Bioscience Reports (2020) **40** BSR20191043
https://doi.org/10.1042/BSR20191043

PORTLAND PRESS

Research Article

MicroRNA files in the prevention of intestinal ischemia/reperfusion injury by hydrogen rich saline

Weifeng Yao[1,*], Xiaoyu Lin[1,*], Xue Han[2], Lanfen Zeng[3], Anshun Guo[1], Yu Guan[1], Ziqing Hei[1], Jianpei Liu[4] and Pinjie Huang[1]

[1]Department of Anesthesiology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510000, China; [2]Department of Anesthesiology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510000, China; [3]Department of Anesthesiology, Affiliated Hospital of Guangdong Medical University, Zhanjiang, Guangdong, China; [4]Department of Gastrointestinal Surgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Correspondence: Pinjie Huang (hpjie@126.com) or Jianpei Liu (kamplau@126.com)

Background: Hydrogen-rich saline (HRS) has been proven effective against ischemia/reperfusion (I/R) injury. However, knowledge on the underlying signaling events remain poor. Having recent highlight of microRNAs (miRNAs) in mediating intestinal I/R injury, we hypothesized that HRS may protect intestine against I/R injury by regulating miRNAs.

Method: Mice were given intraperitoneal injection of saline or HRS once daily for five consecutive days before undergoing intestinal I/R that was induced by 60-min ischemia followed by 180-min reperfusion of superior mesenteric artery. The intestine was collected for histopathological assay, miRNA microarray profiling, Real-Time PCR, and Western blotting. Next, miR-199a-3p mimics or inhibitors were transfected into IEC-6 cells to explore the relationship between HRS treatment and miR-199a-3p.

Results: I/R-induced mucosal injury and epithelial cells apoptosis were attenuated by HRS pretreatment. A total of 64 intestinal I/R-responsive miRNAs were altered significantly by HRS pretreatment, in which we validated four novel miRNAs with top significance by Real-Time PCR, namely miR-199a-3p, miR-296-5p, miR-5126, and miR-6538. Particularly, miR-199a-3p was drastically increased by I/R but reduced by HRS. Computational analysis predicts insulin-like growth factor (IGF)-1, mammalian target of rapamycin (mTOR), and phosphoinositide-3-kinase (PI3K) regulatory subunit 1 as targets of miR-199a-3p, suggesting involvement of the pro-survival pathway, IGF- 1/PI3K/Akt/mTOR. In *in vitro* experiment, HRS treatment reduced miR-199a-3p level, increase IGF-1, PI3K and mTOR mRNA expression, restore IEC-6 cells viability, and this protective effects were reversed under miR-199a-3p mimics treatment.

Conclusion: Collectively, miR-199a-3p may serve a key role in the anti-apoptotic mechanism of HRS that contributes to its protection of the intestine against I/R injury.

Introduction

Ischemia/reperfusion (I/R) injury occurs in a tissue that undergoes a period of blood flow restriction followed by restoration [1]. The intestine is one of the organs that is most susceptible to I/R injury, which is problematical in surgical procedures requiring thoracic or abdominal vascular occlusion such as cardiopulmonary bypass and intestinal transplantation. It can also take place in hemorrhagic or septic shock [2]. The devastating sequelae of intestinal I/R injury includes deteriorated integrity and motility of mucosa, bacterial translocation to the circulation, and overproduction of reactive oxygen species (ROS) [2,3]. These events are strong drive of systemic inflammation and multi-organ failure, imposing high morbidity and mortality in patients subjected to intestinal I/R [4].

*These authors contributed equally to this work.

Received: 25 April 2019
Revised: 18 October 2019
Accepted: 28 October 2019

Accepted Manuscript online: 02 December 2019
Version of Record published: 24 January 2020

Bioscience Reports (2020) **40** BSR20191043
https://doi.org/10.1042/BSR20191043

Saline saturated with hydrogen, a proven antioxidant, has been shown to protect tissue by antioxidation and anti-inflammation in clinic [5], as well as in various animal models of I/R [6,7], including intestinal I/R [8]. Such hydrogen-rich saline (HRS), by selectively reacting with hydroxyl radicals and peroxynitrite, the most toxics of ROS, can effectively reduce cellular oxidative stress and apoptosis [6,9]. Administration of HRS prior to intestinal I/R has been shown to suppress lipid oxidation, proinflammatory cytokines, and crypt cell apoptosis in intestinal mucosa, conferring significant protection of the intestine [3,8]. However, besides these antioxidant qualities, much less is known on the role of HRS in cell signaling.

In recent years, accumulating research has highlighted the functional significance of microRNAs (miRNAs) in control of intestinal I/R injury [10,11]. MiRNAs are small noncoding RNAs that silence gene expression during post-transcription [12]. By targeting miRNAs related to intestinal I/R injury, such as miR-665 and miR-381, studies have demonstrated histological and functional improvements in the intestine following I/R [13,14]. Therefore, we infer that HRS may protect intestine from I/R injury by regulating miRNAs. However, no literature has reported miRNAs expression in HRS-treated intestinal I/R. The present study aims to identify novel miRNAs involved in the protective mechanism of HRS in intestinal I/R injury.

Materials and methods animals

Thirty-two male C57BL/6 mice (age 7–8 weeks and body weight 20–24 g) were used in the present study. Mice were housed in a room illuminated 12-h light–dark cycle. Mice were allowed access to water and food *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee at The Third Affiliated Hospital of Sun Yat-Sen University and performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. The committee is guided by the Care and Use of Laboratory Animals (1996). All of the animal experiments in the present study were carried out in experimental animal center of Sun Yat-sen University, which were approved by Animal Ethics Committee of Sun Yat-sen University (SYSU-IACUC-2017-B432).

Experiment design

Thirty-two mice were randomly divided into four groups ($n = 8$) as the following:

(a) Sham group, mice were given daily intraperitoneal injection (i.p.) of saline (the same volume as HRS) for five consecutive days before sham operation.

(b) I/R group, same administration scheme as sham group before intestinal I/R operation.

(c) HRS group, daily i.p. injection of HRS (3 μmol/kg) for five consecutive days before sham operation.

(d) HRS+I/R group, same administration scheme as HRS group before intestinal I/R operation.

All the mice were killed by high concentration of carbon dioxide. Intestine tissue at 10 cm to the terminal ileum (0.5 cm) was collected and fixed in 10% formaldehyde, followed by embedding in paraffin. Next, the whole small intestine was removed and washed thoroughly with saline (0°C). Intestinal epithelium was exposed by a longitudinal cut, and was rinsed completely with saline and stored at −80°C freezer.

Hydrogen-rich saline preparation

To obtain HRS, hydrogen was dissolved in saline (0.9%) for 6 h under pressure of 0.4 MPa to a supersaturated level using a hydrogen rich saline-producing apparatus (ZhongKeHuiHeng, Beijing, China). The saturated hydrogen saline was stored fully in an aluminum bottle at 4°C under atmospheric pressure and sterilized by gamma radiation and freshly prepared to maintain the saturated concentration at 0.6 mmol/l. The content of hydrogen in HRS was measured by a hydrogen gas concentration measurement instrument (TRUSTLEX ENH-1000, Japan).

Intestinal ischemia/reperfusion model

Intestinal I/R was performed as previously described [15]. Briefly, surgical area of mouse abdomen was sterilized and opened with middle abdominal incisions under anesthesia (ketamine and chlorpromazine). Intestinal I/R were carried out by clipping of the superior mesenteric artery (SMA) completely with a microvascular clamp. After 60 min of occlusion, intestinal blood flow was restored by removing the microvascular clamp. This I/R regimen (60-min ischemia followed by 180-min reperfusion) was chosen because this time course provoked the most severe intestinal inflammation and oxidative stress in our previous study [16]. Sham-operated mice were subjected to identical surgical interventions including laparotomy and vascular microdissection for the same operation period without SMA occlusion.

Intestinal cell hypoxia/reoxygenation

Intestinal cells line IEC-6 was obtained from ATCC (Manassas, VA, U.S.A.). Hypoxia/Reoxygenation (HR) was carried out as described in our previous studies [17]. In brief, cells were cultured under hypoxia gas mixture (5% CO_2, 94% N_2 and 1% O_2, 37°C) in a hypoxia incubator (Eppendorf Company, Hamburg, Germany) for 2 h and after hypoxia, cells were then placed in 5% CO_2 incubator for reoxygenation for 60 min. The viability of IEC-6 was detected using the Cell Counting Kit-8 (CCK-8) method according to the manufacturer's instructions (Roche, U.S.A.).

IEC-6 cells transfection

IEC-6 cells ($1–5 \times 10^5$) were seeded into 24-well plates with 30–50% cell confluence. Negative control, inhibitors or mimics of miR-199a-3p (RiboBio, China) were diluted with riboFECT™ CP buffer (RiboBio, China), respectively. Mix diluted Negative control, inhibitors or mimics of miR-199a-3p with riboFECT™ CP Reagent Transfection Agent (RiboBio, China) and then incubate the mixture for 10 min at room temperature. Add the mixture to the cell culture medium and mix gently. Negative control, inhibitors or mimics of miR-199a-3p were diluted at a final concentration of 50 nM. The transfected IEC-6 cells were cultured in 37°C for 48 h before hypoxia/reoxygenation or HRS treatment.

Hydrogen-rich saline medium preparation

To obtain HRS medium, hydrogen was dissolved in Dulbecco's modified eagle medium (DMEM) for 6 h under pressure of 0.4 MPa to a supersaturated level. The saturated HRS medium was stored fully in an aluminum bottle at 4°C under atmospheric pressure and freshly prepared to maintain the saturated concentration at 0.6 mmol/l. IEC-6 cells were pretreatment with HRS medium for 24 h before cells subjected to hypoxia/reoxygenation.

Evaluation of histological injury

Sections of 5-μm thickness from intestinal paraffin block were prepared. Tissue section was then stained with hematoxylin–eosin (H&E) after dewaxing hydration. Five randomly selected fields (×200) were captured from each slide using a light microscope in a blinded manner. The severity of intestinal injury was graded by two histopathologists who were initially blind to the experiment based on the criteria of *Chiu's* method [18,19].

TUNEL assay

Intestinal paraffin block sections (5 μm) were prepared for terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) detection. TUNEL assay was performed according to the manufacturer's instructions (Roche, U.S.A.). Five randomly selected fields (×200) were captured from each slide using a light microscope in a blinded manner. TUNEL positive cells were counted automatically by an imaging processing software (ImageJ, NIH, U.S.A.).

