Original Paper

Characteristic MicroRNA Expression Induced by δ -Opioid Receptor Activation in the Rat Liver Under Prolonged Hypoxia

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Key Words

δ -opioid receptor • MiRNA • Liver • Hypoxia

Abstract

Background/Aims: Hypoxic/ischemic injury to the liver is a frequently encountered clinical problem with limited therapeutic options. Since microRNAs (miRNAs) are involved in hypoxic/ischemic events, and δ -opioid receptor (DOR) is protective against hypoxic/ischemic injury, we asked if pharmacological activation of DOR can alter hypoxic events by regulating miRNA expression in the liver. As the first step, the present work aimed at testing the effect of DOR activation on hepatic miRNA expression in hypoxia. **Methods:** Male Sprague Dawley rats were exposed to hypoxia (9.5–10% O₂) for 1, 5, or 10 days with or without DOR activation. The target miRNAs were selected according to TaqMan low-density array (TLDA) data and analyzed by quantitative real-time PCR. **Results:** We found that: 1) 1-day hypoxia caused the upregulation of 9 miRNAs (miR-7a-5p, miR-10a-5p, miR-25-3p, miR-26b-5p, miR-122-5p, miR-128a-3p, miR-135b-5p, miR-145-5p, and miR-181a-5p) and the downregulation of 2 miRNAs (miR-34a-5p and miR-182); 2) 5 and 10-days hypoxia altered the expression of 4 miRNAs (miR-34c-5p, miR-184, miR-107-3p and miR192-5p); 3) DOR activation shifted the expression of 8 miRNAs (miR-122-5p, miR-146a-5p, miR-30e-5p, miR-128a-3p, miR-182, miR-192-5p miR-107-3p and miR-184) in normoxic condition; and 4) DOR activation modified hypoxia-induced changes in 6 miRNAs (miR-142-5p, miR-145-5p, miR-146a-5p, miR-204-5p, miR-34a-5p and miR-192-5p). **Conclusion:** Hypoxia significantly modifies the miRNA profile in the liver, while DOR activation can modify the hypoxic modification. Therefore, it is potentially possible to alter hypoxic/ischemic pathophysiology in the liver through DOR pharmacotherapy.

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Introduction

Oxygen is important for the maintenance of liver functions and its insufficiency or deprivation in hepatic microenvironment due to respiratory/circulatory disorders leads to hepatocellular damage. Indeed, hypoxia activates multiple hypoxia mediators and in turn accelerate or antagonize hepatic damage [1, 2]. A variety of pharmacological interventions have been proposed to alleviate hypoxic injury in the liver; however, there is no promising therapy for the prevention and treatment of hypoxic liver injury yet [3-6].

MicroRNAs (miRNAs) are short non-coding RNAs of 17-25 nucleotides in length which regulate gene expression by binding to their mRNA 3'-untranslated regions (3'-UTR) in a wide range of species [7, 8]. It has been widely reported that miRNA activity participates in the control of a wide range of biological functions and processes such as hypoxic/ischemic response, tumorigenesis, cell differentiation, and immune regulation [9-11]. For example, miR-146a attenuates hypoxia/reoxygenation injury *in vitro* by directly suppressing IRAK1 and TRAF6 in liver [12] and miR-192-5p protects liver cell from hepatic injury induced by oxidative stress by targeting Zeb2 [13]. Also, miR-494 protects against hypoxia-induced apoptosis by upregulating HIF1 α expression through activating PI3K/Akt pathway [14]. Moreover, miR-125a-5p prompts the activation and proliferation of hepatic stellate cells by targeting FIH1 [15], while miR-150 is involved in liver regeneration by targeting VEGF-A [16]. These findings suggest a new possibility to treat hypoxic/ischemic liver injury by modulating hepatic miRNAs.

