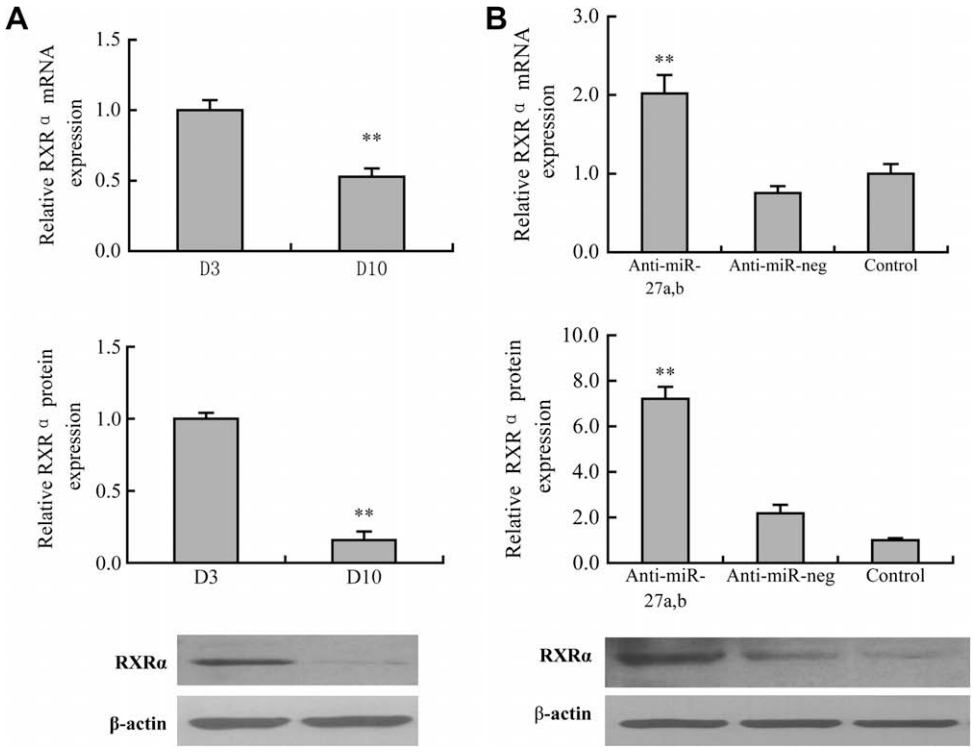

Fig. 5. Cotransfection of anti-miR-27a and anti-miR-27b up-regulated the expression of RXRα in activated HSCs. (A) The expression of RXRα decreased in D10 activated HSCs. $^{**}P < 0.01$ compared with D3 HSCs. (B) The expression of RXRα was up-regulated in passage two HSCs cotransfected with 75 nM anti-miR-27a, 27b for 42 h. The expression of RXRα mRNA was determined by real-time PCR, normalized to β-actin; the expression of RXRα protein was determined by Western blot analyses using whole cell lysates, β-actin served as loading control. $^{**}P < 0.01$ compared with those transfected with anti-miR-neg. D3 and D10 HSCs represent day3 quiescent and day10 activated HSCs; anti-miR-27a, b represents anti-miR-27a and anti-miR-27b.

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