RNA isolation

Total RNA was extracted using Trizol reagent (Invitrogen, California, U.S.A.) according to the protocol of the manufacturer. The quantity and purity of total RNA were monitored using NanoDrop ND-1000 spectral photometer. The integrity of the RNA was assessed with the RNA 6000 Nano Lab Chip Kit (Agilent, California, U.S.A.) in combination with the Bioanalyzer 2100 (Agilent Technologies, California, U.S.A.) with RIN number >8.0.

Small RNA library preparation

An input of 1 μg of total RNA were used to prepare small RNA library according to manufacturer's instructions of TruSeq Small RNA Sample Prep Kit (Illumina, SanDiego, U.S.A.). Briefly, RNA molecules were ligated to 5' and 3' adaptors successively and converted to cDNA by reverse transcription followed by PC amplification. PCR products were purifed using gel purifcation (6% Novex TBE gels). Quality control of each library sample was performed using the High Sensitivity DNA LabChip Kit (Agilent Technologies) on the 2100 Bioanalyzer (Agilent Technologies).

Next-generation sequencing

The purified cDNA libraries were indexed with unique adapters and performed on an Illumina Hiseq 2000 at the BGI (Guangzhou, China) following the manufacturer's instructions for instrument use. Illumina's Sequencing Control Studio software version 2.8 (SCS v2.8) was used to obtain raw sequencing reads, which following real-time sequencing image analysis and base-calling by Illumina's Real-Time Analysis version 1.8.70 (RTAv1.8.70).

Bioscience Reports (2020) **40** BSR20191043
https://doi.org/10.1042/BSR20191043

Quality control, mapping, and differential expression analysis

Following the removal of adapter sequences, low-quality reads, and common RNA families, only reads with the length between 15 and 55 base pairs were selected for further analyses. The reads were then mapped in miRBase 20.0 (http://www.mirbase.org/) by Bowtie [20]. Differential expression analyses including data quality assessment were performed with the DESeq software package based on the reads per sample.

Real-time PCR (RT-PCR) assay

RT-PCR analyzed the expressions of mmu-miR-199a-3p, mmu-miR-296-5p, mmu-miR-5126, mmu-miR-6538, mmu-miR-342-3p, mmu-miR-677-5p, mmu-let-7c- 2-3p, and mmu-miR-574-5p in intestinal tissue and IGF1, PI3K, and mTOR in cultured cells. The total RNA was isolated from intestinal mucosa tissue using Trizol reagent (Gibco-BRL) according to the manufacturers' instruction (Roche). Total RNA (5 μg) was reverse-transcribed into cDNA, and the PCR reaction mixtures were made by SYBR Green qPCR master mix (Toyoho Co., Ltd., Osaka, Japan). U6 snRNA was used as the internal control. Primer sequences were designed as follows: mmu-miR-199a-3p, ACAGUAGUCUGCACAUUGGUUA; mmu-miR-296-5p, AGGGCCCCCCCUC AAUCCUG U; mmu-miR-5126, GGGACCGGACGCCUCCUGCAGCUGCGGGA GCCCGUGGUUCCCCGGGCAACCGCG GGCGGGGCCGGGGGCGGGGCCCA; mmu-miR-6538, CCGUGCUGCCGGGCGGGGACCCCGCGGGCUCC GGGGCG GCGAUGGGGACUAUGCUCCGGGAUUCCGCUGGGUGACGGCCGCGUCCUC CUGGCCGCU UGCACUUGGGGAUC; mmu-miR-342-3p, UCUCACACAGAA AUCGCACCCGU; mmu-miR-677-5p, UUCA GUGAUGAUUAGCUUCUGA; mmu- let-7c-2-3p, UUCAGUGAUGAUUAGCUUCUGA; mmu-miR-574-5p, UG AGUGU GUGUGUGUGAGUGUGU; U6, Forward: CTCGCTTCGGCAGCACAU and Reverse: AACGCTTCAC GAATTTGCGT. IGF1, Forward: GGCACTCTGC TTGCTCACCTTT and Reverse: CACGAATTGAAGAGCGTC CACC; PI3K, Forward: CTCAGGGAAAGCTGGACCAC and Reverse: TGGTTCAGACG AGCTTCTGTG; mTOR, Forward: ACCCAAGCCTGGGACCTCTA and Reverse: GGCTGGTTGGGGTCATATGTT; β-Actin, Forward: TC GTACCACTGGCATTG TGAT and Reverse: CGAAGTCTAGGGCAACATAGCA. The relative expression level of miRNA in the intestine and mRNA in cultured cells were determined by the $2^{-\Delta\Delta C}{}_{\mathrm{T}}$ method.

Computational analysis

MiRNA target prediction was performed on-line using TargetScan (http://www.targetscan.org/vert_71/) and BiBiS-erv2 – RNAhybrid algorithms.

Western blot

Intestine tissues frozen at −80°C were thawed and fully grinded into homogenate in lysis buffer containing protease inhibitors and phosphatase inhibitors in glass grinder. After lysing on ice for 30 min, the samples were centrifuged at 12,000 r/min for 15 min and supernatants were collected whose protein concentrations were detected by BCA Kit. Thirty microgram proteins of each sample were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes. The membranes were then blocked with 5% nonfat milk in TBST at room temperature for 1 h and followed by incubation in primary antibody IGF1 (dilution in 1:1000, Cell Signaling Technology, Inc., U.S.A.), PI3K (dilution in 1:1000, Cell Signaling Technology, Inc., U.S.A.), Akt (dilution in 1:1000, Cell Signaling Technology, Inc., U.S.A.), mTOR (dilution in 1:1000, Cell Signaling Technology, Inc., U.S.A.), or GADPH (dilution in 1:5000, Cell Signaling Technology, Inc., U.S.A.) at 4°C overnight. For secondary antibody incubation, the membranes were first washed with TBST three times, 10 min each time, and then incubated with goat anti-rabbit secondary antibody (1:2000) at room temperature for 1 h. Target bands were illuminated by ECL developer and captured by gel imaging system. Absorbance value was analyzed by ImageJ (NIH, U.S.A.).

Statistical analysis

Data were analyzed by SPSS 13.0 (SPSS Inc., U.S.A.) and are presented as mean ± standard error of the mean (SEM). Differences between multiple groups were compared by one-way ANOVA analysis with Tukey post-test. *P* value less than 0.05 was considered significant.

Results

Hydrogen-rich saline pretreatment attenuated intestinal ischemia reperfusion injury

To test the effects of HRS on intestinal I/R injury, daily i.p. injection of HRS (3 μmol/kg) was given for 5 days before inducing I/R by 60-min ischemia and 180-min reperfusion (Figure 1A). Histological change following I/R in the

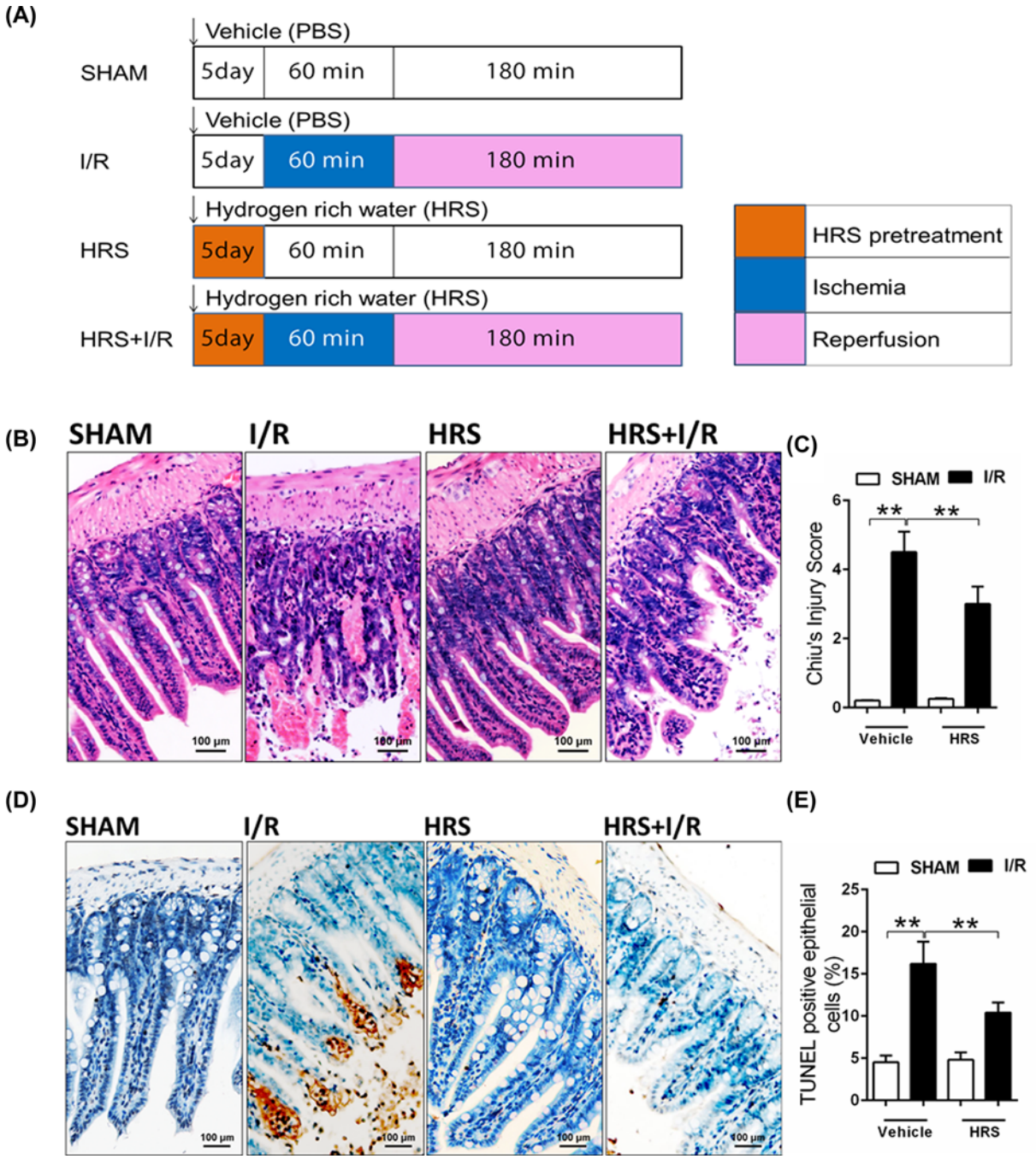

Figure 1. Experiment design and histology of intestinal I/R injury

(**A**) Four experimental groups, SHAM, I/R, HRS, and HRS+I/R were designed in the present study. Mouse I/R model was established by 60 min of intestinal ischemia followed by 180 min of reperfusion. HRS (3 μmol/kg) was injected intraperitoneally once daily for five consecutive days before inducing intestinal I/R. The same volume of vehicle (saline) was given as control in SHAM and I/R groups. (**B**) Hematoxylin–eosin staining (×20) of intestine tissue and (**C**) grading of the observed injury based on Chiu's score. (**D**) Intestinal epithelial cell apoptosis was detected by TUNEL assay (×40), and (**E**) TUNEL positive cells were quantified. Data are expressed as mean ± SEM, $n = 8$/group. $^{**}P < 0.01$. HRS, hydrogen-rich saline; I/R, ischemia reperfusion; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling.