Our recent work and those of others have well demonstrated that the δ -opioid receptor (DOR) is cytoprotective against hypoxic/ischemic injury in various cells [17-19] via complex mechanisms such as regulating ionic homeostasis or enhancing antioxidative capacity [20-24]. Moreover, we found the involvement of DOR in the regulation of miRNA expression in the brain, kidney and heart [25-27]. All the brain, heart and kidney are hypoxia/ischemia sensitive organs. It is unknown whether the liver, a hypoxia/ischemia insensitive organ, behaves differently in terms of its miRNA responses to DOR activation in hypoxic condition. We therefore asked whether an increased DOR activity can regulate mRNA expression/function and thus alter hypoxic/ischemic pathophysiology in the liver. In this work, as the first step, we used the same methodology previously used for the brain, heart and kidney to determine the effect of DOR activation on hepatic miRNA expression in hypoxia.

Materials and Methods

Animals

All animal procedures were performed in accordance with the NIH guidelines and were approved by the Animal Care and Use Committee of Shanghai Research Center for Acupuncture and Meridians and the Animal Experimental Committee of the Soochow University. Sprague Dawley male rats at 21 days of age were purchased from the Shanghai Experimental Animal Center of Chinese Academy of Sciences (China). Immediately after their arrival, all rats were randomly divided into 4 groups: (A) control, (B) DOR agonist (UFP-512), (C) chronic anoxia, and (D) chronic anoxia+DOR agonist (UFP-512). Each group had at least 6 animals for further experiments.

Hypoxia induction and DOR activation

Groups A and B were raised in normal conditions, while Groups C and D were raised under hypoxic conditions. Chronic hypoxia was induced as described in our previous work [25-27]; the O₂ level was maintained at 9.5%–10% in the plexiglass box to induce hypoxia. The rats of Groups C and D were kept in the hypoxic box for 1, 5, or 10 days. The box was rapidly cleaned daily when the animals were removed to have their body weights recorded. The rats of Groups B and D were subjected to an intraperitoneal injection of UFP-512 (H-Dmt-Tic-NH-CH[CH₂-COOH]-Bid), a specific and potent DOR agonist synthesized by our team [28]. The injections (1 mg/kg in <1 ml) were performed on day 0 (immediately before the onset of hypoxia), day 4, and day 8. As a control, Groups A and C received the same amount of saline.

Tissue Collection

After 1, 5, or 10 days of hypoxia, the rats were decapitated following deep anesthesia. Their livers were rapidly removed, weighed, frozen in liquid nitrogen, and stored at -80°C until later use.

RNA Extraction and qRT-PCR analysis

Whole liver homogenization was carried out under liquid nitrogen to completely disrupt all of the cells. The total RNA was extracted from the collected homogenate using TRIzol Reagent (Thermo Fisher, USA) according to the manufacturer's instructions. The miRNA expression levels were quantified using TaqMan microRNA assay (Thermo Fisher, USA) on the Applied Biosystems 7500 (Thermo Fisher, USA) as previously reported [26]. U6 served as an internal control. In brief, $1\text{ }\mu\text{g}$ of total RNA was reverse transcribed to cDNA using an AMV reverse transcriptase (TaKaRa, Dalian, China) and a stem-loop primer (Applied Biosystems). The mixture was incubated at 16°C for 15 min, 42°C for 60 min, and 85°C for 5 min to generate a library of miRNA cDNAs. qRT-PCR was then performed at 95°C for 10 min, followed by 40 cycles at 95°C for 15s and at 60°C for 60s. After the reactions were completed, the threshold cycle (Ct) values were determined using the default threshold settings for analyzing the data. The relative amount of one miRNA to internal control U6 transcript was calculated using the equation $2^{-\Delta\text{Ct}}$ in which $\Delta\text{Ct} = \text{Ct}_{\text{miRNA}} - \text{Ct}_{\text{U6}}$. All reactions, including the controls that contained no template RNA, were performed in triplicate.

Statistical analysis

The results are presented as the mean $\pm$ SD with at least 6 animals in each group ($n \geq 6$). The mean value for Group A (the control) for each time point is set at one. Statistical significance was determined using either a student's t-test or a one-way ANOVA following Turkey's Multiple Comparison Test on paired columns as appropriate.

Results

We monitored hypoxia-induced changes in the liver and body weights and investigated 20 miRNAs (miR-7a-5p, miR-10a-5p, miR-25-3p, miR-26b-5p, miR-30e-5p, miR-34a-5p, miR-34c-5p, miR-107-3p, miR-122-5p, miR-125a-5p, miR-128a-3p, miR-135b-5p, miR-142-5p, miR-145-5p, miR-146a-5p, miR-181a-5p, miR-182, miR-184, miR-192-5p, and miR-204-5p) that differentially respond to hypoxic stress based on our microarray and PCR analyses of miRNAs in the hypoxic kidney [26], cortex [25], and heart [27].

Hypoxia-induced changes in body and liver weights

We monitored the body and liver weights at all time points. The liver weight increased from day 1 to day 10. Hypoxic exposure slightly, though not statistically, reduced the liver weight at 5 days and significantly it after 10-day hypoxia (Fig. 1A). In addition, significant weight differences were noted between normoxic and hypoxic animals with UFP-512 treatment for at 5 and 10 days (Fig. 1A). The liver/body weight ratio maintained at a relative stable level, except a small but significant difference between normoxic and hypoxic animals with UFP-512 treatment at 5 days (Fig. 1B).

Early longitudinal miRNA changes in response to hypoxia

Hypoxia alone significantly increased the expression of miR-7a-5p, miR-10a-5p, miR-25-3p, miR-26b-5p, miR-122-5p, miR-128a-3p, miR-135b-5p,

Fig. 1. Effect of hypoxia and DOR activation on the liver weight. (A) Liver weight ($n = 9$). (B) Ratio of liver to body weight for each study group ($n = 9$). C: control; C+DOR: control+DOR agonist UFP-512; H: hypoxia; H+DOR: hypoxia+ DOR agonist UFP-512. * $p < 0.05$, ** $p < 0.01$.

Fig. 2. Hypoxia-induced changes in hepatic miRNAs. The expression levels of miR-7a-5p, miR-10a-5p, miR-25-3p, miR-26b-5p, miR-122-5p, miR-128a-3p, miR-135b-5p, miR-145-5p, and miR-181a-5p in the liver following 1, 5, or 10 days of hypoxia are determined by qRT-PCR. C: control; H: hypoxia. *p<0.05, **p<0.01, ***p<0.001.

miR-145-5p, and miR-181a-5p in the liver after exposure for one day (Fig. 2). This increase in these miRNAs was attenuated after 5 days of exposure to hypoxia. At day 10, some of them (miR-10a-5p and miR-181a-5p) even showed a statistically lower level in hypoxic livers than in normoxic ones (Fig. 2B and 2I). Hypoxia also increased the level of miR-128a-3p after one day, but the change was relatively constant at day 5 and day 10 (Fig. 2F).

The levels of miR-7a-5p, miR-26b-5p, miR-122-5p, and miR-145-5p tended to be lower after 10-day hypoxia though there were no statistical differences when compared to the control (Fig. 2A, 2D, 2E, 2H). In contrast, hypoxia significantly decreased the expression of miR-34a-5p and miR-182 in the liver after exposure for one day (Fig. 3A and 3B). These two miRNAs continued to decrease after 5 days and 10 days exposure to hypoxia. At 10 days exposure, the relative expression of miR-34-5p and miR-182 was 0.19 and 0.17 compared with their respective control. Though miR-146a-5p did not decrease after 1 day exposure to hypoxia, it decreased after 5 days and 10 days exposure to hypoxia (Fig. 3C).