Bioscience Reports (2020) **40** BSR20191043
https://doi.org/10.1042/BSR20191043

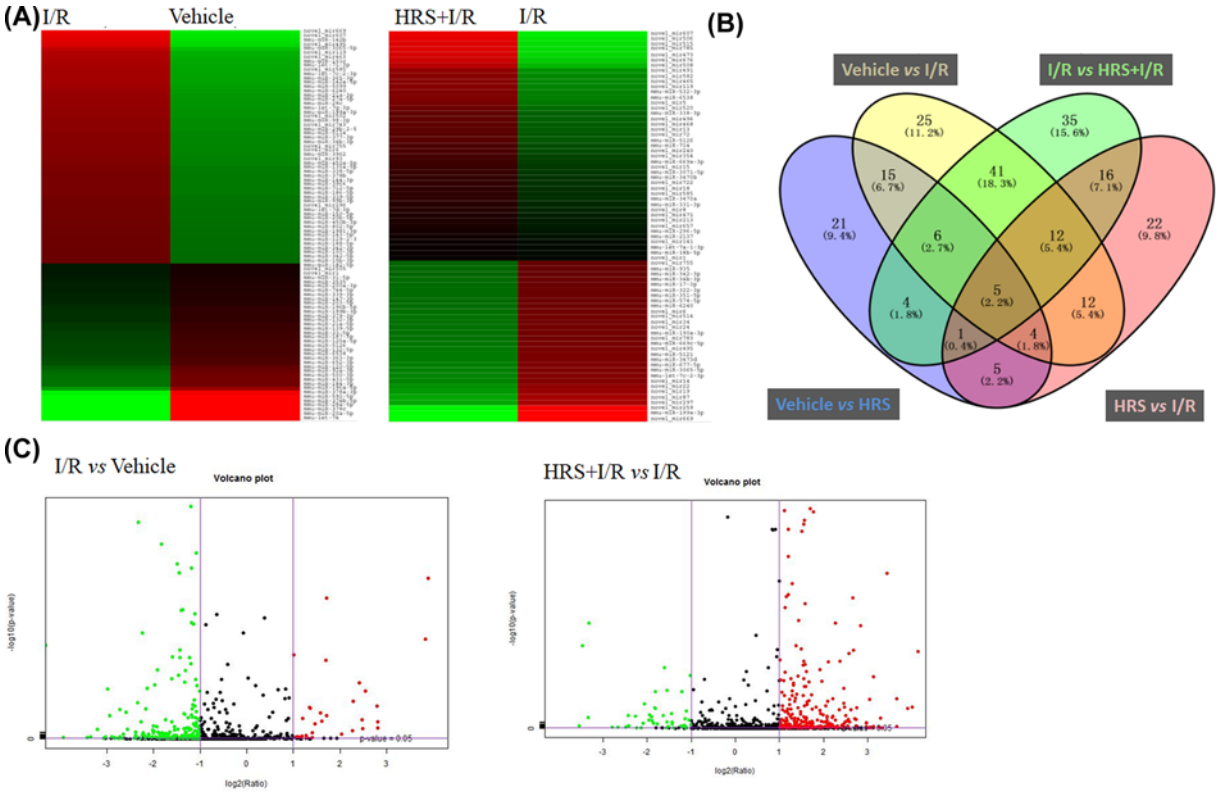

Figure 2. MiRNA sequencing analysis of intestinal mucosa

Heat map (**A**) and volcano plots (**C**) present expression profiles of significantly altered miRNAs with fold change ≥2.0 ($P < 0.05$; false discovery rate <0.05) in response to I/R and HRS in the intestine. Green indicates down-regulation and red indicates up-regulation. Color brightness depicts the relative miRNAs expression level as brighter color represents higher expression. (**B**) Venn diagram indicates number of miRNAs counts in each group or shared between groups (overlapped area). HRS, hydrogen-rich saline; I/R, ischemia/reperfusion.

intestine visualized by hematoxylin–eosin staining demonstrated severe mucosal injury (Figure 1B). Compared with the normal mucosal villi in sham, I/R-injured intestine showed mucosal edema, small hemorrhagic spot on superficial mucosa, as well as necrotic and scaled superficial epithelium. HRS pretreatment did not disturb the normal mucosal structures in sham and preserved its integrity after I/R injury. Grading of mucosal damage by Chiu's method also showed significantly lower score in the HRS-pretreated animals than the ones that had vehicle following I/R ($P < 0.01$, Figure 1C).

In addition, TUNEL assay revealed significantly increased apoptosis of mucosal epithelial cells in the I/R-injured intestine ($P < 0.01$ versus sham, Figure 1D,E). In contrast, HRS pretreatment markedly reduced the number of TUNEL positive epithelial cells (stained in brown) in the intestine after I/R ($P < 0.01$ versus I/R).

Profiling of intestinal miRNAs in response to ischemia/reperfusion injury and pretreatment of hydrogen-rich saline

MiRNAs play a critical role in the pathophysiological process of intestinal I/R injury. To investigate novel targets for preventing mucosal injury after intestinal I/R, we performed miRNA sequencing of the I/R-injured intestine with or without HRS pretreatment. According to our cut-off criteria of $P < 0.05$ and fold-change ≥ 2.0, 120 differentially expressed miRNAs were revealed between I/R and sham groups, and between HRS+I/R and I/R groups, respectively (Figure 2A, data details see attachment Supplementary Tables S1 and S2). In comparison of I/R and sham groups, the number of down-regulated miRNAs was greater than the up-regulated ones, while the opposite case was seen in the comparison of HRS+I/R and I/R groups (Figure 2C). Of these 120 differentially expressed miRNAs found in each pair of comparison, 64 were shared between them (Figure 2B), which indicates the intestinal I/R injury-specific miRNAs altered by HRS pretreatment. We then identified the top 10 most diversely expressed miRNAs out of these

64 miRNAs and further selected 8 of them whose expression levels reached over 100 copies. These 8 miRNAs may be candidate targets for protection of intestine from IR injury: miR-199a-3p, miR-296-5p, miR-5126, miR- 6538, miR-342-3p, miR-677-5p, let-7c-2-3p, and miR-574-5p.

RT-PCR validation of differentially expressed miRNAs

The eight chosen candidate miRNAs underwent validation of RT-PCR assay. Expressions of miR-199a-3p, miR-296-5p, miR-342-3p, miR-677-5p, and let-7c-2-3p were up-regulated ($P < 0.05$ versus sham, Figure 3A,B,E–G), while that of miR-5126 and miR- 6538 were down-regulated ($P < 0.05$ versus sham, Figure 3C,D) significantly after intestinal I/R. On the other hand, HRS pretreatment markedly down-regulated miR-199a-3p and miR-296-5p ($P < 0.05$ versus I/R, Figure 3A,B), but up-regulated miR-5126 and miR-6538 ($P < 0.05$ versus I/R, Figure 3C,D) following intestinal I/R; especially miR-5126 and miR- 6538 that were also up-regulated by HRS in sham animals. It's worth noting that these four differentially expressed miRNAs in response to I/R with HRS pretreatment was oppositely regulated during I/R injury, indicating HRS could reverse these I/R-induced alterations. Expressions of miR-342-3p, miR-677-5p, mmu-let-7c-2-3p, and miR-574- 5p were not significantly altered between I/R and HRS+I/R groups (Figure 3E–H), denoting inconsistency with the miRNA microarray results.

Predicted targets of miR-199a-3p is related to the IGF-1/PI3K/Akt/mTOR pathway

Since miR-199a-3p displayed the most drastic change in response to I/R and HRS pretreatment among our validated miRNAs, we performed computational analysis of its potential targets and related pathways. Using RNAhybrid (Figure 4A) and TargetScan (Figure 4B) programs, we found that miR-199a-3p could target insulin-like growth factor (IGF)-1, mammalian target of rapamycin (mTOR), and phosphoinositide-3-kinase regulatory subunit 1 (PIK3r1), which is a gene coded for a key part of the phosphatidylinositol 3-kinase (PI3K). Interestingly, these three targets of miR-199a-3p form a well-known pathway that promotes cell growth and division, namely the IGF-1/PI3K/Akt/mTOR pathway [21,22]. Therefore, we examined the protein expression of this targeted pathway and found a general decrease in all four of its members' expressions after intestinal I/R injury ($P < 0.05$ versus sham, Figure 4C–G). However, HRS pretreatment reduced these changes, significantly increasing protein levels of IGF-1, PI3K, Akt, and mTOR ($P < 0.05$ versus I/R).

HRS protected intestinal epithelial cell from hypoxia/reoxygenation injury through down-regulating miR-199a-3p expression and reactivating mTOR-related pro-survival pathway

In order to confirm the role of miR-199a-3p in intestinal I/R injury, we created intestinal epithelial cell IEC-6 hypoxia/reoxygenation to mimic *in vivo* intestinal I/R model. miR-199a-3p mimics or inhibitor were transfected into IEC-6 cells to perform the gain and loss functional experiments. As shown in Figure 5A–C, we found IEC-6 cells viability was significantly decreased after HR injury, accompany with decrease of IGF-1, PIK3r1, and mTOR mRNA expression. HRS treatment was found to reduce miR-199a-3p level, increase IGF-1, PIK3r1 and mTOR mRNA expression, restore IEC-6 cells viability, and this protective effects were similar to miR-199a-3p inhibitor treatment. Furthermore, the protective effects of HRS were reversed under miR-199a-3p mimics treatment. Taken together, aforementioned results suggest HRS treatment may protect intestinal epithelial cell from HR injury through down-regulating miR-199a-3p expression and activating IGF-1/PIK3r1/mTOR-related cell survival pathway.

Discussion

Our study identified and validated four novel miRNAs, miR-199a-3p, miR-296-5p, miR-5126, and miR-6538, which may mediate the protective effects of HRS in intestinal I/R injury. Further, we found that miR-199a-3p, the most significantly altered miRNA among these four, has its putative target gene linked to a cell growth-promoting pathway IGF-1/PI3K/Akt/mTOR [21], predicted by both RNAhybrid and TargetScan algorithms. The fact that miR-199a-3p was markedly increased by intestinal I/R is consistent with the significant decrease of IGF-1/PI3K/Akt/mTOR pathway protein expression following I/R, indicating potential suppression of the pro-survival pathway by miR-199a-3p. This is in line with the marked epithelial cell death and deteriorated mucosa in the intestine after I/R. HRS pretreatment effectively reduced miR-199a-3p and reversed these changes, suggesting a putative anti-apoptotic mechanism induced by HRS (Figure 6).