Late longitudinal miRNA changes in response to hypoxia

Some miRNAs kept stable during the period we observed. The expression of miR-34c-5p significantly increased to about 2.0 fold after exposure to hypoxia for one day, and this change persisted at a steady state after 10 days of exposure to hypoxia (Fig. 4A). The expression of miR-184 showed no appreciable change following exposure to hypoxia for 1 day and tended to decrease after 10 days of exposure to hypoxia though there is no statistical significance (Fig. 4B). Some miRNAs kept to increase during the hypoxic period. For example, miR-107-3p and miR192-5p significantly increased after 1-day hypoxic exposure, and they continued to increase after exposure to hypoxia for 5 and 10 days (Fig. 4C and 4D).

Fig. 3. Differential changes in various miRNAs in hypoxia. The expression levels of miR-34a-5p, miR-182, and miR-146a-5p in the liver following 1, 5, or 10 days of hypoxia are determined by qRT-PCR. C: control; H: hypoxia. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. 4. Late longitudinal miRNA changes in response to hypoxia. The expression levels of miR-34c-5p, miR-184, miR-107-3p, and miR-192-5p in the liver following 1, 5, or 10 days of hypoxia are determined by qRT-PCR. C: control; H: hypoxia. ** $p < 0.01$, *** $p < 0.001$.

Discrete miRNA changes in response to hypoxia

Some miRNAs were altered by hypoxia at discrete time points. Hypoxia significantly induced miR-142-5p, miR-204-5p and miR-30e-5p over 200% within the first 24 hours (Fig. 5). However, miR-142-5p was sharply reduced by 50% at 5 days and continued to decrease at 10 days (Fig. 5A), while miR-204-5p was reduced to its control level at 5 days and tended to further reduce at 10 days (Fig. 5B). On the other hand, miR-30e-5p was reduced to 77% of its control level at 5 days and then tended to increase at 10 days of hypoxia (Fig. 5C).

MicroRNA changes in response to DOR activation in normoxia

DOR activation with UFP-512 significantly altered the level of some miRNAs. The activation of DOR significantly decreased miR-122-5p expression after one day treatment

Fig. 5. Discrete miRNA changes in response to hypoxia. The expression levels of miR-142-5p, miR-204-5p, and miR-30e-5p in the liver following 1, 5, or 10 days of hypoxia are determined by qRT-PCR. C: control; H: hypoxia. **p<0.01, ***p<0.001.

Fig. 6. Effects of DOR activation on the expression of miR-122-5p, miR-146a-5p, miR-30e-5p, and miR-128a-3p in the liver under normoxia. C: control; C+DOR: control+DOR agonist UFP-512. *p<0.05, **p<0.01, ***p<0.001.

with UFP-512 (one time treatment) (Fig. 6A). This decrease was aggravated after 5 days (2-time treatments) and 10 days (3-time treatments) (Fig. 6A). In contrast, DOR activation tended to increase the expression of miR-146a-5p at one day after treatment using UFP-512 (one time treatment) with no statistical significance in the liver (Fig. 6B) and this increase became significantly appreciable after 5 days (2-time treatments) and 10 days (3-time treatments) (Fig. 6B) of DOR activation. MiR-30e-5p and miR-128a-3p were the only two miRNAs whose expression was unaltered during the whole experimental period (Fig. 6C and 6D).

MiR-182 and miR-192-5p were significantly upregulated after DOR activation with UFP-512 for one day (1-time treatment) and their expression levels were maintained at a relative stable level at day 5 (2-time treatments) and day 10 (3-time treatments) (Fig. 7A and 7B). In sharp contrast, miR-107-3p and miR-184 were in an opposite manner. Their expression was unaltered at day 1 (one time treatment), but significantly downregulated by day 5 (2-

Fig. 7. Effects of DOR activation on the expression of miR-182, miR-192-5p, miR-107-3p, and miR-184 in the liver under normoxia. C: control; C+DOR: control+DOR agonist UFP-512. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

time treatments) and day 10 (3-time treatments) (Fig. 7C and 7D). All other miRNAs studied in this work remained unaltered or showed no statistical significance in response to DOR activation in the liver.