From hydrogen gas to HRS, hydrogen as a potent and selective antioxidant has been applied in clinic and studied in various animal disease models, especially in the setting of I/R injury [23–25]. As a well-established key feature

Bioscience Reports (2020) **40** BSR20191043
https://doi.org/10.1042/BSR20191043

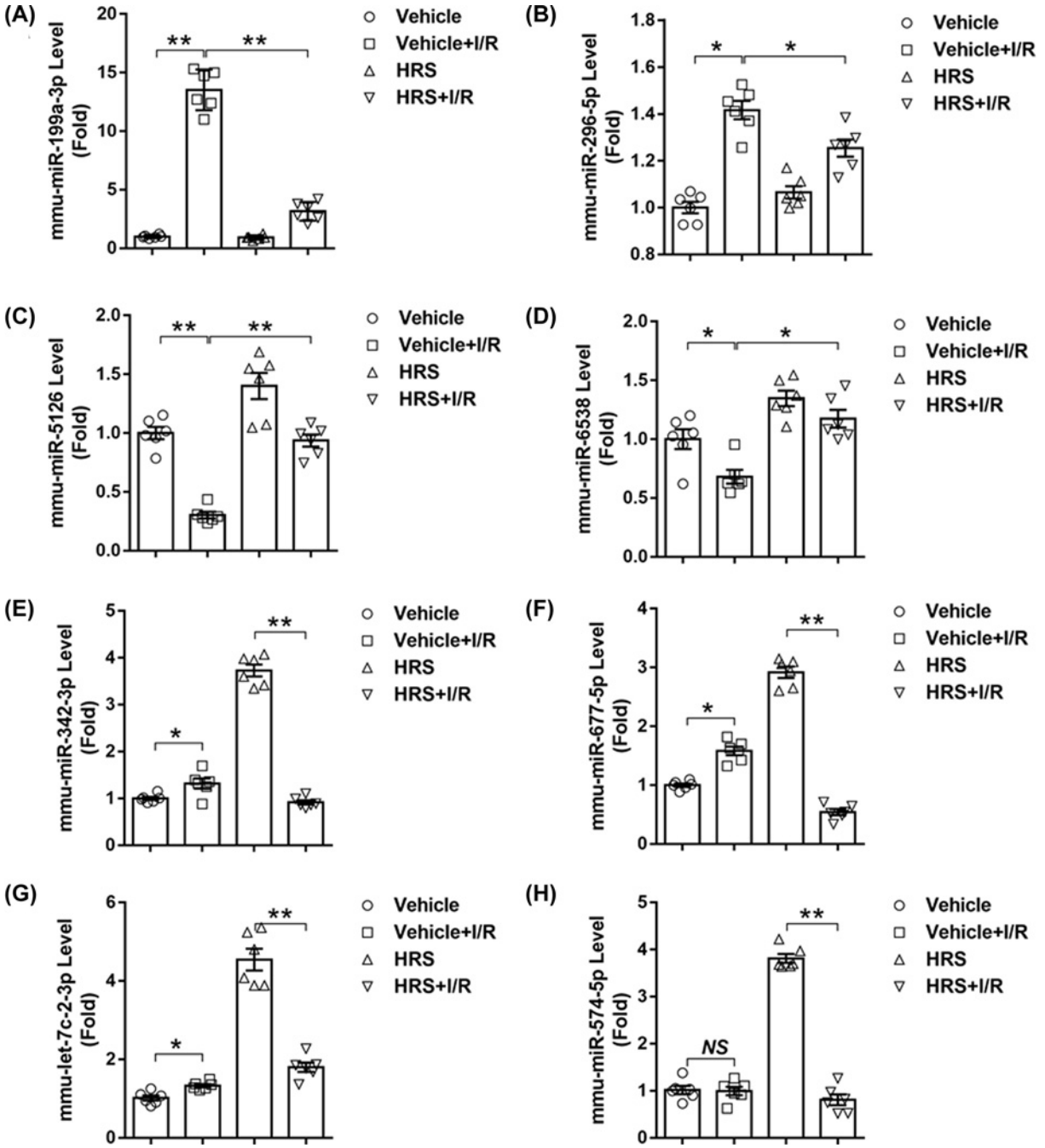

Figure 3. RT-PCR validation of candidate miRNAs responsive to HRS in intestinal I/R injury

Expression levels of (**A**) mmu-miR-199a-3p, (**B**) mmu-miR-296-5p, (**C**) mmu-miR-5126, (**D**) mmu-miR-6538, (**E**) mmu-miR-342-3p, (**F**) mmu-miR-677-5p, (**G**) mmu-let-7c-2-3p, and (**H**) mmu-miR-574-5p are presented. Data are expressed as mean ± SEM, n = 6/group. $^{*}P < 0.05$, $^{**}P < 0.01$.

of I/R injury, oxidative stress mediated by highly toxic ROS like hydroxyl radicals and peroxynitrite has been a main therapeutic target in interventions for I/R injury. For oxidative stress not only exaggerates host inflammatory response to I/R, but also damages cellular components indiscriminately, leading to escalated cellular apoptosis and thus tissue deterioration [5]. In line with previous studies [3,9], we demonstrated advanced disruption of intestinal mucosa in mice subjected to I/R, evidenced by massive apoptosis of epithelial cells and lifting of epithelium down the villi. However, mice pretreated with HRS exhibited significantly lower level of epithelial apoptosis and preserved tissue

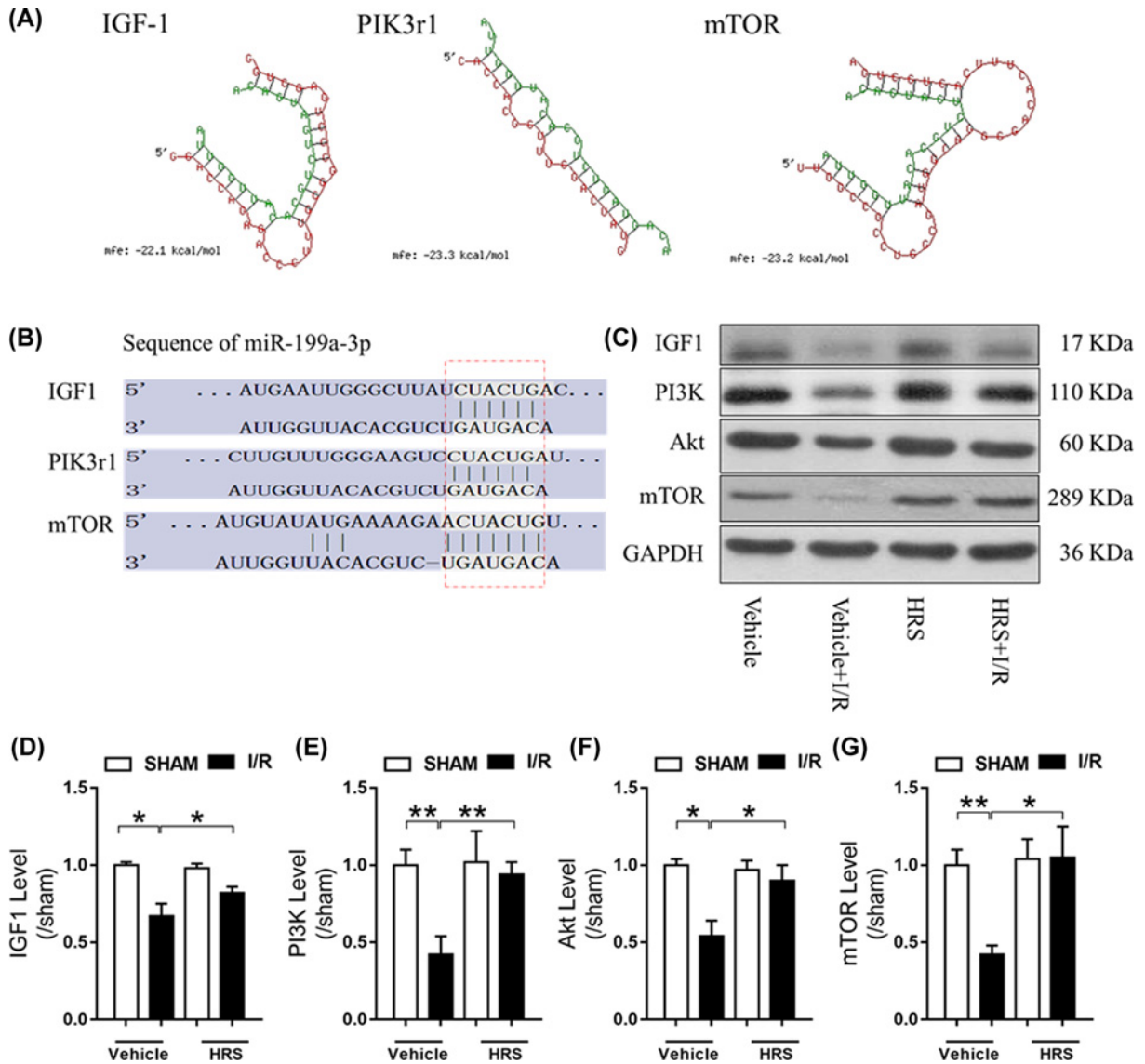

Figure 4. Predictive targets of mmu-miR-199a-3p and their related pathway

(**A**) Schematic of miR-199a-3p binding to its target genes predicted by RNAhybrid. Green sequence represents miR-199a-3p mature sequence and the red represents its target gene. (**B**) Schematic of miR-199a-3p binding to its target genes predicted by TargetScan. (**C**) Representative images of IGF-1/PI3K/Akt/mTOR pathway proteins expressions and their quantification shown in (**D**) IGF-1, (**E**) PI3K, (**F**) Akt, and (**G**) mTOR. Data are expressed as mean ± SEM, $n = 6$/group. $^{*}P < 0.05$, $^{**}P < 0.01$. HRS, hydrogen-rich saline; I/R, ischemia/reperfusion; IGF-1, insulin-like growth factor-1; mTOR, mammalian target of rapamycin; PIK3r1, phosphoinositide-3-kinase regulatory subunit 1; PI3K, phosphoinositide-3-kinase.

integrity in the intestine mucosa. These benefits have been documented previously and are attributable to the selective scavenging ability of HRS for hydroxyl radicals and peroxynitrite, effectively reducing oxidative stress and associated cell death [5,6]. Nonetheless, the prominent anti-apoptotic property of HRS may be mediated by more than just antioxidation.

Recent highlights of miRNAs in intestinal I/R injury pointed out a close link between miRNAs and I/R-induced oxidative stress, inflammation, and cell death [10,11,14]. Indeed, by negatively regulate gene expressions, miRNAs stay in controls of highly regulated events in cell, such as cellular proliferation, differentiation, and apoptosis [12]. Therefore, we profiled miRNAs that were expressed differentially in intestinal I/R and validated the ones that were most responsive to HRS pretreatment. We found that intestinal I/R significantly increased miR-199a-3p and miR-296-5p levels, and decreased miR-5126 and miR-6538 levels, which can be prevented by HRS. Of these four, miR-296-5p,

Bioscience Reports (2020) **40** BSR20191043
https://doi.org/10.1042/BSR20191043

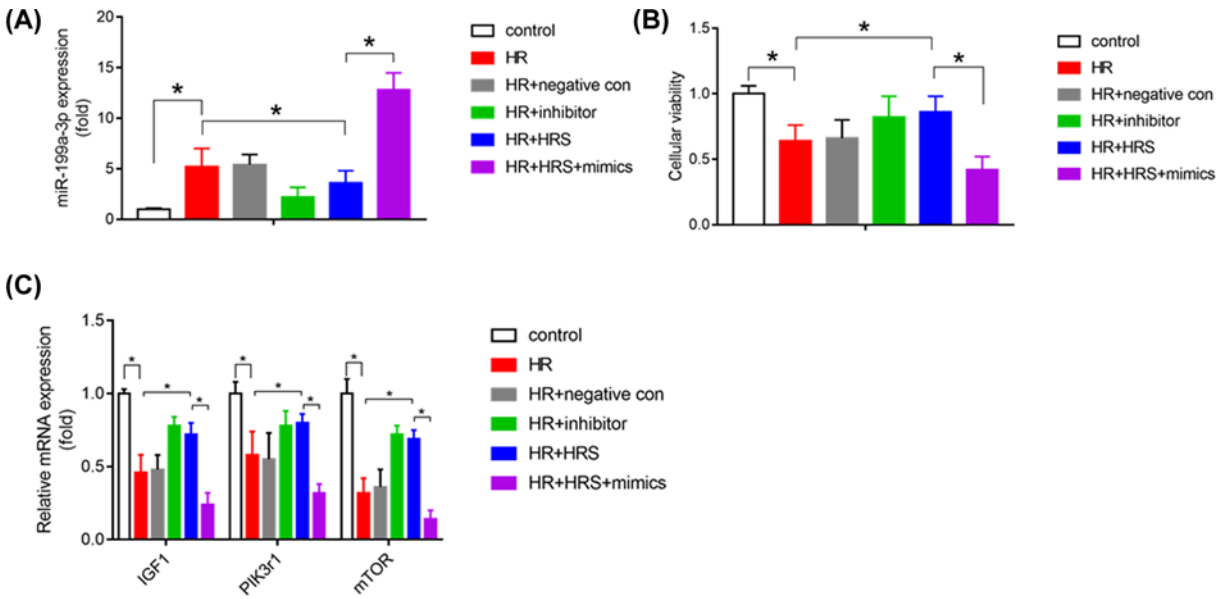

Figure 5. HRS protected intestinal epithelial cell from HR injury through down-regulating miR-199a-3p expression and reactivating mTOR-related pro-survival pathway

(**A**) Relative miR-199a-3p expression was measured after HR with or without HRS treatment or transfecting with miR-199a-3p mimics or inhibitor in intestinal epithelial cell IEC-6. Cellular viability of IEC-6 was detected using CCK-8 method (**B**), and mRNA expression of IGF1, PIK3r1 and mTOR (**C**) were detected after HR with or without HRS or transfecting with miR-199a-3p mimics or inhibitor in IEC-6; $n = 6$. $^*P < 0.05$. HR: hypoxia for 2 h/reoxygenation for 60 min; HR+negative con: miR- 199a-3p negative control was transfected to IEC-6 24 h before HR performed; HR+inhibitor: miR-199a-3p inhibitor was transfected to IEC-6 24 h before HR performed; HR+HRS: IEC-6 cells were pretreatment with HRS medium for 24 h before cells subjected to hypoxia/reoxygenation; HR+HRS+mimics: miR-199a-3p mimics was transfected to IEC-6 24 h before HRS treatment and subsequent HR performed.

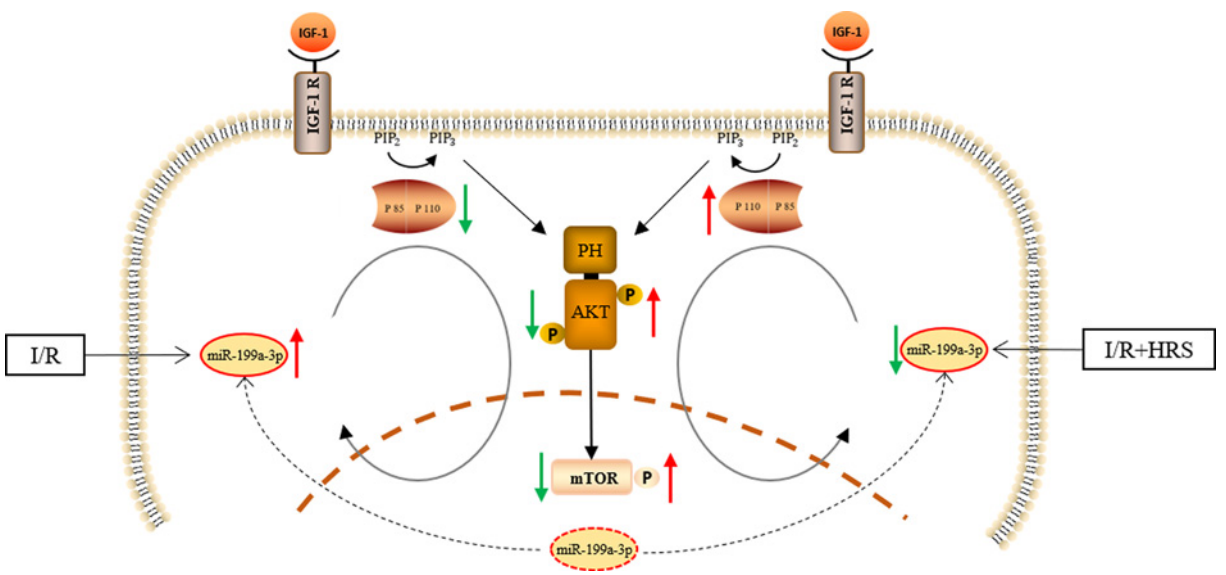

Figure 6. Schematic representation about the effect of HRS in intestinal I/R injury

IGF-1/PI3K/Akt/mTOR pathway was downregulated during intestine I/R injury with miR-199a-3p significantly increase. HRS activated the IGF-1/PI3K/Akt/mTOR pathway via stimulating miR-199a-3p generation and attenuated the intestine I/R injury.

miR-5126, and miR-6538 has been reported in intestinal-related illness. They were found significantly altered in the intestinal epithelial cells during inflammatory bowel diseases [26]. It is also known that miR-296-5p regulates genes involved in inflammation, angiogenesis, hypertension, cholesterol metabolism, cellular proliferation, and apoptosis. Previous literature suggested it to be a promising target of atherosclerosis, an inflammatory disease in the vascular wall [27]. Given the disturbances in blood circulation and the inflammatory nature of I/R injury, it is reasonable to defer that miR-296-5p may be a key contributor to the pathogenesis of intestinal I/R injury.

On the other hand, miR-5126 and miR-6538 has been demonstrated responsive to blood-stage malaria infection, while miR-5126 was confirmed down-regulated in macrophage infected with bacteria *Brucella* [28]. Of note, they were both down-regulated in exosome extracted from plasma after I/R preconditioning in the abdominal aorta [29], which resembles a mild version of I/R injury in the abdominal circulation. These findings support our result that miR-5126 and miR-6538 decreased in response to intestinal I/R injury and may also be associated with the I/R-induced bacterial translocation in the circulation. Therefore, HRS may prevent intestinal I/R injury by prophylactically up-regulate miR-5126 and miR-6538, as seen in HRS-pretreated sham animals, which then afforded them the later decrease by I/R, hence reduced injury.

Regarding miR-199a-3p, the most drastically altered miRNA in the miRNomes in HRS-pretreated intestinal I/R injury, has never been reported in intestinal diseases. Interestingly, miR-199a-3p, miR-5126, and miR-6538 have been listed together among the top differentially expressed miRNAs in mitochondria from the failing heart at late stage, suggesting link to energy metabolism, oxidative stress, and apoptosis [30]. Specifically, study has highlighted that miR-199a-3p inhibits cell proliferation and induces apoptosis in human hepatocellular carcinoma [31]. These are in line with our current finding. Target prediction of miR-199a-3p revealed involvement of the cell survival pathway, IGF-1/PI3K/Akt/mTOR [21,22], suggesting miR-199a-3p may suppress the pathway and induce apoptosis. Further pathway protein analysis showed decreases in IGF-1/PI3K/Akt/mTOR levels after intestinal I/R, which agreed with the up-regulation of miR-199a-3p following the injury, supporting the notion on the inhibition of IGF-1/PI3K/Akt/mTOR by miR-199a-3p. In *in vitro* experiment, IEC-6 HR model was created to mimic *in vivo* intestinal I/R, HRS or miR-199a-3p inhibitor treatment were found to decrease miR-199a-3p level, increase IGF-1, PIK3r1, and mTOR mRNA expression. However, the effects of HRS were reversed under miR- 199a-3p mimics treatment. The fact that these changes together with epithelial cell apoptosis in the I/R-injured intestine can be reversed by HRS-pretreatment indicated the anti-apoptotic effect of HRS may be mediated by regulating miR-199a-3p.

Conclusion

In conclusion, our results revealed novel miRNAs involved in the protective mechanism of HRS in intestinal I/R injury. In particular, we demonstrated that miR- 199a-3p may serve a key role in the anti-apoptotic mechanism of HRS that contributes to its protection of the intestine against I/R injury.

Funding

This work was supported by the Natural Science Foundation of China [grant numbers 81974081, 81601724 (to Weifeng Yao), 81501693 (to Pinjie Huang), 81974296, 81772127, 81571926 (to Ziqing Hei), 81900578 (to Xue Han)]; and the Natural Science Foundation of Guangdong Province [grant number 2016A030313232 (to Pinjie Huang)].