MicroRNA changes in response to DOR activation under hypoxia

Next we investigated whether DOR activation could modify hypoxia-induced changes in miRNA expression. Some miRNAs were significantly altered at the earliest time point (1 day) when UFP-512 treatment was combined with hypoxia. The combination of hypoxia and DOR activation significantly increased the expression of miR-142-5p, miR-145-5p, miR-146a-5p, and miR-204-5p in the liver after treatment using UFP-512 for one day. The relative levels of these miRNAs were then attenuated after 5 days of hypoxia and reached their lowest point at day 10 in response to DOR activation under hypoxia (Fig. 8). At day 10, the relative levels of these miRNAs were still higher than in the group of hypoxia alone (Fig. 8). It is interesting to note that the relative level of miR-145-5p was nearly the same in hypoxia group and hypoxia plus DOR group (Fig. 8B).

MiR-34a-5p and miR-192-5p were the only two miRNAs whose expression levels were increased or decreased throughout the entire time course after DOR activation while others were not (Fig. 9). The effects of DOR activation on miRNAs in the liver exposed to prolonged hypoxia are summarized in Table 1.

Discussion

Hypoxia is associated with various clinical conditions including liver disorders such as fatty liver disease [29], liver-stage malaria [30], and hepatocellular carcinoma [31].

Fig. 8. Effects of DOR activation on the expression of miR-7a-5p, miR-107-3p, miR-200b-3p, and miR-376a-3p in the liver under hypoxia. C: control; H: hypoxia; H+DOR: hypoxia+ DOR agonist UFP- 512. *p<0.05, **p<0.01, ***p<0.001.

Hepatocellular cells need to activate specific molecular programs to overcome hypoxic challenges in these conditions [32-34]. As a key player in almost every aspect of liver diseases, miRNAs modulate various cellular and organ functions by modulating their target genes under different conditions [35]. In this study, we present the first data to identify several liver miRNAs showing a major alteration in their expression in response to chronic hypoxia and DOR activation. Our novel data suggest that some miRNAs are very sensitive to oxygen deprivation and DOR activation.

Some miRNAs were significantly altered after only one day of hypoxia, i.e., 9 miRNAs upregulated and 3 miRNAs downregulated, indicating that they are more sensitive to hypoxia than other miRNAs and involved in hypoxic regulation of cellular and molecular processes in the liver at relatively early stage of hypoxic stress. Though none of these miRNAs are reported in current literature to be directly involved in hypoxia regulation in the liver, they do take part in functional regulation in other organs in hypoxia. For example, miR-7a-5p attenuates post-myocardial infarction remodeling and protects cardiac myocyte from hypoxia-induced apoptosis involving Sp1 and PARP-1 in mice [36-38] and miR-25 protects against hypoxia/reoxygenation-induced fibrosis and apoptosis in cardiomyocytes by targeting HMGB1 [39]. Also, miR-26 is induced in response to low oxygen and decreases proapoptotic signaling in a hypoxic environment [40], while miR-122-5p inhibition attenuates hypoxia/reoxygenation-induced myocardial cell apoptosis by targeting GATA4 [41]. There is also evidence showing that miR-145-5p is downregulated by ischaemia in acute myocardial infarction via targeting Dab2 [42] and miR-181a-5p is involved in hypoxia-induced chemoresistance in gastric cancer [43]. Other studies showed that miR-34a-5p, miR-182, and miR-146a-5p were the three downregulated miRNAs upon hypoxia and miR-34a-5p inhibition protects against

Fig. 9. Effects of DOR activation on the expression of miR-34a-5p and miR-192-5p in the liver under hypoxia. C: control; H: hypoxia; H+DOR: hypoxia+ DOR agonist UFP- 512. ***p<0.001.