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contribution

Weifeng Yao and Pinjie Huang devised the study. Weifeng Yao, Lanfen Zeng, Anshun Guo, and Yu Guan carry out the experiment. Weifeng Yao, Xiaoyu Lin, Jianpei Liu, and Ziqing Hei extracted, analyzed, and interpreted the data. Weifeng Yao, Xiaoyu Lin, and Xue Han wrote the first draft. All authors contributed to subsequent versions and approved the final article. Pinjie Huang and Jianpei Liu is the corresponding authors. All authors read and approved the final manuscript.

Abbreviations

H&E, hematoxylin–eosin; HRS, hydrogen-rich saline; IGF, insulin-like growth factor; I/R, ischemia/reperfusion; miRNA, microRNA; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide-3-kinase; PIK3r1, phosphoinositide-3-kinase regulatory subunit 1; ROS, reactive oxygen species; SMA, superior mesenteric artery; TUNEL, (TdT)-mediated dUTP nick end labeling.

References

1 Schoenberg, M.H. and Beger, H.G. (1993) Reperfusion injury after intestinal ischemia. *Crit. Care Med.* **21**, 1376–1386, https://doi.org/10.1097/00003246-199309000-00023
2 Grootjans, J., Lenaerts, K., Derikx, J.P., Matthijsen, R.A., de Bruine, A.P., van Bijnen, A.A. et al. (2010) Human intestinal ischemia-reperfusion-induced inflammation characterized: experiences from a new translational model. *Am. J. Pathol.* **176**, 2283–2291, https://doi.org/10.2353/ajpath.2010.091069
3 Chen, H., Sun, Y.P., Hu, P.F., Liu, W.W., Xiang, H.G., Li, Y. et al. (2011) The effects of hydrogen-rich saline on the contractile and structural changes of intestine induced by ischemia-reperfusion in rats. *J. Surg. Res.* **167**, 316–322, https://doi.org/10.1016/j.jss.2009.07.045
4 Mallick, I.H., Yang, W., Winslet, M.C. and Seifalian, A.M. (2004) Ischemia-reperfusion injury of the intestine and protective strategies against injury. *Dig. Dis. Sci.* **49**, 1359–1377, https://doi.org/10.1023/B:DDAS.0000042232.98927.91
5 Hong, Y., Chen, S. and Zhang, J.M. (2010) Hydrogen as a selective antioxidant: a review of clinical and experimental studies. *J. Int. Med. Res.* **38**, 1893–1903, https://doi.org/10.1177/147323001003800602
6 Chang, W.J. and Toledo-Pereyra, L.H. (2013) The potential benefits of hydrogen-rich saline in ischemia and reperfusion injury. *J. Surg. Res.* **180**, 248–249, https://doi.org/10.1016/j.jss.2011.12.025
7 Li, J., Hong, Z., Liu, H., Zhou, J., Cui, L., Yuan, S. et al. (2016) Hydrogen-Rich Saline Promotes the Recovery of Renal Function after Ischemia/Reperfusion Injury in Rats via Anti-apoptosis and Anti- inflammation. *Front. Pharmacol.* **7**, 106, https://doi.org/10.3389/fphar.2016.00106
8 Zheng, X., Mao, Y., Cai, J., Li, Y., Liu, W., Sun, P. et al. (2009) Hydrogen-rich saline protects against intestinal ischemia/reperfusion injury in rats. *Free Radic. Res.* **43**, 478–484, https://doi.org/10.1080/10715760902870603
9 Wu, M.J., Chen, M., Sang, S., Hou, L.L., Tian, M.L., Li, K. et al. (2017) Protective effects of hydrogen rich water on the intestinal ischemia/reperfusion injury due to intestinal intussusception in a rat model. *Med. Gas. Res.* **7**, 101–106, https://doi.org/10.4103/2045-9912.208515
10 Li, Y., Wen, S., Yao, X., Liu, W., Shen, J., Deng, W. et al. (2017) MicroRNA-378 protects against intestinal ischemia/reperfusion injury via a mechanism involving the inhibition of intestinal mucosal cell apoptosis. *Cell Death. Dis.* **8**, e3127, https://doi.org/10.1038/cddis.2017.508
11 Hu, Y., Tao, X., Han, X., Xu, L., Yin, L., Sun, H. et al. (2018) MicroRNA-351-5p aggravates intestinal ischaemia/reperfusion injury through the targeting of MAPK13 and Sirtuin-6. *Br. J. Pharmacol.* **175**, 3594–3609, https://doi.org/10.1111/bph.14428
12 Mendell, J.T. and Olson, E.N. (2012) MicroRNAs in stress signaling and human disease. *Cell* **148**, 1172–1187, https://doi.org/10.1016/j.cell.2012.02.005
13 Li, Z., Wang, G., Feng, D., Zu, G., Li, Y., Shi, X. et al. (2018) Targeting the miR-665-3p-ATG4B-autophagy axis relieves inflammation and apoptosis in intestinal ischemia/reperfusion. *Cell Death Dis.* **9**, 483, https://doi.org/10.1038/s41419-018-0518-9
14 Liu, L., Yao, J., Li, Z., Zu, G., Feng, D., Li, Y. et al. (2018) miR-381- 3p knockdown improves intestinal epithelial proliferation and barrier function after intestinal ischemia/reperfusion injury by targeting nurr1. *Cell Death Dis.* **9**, 411, https://doi.org/10.1038/s41419-018-0450-z
15 Zhao, W., Huang, X., Han, X., Hu, D., Hu, X., Li, Y. et al. (2018) Resveratrol Suppresses Gut- Derived NLRP3 Inflammasome Partly through Stabilizing Mast Cells in a Rat Model. *Mediat. Inflamm.* **2018**, 6158671, https://doi.org/10.1155/2018/6158671
16 Huang, X., Zhao, W., Hu, D., Han, X., Wang, H., Yang, J. et al. (2017) Resveratrol efficiently improves pulmonary function via stabilizing mast cells in a rat intestinal injury model. *Life Sci.* **185**, 30–37, https://doi.org/10.1016/j.lfs.2017.07.018
17 Luo, C., Yuan, D., Yuan, W., Cai, J., Zhou, S., Zhang, Y. et al. (2015) Dexmedetomidine protects against apoptosis induced by hypoxia/reoxygenation through the inhibition of gap junctions in NRK-52E cells. *Life Sci.* **122**, 72–77, https://doi.org/10.1016/j.lfs.2014.12.009
18 Han, X., Yao, W., Liu, Z., Li, H., Zhang, Z.J., Hei, Z. et al. (2016) Lipoxin A4 Preconditioning Attenuates Intestinal Ischemia Reperfusion Injury through Keap1/Nrf2 Pathway in a Lipoxin A4 Receptor Independent Manner. *Oxidative Med. Cell. Longev.* **2016**, 9303606, https://doi.org/10.1155/2016/9303606
19 Chi, X., Yao, W., Xia, H., Jin, Y., Li, X., Cai, J. et al. (2015) Elevation of HO-1 Expression Mitigates Intestinal Ischemia-Reperfusion Injury and Restores Tight Junction Function in a Rat Liver Transplantation Model. *Oxidative Med. Cell. Longev.* **2015**, 986075, https://doi.org/10.1155/2015/986075
20 Langmead, B., Trapnell, C., Pop, M. and Salzberg, S.L. (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10**, R25, https://doi.org/10.1186/gb-2009-10-3-r25
21 Feng, Z., Zhang, H., Levine, A.J. and Jin, S. (2005) The coordinate regulation of the p53 and mTOR pathways in cells. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 8204–8209, https://doi.org/10.1073/pnas.0502857102
22 Floyd, S., Favre, C., Lasorsa, F.M., Leahy, M., Trigiante, G., Stroebel, P. et al. (2007) The insulin-like growth factor-I-mTOR signaling pathway induces the mitochondrial pyrimidine nucleotide carrier to promote cell growth. *Mol. Biol. Cell* **18**, 3545–3555, https://doi.org/10.1091/mbc.e06-12-1109
23 Ge, L., Yang, M., Yang, N.N., Yin, X.X. and Song, W.G. (2017) Molecular hydrogen: a preventive and therapeutic medical gas for various diseases. *Oncotarget* **8**, 102653–102673, https://doi.org/10.18632/oncotarget.21130
24 Ostojic, S.M. (2015) Molecular hydrogen: An inert gas turns clinically effective. *Ann. Med.* **47**, 301–304, https://doi.org/10.3109/07853890.2015.1034765
25 Luo, Z.L., Cheng, L., Ren, J.D., Fang, C., Xiang, K., Xu, H.T. et al. (2015) Hydrogen- rich saline protects against ischemia/reperfusion injury in grafts after pancreas transplantations by reducing oxidative stress in rats. *Mediat. Inflamm.* **2015**, 281985, https://doi.org/10.1155/2015/281985
26 Lee, J., Park, E.J., Yuki, Y., Ahmad, S., Mizuguchi, K., Ishii, K.J. et al. (2015) Profiles of microRNA networks in intestinal epithelial cells in a mouse model of colitis. *Sci. Rep.* **5**, 18174, https://doi.org/10.1038/srep18174
27 Li, H., Ouyang, X.P., Jiang, T., Zheng, X.L., He, P.P. and Zhao, G.J. (2018) MicroRNA-296: a promising target in the pathogenesis of atherosclerosis? *Mol. Med.* **24**, 12, https://doi.org/10.1186/s10020-018-0012-y
28 Dkhil, M.A., Al-Quraishy, S.A., Abdel-Baki, A.S., Delic, D. and Wunderlich, F. (2016) Differential miRNA Expression in the Liver of Balb/c Mice Protected by Vaccination during Crisis of Plasmodium chabaudi Blood-Stage Malaria. *Front. Microbiol.* **7**, 2155

29 Ueno, K., Samura, M., Nakamura, T., Tanaka, Y., Takeuchi, Y., Kawamura, D. et al. (2016) Increased plasma VEGF levels following ischemic preconditioning are associated with downregulation of miRNA-762 and miR-3072-5p. *Sci. Rep.* **6**, 36758, https://doi.org/10.1038/srep36758
30 Wang, X., Song, C., Zhou, X., Han, X., Li, J., Wang, Z. et al. (2017) Mitochondria Associated MicroRNA Expression Profiling of Heart Failure. *Biomed. Res. Int.* **2017**, 4042509
31 Ren, K., Li, T., Zhang, W., Ren, J., Li, Z. and Wu, G. (2016) miR-199a-3p inhibits cell proliferation and induces apoptosis by targeting YAP1, suppressing Jagged1-Notch signaling in human hepatocellular carcinoma. *J. Biomed. Sci.* **23**, 79, https://doi.org/10.1186/s12929-016-0295-7