Table 1. Effects of DOR activation on miRNAs in the liver exposed to prolonged hypoxia. Note: ↑, upregulation; ↓, Downregulation; –, No statistical difference as compared to the control. C, normoxic control. H, Hypoxia. DOR, DOR activation

miRNA	1 Day			5 Days			10 Days		
	Hypoxia	C+DOR	H+DOR	Hypoxia	C+DOR	H+DOR	Hypoxia	C+DOR	H+DOR
miR-7a-5p	↑			–			–		
miR-10a-5p	↑			↑			↓		
miR-25-3p	↑			↑			–		
miR-26b-5p	↑			↑			–		
miR-30e-5p	↑	–		↓	–		–	–	
miR-34a-5p	↓		↑	↓		↑	↓		↑
miR-34c-5p	↑			↑			↑		
miR-107-3p	↑	–		↑	↓		↑	↓	
miR-122-5p	↑	↓		↑	↓		–	↓	
miR-128a-3p	↑	–		↑	–		↑	–	
miR-135b-5p	↑			–			–		
miR-142-5p	↑		↑	↓		↑	↓		↑
miR-145-5p	↑		↑	↑		–	–		↑
miR-146a-5p	–	–	↑	↓	↑	↑	↓	↑	↑
miR-181a-5p	↑			–			↓		
miR-182	↓	↑		↓	↑		↓	–	
miR-184	–	–		–	↓		–	↓	
miR-192-5p	↑	↑	↓	↑	↑	↓	↑	↑	↓
miR-204-5p	↑		↑	–		↑	–		↑

anoxia/reoxygenation injury in cardiomyocytes *in vitro* [44, 45]. Furthermore, miR-34a-5p inhibition protects cardiomyocyte against apoptosis post myocardial infarction via negatively regulating ALDH2 [46]. MiR-34a-5p inhibition alleviates intestinal ischemia/reperfusion-induced reactive oxygen species accumulation and apoptosis via activation of SIRT1 signaling [47]. In addition, miR-146a-5p has a protective effect against cardiac ischemia/hypoxia-induced cardiac dysfunction and apoptosis through the TRAF6-p-p38-caspase-3 signal pathway [48]. MiR-182 is also a hypoxia-responsive miRNA which is directly regulated by HIF1 α at transcriptional level in prostate cancer [49]. All these studies suggest that the miRNAs investigated in this work are involved in hypoxic response in the liver, similarly as in other organs.

In contrast to the above-mentioned miRNA, some miRNAs, e.g., miR-34c-5p, miR-184, miR-107-3p and miR192-5p. changed only after prolonged hypoxia. MiR-34c-5p, directly targets sGC β 1 in hypoxia and is a crucial factor in the control of various physiological functions [50]. MiR-184 can inhibit hypoxia-induced apoptosis due to activation of caspase-3 and caspase-9 in cyanotic congenital heart disease [51]. MiR-107-3p inhibition can reduce

capillary density in the ischemic boundary zone after stroke and regulates post-stroke angiogenesis by targeting Dicer1 [52]. MiR-192-5p is also a hypoxia responsive miRNA under different cellular stress [53]. miR-204-5p promotes the apoptosis of neuronal cells under hypoxia by targeting Bcl-2 [54]. Therefore, their changes in the hypoxic liver may represent a delayed action for the hepatocytes to respond to hypoxic stress.