Supple Table 1: Differential miRNAs between Sham and IR groups

miRNA id	Read count(Sham)	Read count(IR)	Expression(Sham)	Expression(IR)	log2Ratio(IR/Sham)	Up down regulation
mmu-miR-21a-5p	757386	519731	9599.666667	5186.77	-1.431266883	DOWN
mmu-miR-378a-3p	87506	7355	1138.303333	80.32333333	-4.460587937	DOWN
mmu-miR-200a-3p	141382	122238	1757.086667	1217.28	-1.097908854	DOWN
mmu-miR-378b	46622	190913	498.2366667	1909.353333	1.145829303	UP
mmu-miR-92a-3p	29394	11326	376.4833333	113.8133333	-2.263886504	DOWN
mmu-miR-652-3p	34257	18111	437.1366667	181.91	-1.80753585	DOWN
novel_mir1	40097	32918	466.01	349.0333333	-1.172620858	DOWN
mmu-miR-199b-3p	29718	20907	374.47	221.6166667	-1.395354208	DOWN
novel_mir93	14922	64824	168.2633333	699.6566667	1.23108389	UP
mmu-miR-3074-5p	6140	57	79.08666667	0.58	-7.639136168	DOWN
mmu-miR-5126	12467	6918	151.46	71.11	-1.737690667	DOWN
mmu-miR-150-5p	10112	41722	126.07	433.9633333	1.156736706	UP
mmu-miR-142a-5p	2679	18187	31.57666667	187.3133333	1.875137858	UP
mmu-miR-142b	877	11095	9.563333333	114.01	2.773185773	UP
mmu-miR-31-5p	22781	20572	278.1833333	203.6466667	-1.03515226	DOWN
mmu-miR-199a-3p	3984	21854	43.25666667	210.5633333	1.56760457	UP
novel_mir755	4959	24062	53.71666667	233.72	1.39063219	UP
novel_mir807	4123	661	54.72	6.743333333	-3.528975526	DOWN
mmu-miR-33-5p	8622	5413	108.0266667	54.32333333	-1.559597416	DOWN
mmu-miR-28c	2840	15292	30.24033333	154.94	1.540811028	UP
mmu-miR-28a-5p	2448	82	31.82333333	0.833333333	-5.78783908	DOWN
mmu-miR-125a-5p	6866	4086	86.67666667	41.34666667	-1.636783895	DOWN
mmu-let-7k	2189	43	28.03333333	0.426666667	-6.557794726	DOWN
mmu-miR-378d	2141	10	28.557	0.110666667	-8.630144227	DOWN
mmu-miR-802-5p	4532	17434	51.86	175.21	1.055680606	UP
mmu-miR-20a-5p	1620	42	20.71	0.423333333	-6.157463917	DOWN
novel_mir669	273	4141	2.936666667	39.70666667	3.035003104	UP
mmu-let-7d-3p	3744	14926	43.47666667	149.9133333	1.107172009	UP
mmu-miR-182-5p	8246	7246	96.61666667	73.39	-1.074512897	DOWN
mmu-miR-5099	1103	6953	12.16666667	70.71	1.768199557	UP
mmu-miR-139-5p	4757	3159	59.55333333	31.86666667	-1.47858733	DOWN
mmu-miR-374c-5p	4	2938	0.050333333	28.85666667	8.632615439	UP
mmu-miR-196a-5p	1761	408	22.77666667	4.166666667	-2.997757094	DOWN
mmu-miR-6538	3255	1784	39.63666667	17.96666667	-1.75554517	DOWN
novel_mir296	3432	12944	36.73666667	127.98	1.027158813	UP
mmu-let-7c-2-3p	746	4722	7.953333333	48.01333333	1.774147264	UP

mmu-miR-342-3p	2368	9281	28.72	93.40666667	1.082607937	UP
mmu-miR-20b-5p	2076	8446	24.72	84.74333333	1.136458561	UP
mmu-miR-374b-5p	701	33	9.136666667	0.336666667	-5.296879757	DOWN
novel_mir495	160	1872	1.82	19.73	2.660433383	UP
mmu-let-7i-3p	288	2174	3.496666667	22.20333333	2.028207982	UP
novel_mir14	23	900	0.244	9.536666667	4.402215993	UP
novel_mir502	505	2655	5.736666667	27.74333333	1.506353326	UP
novel_mir13	478	53	5.087	0.507333333	-4.060949595	DOWN
novel_mir119	204	1627	2.373333333	16.89666667	2.107569952	UP
mmu-miR-378c	409	13	5.320333333	0.13	-5.863520557	DOWN
novel_mir785	378	44	4.876666667	0.443666667	-3.990814048	DOWN
mmu-miR-101c	208	1426	2.263333333	14.46	1.889315306	UP
novel_mir506	39	740	0.463666667	8.14	3.357976	UP
mmu-miR-365-3p	201	1373	2.38	14.20333333	1.884060977	UP
mmu-miR-21a-3p	233	1409	2.773333333	14.37	1.70826651	UP
novel_mir6	383	1867	4.4	18.94666667	1.397302388	UP
novel_mir151	481	189	5.556666667	1.826666667	-2.235653902	DOWN
mmu-miR-186-5p	465	2016	5.413333333	20.35333333	1.228189776	UP
mmu-let-7j	256	2	3.297	0.020666667	-7.888003242	DOWN
mmu-miR-187-3p	605	346	7.513333333	3.696666667	-1.694166346	DOWN
novel_mir297	71	696	0.757333333	6.663666667	2.405193134	UP
novel_mir8	718	494	8.23	5.066666667	-1.427476044	DOWN
mmu-miR-135a-5p	362	1583	4.173333333	16.19	1.240596411	UP
mmu-miR-15b-3p	599	2219	7.05	22.41666667	1.001278517	UP
novel_mir259	135	826	1.437333333	7.917	1.725179132	UP
novel_mir12	450	262	4.91	2.540333333	-1.668361432	DOWN
mmu-miR-122-5p	330	146	4.296666667	1.473333333	-2.064500897	DOWN
mmu-miR-500-3p	274	99	3.51	1.003333333	-2.356678705	DOWN
mmu-miR-363-3p	385	205	4.806666667	2.08	-1.797237778	DOWN
novel_mir496	14	289	0.150666667	3.12	3.479567518	UP
mmu-miR-592-5p	126	7	1.633333333	0.073333333	-5.057928243	DOWN
novel_mir743	167	822	1.813666667	8.366666667	1.411287049	UP
novel_mir607	28	344	0.297333333	3.743333333	2.730906591	UP
mmu-miR-3065-5p	48	423	0.513666667	4.243333333	2.25154811	UP
mmu-miR-467e-5p	123	1	1.560333333	0.010666667	-7.830517747	DOWN
novel_mir228	0	228	0.001	2.263666667	7.944886772	UP
mmu-miR-3962	188	850	2.01	8.596666667	1.288726937	UP
mmu-miR-132-3p	425	311	5.216666667	3.08	-1.338551503	DOWN

mmu-miR-6240	80	501	0.91	4.993333333	1.758735456	UP
novel_mir418	148	39	1.577333333	0.377	-2.812054389	DOWN
novel_mir656	0	183	0.001	1.744	7.627696596	UP
mmu-miR-450a-5p	136	646	1.586666667	6.543333333	1.359924271	UP
novel_mir657	14	220	0.150666667	2.416666667	3.08600155	UP
novel_mir5	81	467	0.886666667	4.93	1.639425495	UP
mmu-miR-99b-3p	212	844	2.416666667	8.576666667	1.105175492	UP
mmu-miR-184-3p	135	41	1.446666667	0.406666667	-2.607266834	DOWN
novel_mir463	56	375	0.597333333	4.040333333	1.855388621	UP
novel_mir514	12	200	0.127333333	1.910333333	3.170890447	UP
mmu-miR-431-5p	143	51	1.853333333	0.526666667	-2.375449237	DOWN
mmu-miR-27a-5p	65	392	0.766666667	3.93	1.704338789	UP
mmu-miR-744-5p	336	264	3.9	2.68	-1.235926545	DOWN
novel_mir585	50	338	0.563666667	3.463333333	1.869020005	UP
mmu-miR-712-5p	168	686	1.9	7.193333333	1.141744101	UP
novel_mir141	331	262	3.996666667	2.676666667	-1.225267647	DOWN
mmu-miR-3535	315	243	3.476666667	2.416666667	-1.262398757	DOWN
mmu-miR-132-5p	205	122	2.606666667	1.213333333	-1.636746004	DOWN
novel_mir392	74	0	0.817	0.001	-8.097456608	DOWN
novel_mir508	223	149	2.666666667	1.546666667	-1.469734621	DOWN
novel_mir722	0	111	0.001	1.16	6.906412624	UP
mmu-miR-376b-5p	131	61	1.683333333	0.616666667	-1.990688906	DOWN
mmu-miR-144-3p	104	464	1.253333333	4.78	1.269538035	UP
novel_mir22	99	440	1.054	4.197333333	1.263999851	UP
novel_mir473	90	28	1.150333333	0.300333333	-2.572501416	DOWN
novel_mir51	0	103	0.001	0.993666667	6.798497285	UP
novel_mir452	0	100	0.001	0.954	6.755852948	UP
novel_mir663	42	261	0.447	2.523333333	1.747585332	UP
novel_mir49	95	34	1.010666667	0.324	-2.370396009	DOWN
mmu-miR-196b-5p	169	109	1.873333333	1.12	-1.520698353	DOWN
novel_mir619	0	96	0.001	0.930333333	6.696959259	UP
mmu-miR-380-3p	72	17	0.91	0.17	-2.970465402	DOWN
novel_mir600	0	94	0.001	0.897333333	6.66658561	UP
mmu-miR-107-3p	74	20	0.946666667	0.196666667	-2.775528513	DOWN
mmu-miR-98-3p	62	314	0.7	3.23	1.452421197	UP
novel_mir640	0	89	0.001	0.896666667	6.587730189	UP
novel_mir87	79	362	0.947	4.010333333	1.308061897	UP
mmu-miR-29a-5p	80	27	1.023333333	0.273333333	-2.455043835	DOWN