Liver regeneration is a commonly turning up phenomenon when adult liver is undergoing damage from circumstances, which could prevent further damage or inflammation to adjacent tissues. MicroRNAs are an important player in this process. For example, miR-21-5p is required for local and remote ischemic preconditioning in multiple organ protection against sepsis, and an up-regulation of miR-21-5p may be a potential therapy for sepsis [55]. Some studies show that miR-125a-5p prompted the activation and proliferation of hepatic stellate cell by partially down-regulating FIB1 in liver fibrosis [15], and miR-133a-5p was protective against hypoxia/reoxygenation (H/R) injury *in vitro* by targeting MAPK6 in hepatocytes [56]. Moreover, miR-146a could ameliorate liver hypoxia/reoxygenation injury *in vitro* by directly suppressing IRAK1 and TRAF6 [12] and miR-494 was protective against hypoxia-induced apoptosis in liver through HIF-1 α upregulation by activating PI3K/Akt pathway [14]. In addition, there is accumulating evidence suggesting that many other miRNAs are involved in liver reactions to hypoxic stress, such as miR-150 downregulation under hypoxia by targeting VEGF-A and HIF1 α during liver regeneration [16, 57], miR-192-5p upregulation in hepatocyte injury [13] and increased miR-462/731 cluster in the hypoxia-exposed liver of *Megalobrama amblycephala* [58] and zebrafish [59].

DOR has been established as an effective regulator against hypoxia-induced injury [20, 60-62] via multiple mechanisms including the regulation of survival/death signals [60, 62]. DOR activation inhibits serum deprivation-induced apoptosis via the activation of PKC and the mitochondrial pathway in human liver cells [63]. DOR activation inhibited mitochondria-mediated apoptosis in liver cancer cells through the PKC/ERK signaling pathway [64], which is consistent with the observation made in neuronal cells [60, 62]. Apparently, DOR actively participates in the regulation of hepatic cell survival.

In this study, several miRNAs were identified to respond to DOR activation under normoxic and/or hypoxic conditions, either increase or decrease after DOR activation at various time points, suggesting that they are sensitive to DOR signaling in the liver. Among them, miR-146a-5p and miR-182 were significantly downregulated upon hypoxia, while their expression could be significantly enhanced under hypoxia. Moreover, they were upregulated even in normoxic condition. There is evidence showing that miR-182 is also downregulated after hypoxia-ischemia brain injury in neonatal rats [65]. Jiang et al. reported that miR-146a-5p could ameliorate ischemia/reperfusion injury *in vivo* and hypoxia/reoxygenation injury *in vitro* by directly suppressing IRAK1 and TRAF6 in liver [12]. Therefore, it is likely that hypoxia injures the hepatocytes through, at least partially, an inhibitory negative regulation of these two miRNAs, while DOR signaling is able to reverse such negative regulation. In other word, the DOR-induced upregulation of these two miRNAs is of benefit to the liver in hypoxia.

More interestingly, miR-107-3p and miR-192-5p were upregulated by about 3 fold after 10 days of exposure to hypoxia, while DOR activation could downregulate the expression of miR-107-3p and miR-192-5p in both hypoxic and normoxic conditions. Yang et al. reported that miR-107-3p inhibition is able to protect the rat cerebrum from excitatory neurotoxicity during ischemia-reperfusion injury by targeting GLT1 [66, 67]. Roy et al. reported that miR-192-5p inhibition protects from oxidative stress-induced acute liver injury by targeting Zeb2 and might represent a potent marker of hepatic injury [13]. Based on these early studies and our new observations, we are confident that DOR signaling is able to increase hepatic tolerance to hypoxic stress by increasing the activities of miR-107-3p and miR-192-5p.

Conclusion

In summary, we have identified a subset of hepatic miRNAs that are sensitive to hypoxic stress. Some of them are injury factors in the liver under hypoxic condition, while others may play a protective role against hypoxic insult. DOR activation can target some of these hypoxia-sensitive miRNAs, therefore attenuating the injury factors and strengthening the protective factors, which leads to hepatic protection against hypoxic injury. Further studies for in-depth understanding of the multifaceted effects of these miRNAs on gene modulation and post-transcriptional modulation in the liver may provide a novel insight into new strategies for liver protection against hypoxic/ischemic injury.

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Disclosure Statement

The authors have declared no conflicts of interest.

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