novel_mir729	0	85	0.001	0.810666667	6.521387694	UP
novel_mir350	48	0	0.510666667	0.001	-7.472965743	DOWN
mmu-miR-29b-2-5	52	274	0.596666667	2.743333333	1.509589123	UP
novel_mir48	70	327	0.747333333	3.140333333	1.335860567	UP
mmu-miR-411-3p	50	6	0.64	0.063666667	-3.946896931	DOWN
novel_mir766	0	82	0.001	0.784	6.469548763	UP
novel_mir38	0	81	0.001	0.774	6.451846761	UP
novel_mir599	0	80	0.001	0.777	6.433924853	UP
novel_mir460	44	0	0.587333333	0.001	-7.347434861	DOWN
novel_mir237	0	76	0.001	0.753333333	6.359924271	UP
novel_mir802	43	0	0.496666667	0.001	-7.314267997	DOWN
novel_mir39	11	121	0.117333333	1.154	2.571428377	UP
novel_mir162	41	0	0.47	0.001	-7.245555247	DOWN
novel_mir231	95	49	1.047	0.477	-1.843149006	DOWN
mmu-miR-92a-1-5	18	145	0.216666667	1.42	2.121980847	UP
novel_mir771	137	96	1.49	0.923666667	-1.401072824	DOWN
novel_mir31	28	178	0.297333333	1.753333333	1.780375267	UP
novel_mir34	20	150	0.214	1.430666667	2.018887354	UP
mmu-miR-7662-3p	36	2	0.473666667	0.020333333	-5.057928243	DOWN
novel_mir573	37	0	0.394	0.001	-7.097456608	DOWN
novel_mir751	0	65	0.001	0.620666667	6.134364571	UP
novel_mir649	36	0	0.384	0.001	-7.057928243	DOWN
mmu-miR-669o-5p	34	1	0.437	0.010666667	-5.975466083	DOWN
novel_mir511	0	63	0.001	0.600666667	6.089276682	UP
mmu-miR-339-5p	77	317	0.903333333	3.266666667	1.153549247	UP
novel_mir481	35	0	0.374	0.001	-7.017286259	DOWN
novel_mir689	37	199	0.394	1.897333333	1.539168013	UP
novel_mir96	0	61	0.001	0.580666667	6.042734096	UP
mmu-miR-338-3p	85	45	1.02	0.493333333	-1.805541082	DOWN
novel_mir795	53	16	0.597	0.154	-2.615923697	DOWN
novel_mir571	0	60	0.001	0.574	6.018887354	UP
mmu-miR-147-3p	160	132	1.98	1.313333333	-1.165537217	DOWN
novel_mir482	0	59	0.001	0.600666667	5.994639807	UP
mmu-miR-690	50	15	0.566666667	0.153333333	-2.624968836	DOWN
novel_mir444	0	57	0.001	0.544	5.944886772	UP
mmu-miR-541-5p	92	56	1.126666667	0.566666667	-1.604210276	DOWN
novel_mir109	61	261	0.650666667	2.753666667	1.209165417	UP
novel_mir445	31	0	0.330666667	0.001	-6.842199552	DOWN

novel_mir465	0	55	0.001	0.597	5.893356472	UP
novel_mir30	29	0	0.310666667	0.001	-6.745984237	DOWN
mmu-miR-338-5p	52	230	0.603333333	2.33	1.257047091	UP
novel_mir247	28	0	0.297333333	0.001	-6.695358164	DOWN
novel_mir621	21	133	0.224	1.277	1.774961771	UP
novel_mir190	0	50	0.001	0.477333333	5.755852948	UP
mmu-miR-143-5p	59	246	0.746666667	2.496666667	1.171868214	UP
mmu-miR-203-5p	117	90	1.426666667	0.906666667	-1.266514865	DOWN
novel_mir405	82	50	0.874	0.490333333	-1.601699057	DOWN
mmu-miR-23b-5p	37	182	0.433333333	1.803333333	1.410338033	UP
novel_mir545	88	57	0.973666667	0.557	-1.514544846	DOWN
novel_mir275	27	0	0.287333333	0.001	-6.642890744	DOWN
novel_mir114	0	48	0.001	0.457333333	5.696959259	UP
novel_mir386	20	126	0.214	1.200666667	1.767348587	UP
novel_mir746	26	0	0.277	0.001	-6.58844296	DOWN
mmu-miR-342-5p	74	281	0.89	2.846666667	1.036969713	UP
mmu-miR-1843a-3	51	21	0.636666667	0.216666667	-2.168111161	DOWN
novel_mir333	25	0	0.334	0.001	-6.531859432	DOWN
novel_mir410	88	60	0.937333333	0.574	-1.440544265	DOWN
mmu-miR-330-5p	99	73	1.24	0.726666667	-1.327535303	DOWN
novel_mir559	17	113	0.180666667	1.087	1.844712879	UP
novel_mir576	0	45	0.001	0.480333333	5.603849854	UP
novel_mir54	49	20	0.557	0.207	-2.180784991	DOWN
mmu-miR-5113	37	175	0.436666667	1.75	1.353754504	UP
novel_mir154	0	44	0.001	0.420666667	5.571428377	UP
novel_mir158	24	0	0.290333333	0.001	-6.472965743	DOWN
novel_mir107	24	0	0.257333333	0.001	-6.472965743	DOWN
novel_mir712	0	43	0.001	0.413666667	5.538261513	UP
novel_mir441	0	43	0.001	0.410666667	5.538261513	UP
novel_mir258	0	43	0.001	0.410666667	5.538261513	UP
novel_mir149	0	42	0.001	0.400666667	5.504314181	UP
novel_mir491	0	41	0.001	0.457333333	5.469548763	UP
novel_mir588	22	0	0.234	0.001	-6.347434861	DOWN
mmu-miR-466f	10	82	0.106666667	0.836666667	2.147620668	UP
mmu-miR-188-5p	57	223	0.686666667	2.243333333	1.080006644	UP
novel_mir680	71	46	0.810333333	0.483666667	-1.514188405	DOWN
mmu-miR-100-3p	66	246	0.783333333	2.52	1.010117144	UP
novel_mir312	10	81	0.107333333	0.843333333	2.129918666	UP

novel_mir246	92	71	1.013666667	0.707	-1.261818079 DOWN
mmu-miR-183-3p	35	11	0.453333333	0.107	-2.55785464 DOWN
novel_mir811	21	0	0.224	0.001	-6.280320665 DOWN
novel_mir759	21	0	0.224	0.001	-6.280320665 DOWN
novel_mir284	21	0	0.224	0.001	-6.280320665 DOWN
mmu-miR-329-3p	62	234	0.753333333	2.356666667	1.028165167 UP
novel_mir505	125	113	1.447	1.090333333	-1.033608564 DOWN
novel_mir770	0	38	0.001	0.364	5.359924271 UP
novel_mir321	0	38	0.001	0.364	5.359924271 UP
novel_mir534	0	38	0.001	0.364	5.359924271 UP
novel_mir41	33	10	0.350666667	0.097333333	-2.610469266 DOWN
mmu-miR-450b-3p	56	216	0.64	2.166666667	1.059529338 UP
novel_mir800	20	0	0.214	0.001	-6.209931337 DOWN
novel_mir736	20	0	0.214	0.001	-6.209931337 DOWN
novel_mir772	20	0	0.214	0.001	-6.209931337 DOWN
novel_mir428	0	37	0.001	0.414	5.321450124 UP
mmu-miR-335-3p	30	142	0.346666667	1.466666667	1.354853282 UP
novel_mir697	19	0	0.204	0.001	-6.135930755 DOWN
mmu-miR-467h	19	0	0.254	0.001	-6.135930755 DOWN
novel_mir609	19	0	0.204	0.001	-6.135930755 DOWN
mmu-miR-377-3p	25	125	0.286666667	1.263333333	1.433924853 UP
novel_mir252	0	35	0.001	0.360333333	5.241279775 UP
novel_mir101	0	35	0.001	0.334	5.241279775 UP
novel_mir652	0	35	0.001	0.334	5.241279775 UP
novel_mir557	0	35	0.001	0.356666667	5.241279775 UP
novel_mir403	0	35	0.001	0.334	5.241279775 UP
mmu-miR-1981-3p	54	206	0.613666667	2.056666667	1.043609783 UP
novel_mir388	31	10	0.330666667	0.097333333	-2.520271457 DOWN
novel_mir400	0	34	0.001	0.324	5.199459599 UP
novel_mir148	0	34	0.001	0.324	5.199459599 UP
mmu-miR-339-3p	85	68	1.03	0.693333333	-1.209931337 DOWN
novel_mir574	82	65	0.943666667	0.647	-1.223187434 DOWN
novel_mir40	15	91	0.160666667	0.880333333	1.712900803 UP
mmu-miR-331-3p	55	34	0.723333333	0.37	-1.581900114 DOWN
novel_mir198	0	33	0.001	0.314	5.156390877 UP
novel_mir60	0	33	0.001	0.314	5.156390877 UP
novel_mir61	0	33	0.001	0.314	5.156390877 UP
novel_mir346	0	33	0.001	0.314	5.156390877 UP

novel_mir562	0	33	0.001	0.314	5.156390877	UP
novel_mir52	51	31	0.544	0.297333333	-1.606232274	DOWN
mmu-miR-467b-5p	35	15	0.433333333	0.160333333	-2.110395663	DOWN
novel_mir666	0	31	0.001	0.314	5.066193068	UP
novel_mir435	16	0	0.170666667	0.001	-5.888003242	DOWN
novel_mir408	16	0	0.170666667	0.001	-5.888003242	DOWN
novel_mir731	0	30	0.001	0.287333333	5.018887354	UP
novel_mir715	0	30	0.001	0.287333333	5.018887354	UP
novel_mir782	0	30	0.001	0.287333333	5.018887354	UP
novel_mir99	0	30	0.001	0.287333333	5.018887354	UP
novel_mir568	0	30	0.001	0.287333333	5.018887354	UP
mmu-miR-379-3p	59	42	0.733333333	0.433333333	-1.378328869	DOWN
mmu-miR-34b-3p	19	98	0.226666667	0.993333333	1.478779089	UP
novel_mir421	0	29	0.001	0.277333333	4.969977753	UP
novel_mir95	0	29	0.001	0.297	4.969977753	UP
novel_mir629	0	29	0.001	0.277333333	4.969977753	UP
novel_mir230	0	29	0.001	0.277333333	4.969977753	UP
novel_mir188	0	29	0.001	0.277333333	4.969977753	UP
novel_mir477	0	29	0.001	0.324	4.969977753	UP
novel_mir425	0	29	0.001	0.277333333	4.969977753	UP
mmu-miR-129-2-3	41	160	0.496666667	1.623333333	1.076372848	UP
novel_mir612	15	0	0.160666667	0.001	-5.794893838	DOWN
novel_mir719	15	0	0.160666667	0.001	-5.794893838	DOWN
novel_mir301	15	0	0.160666667	0.001	-5.794893838	DOWN
novel_mir337	15	0	0.160666667	0.001	-5.794893838	DOWN
novel_mir78	0	28	0.001	0.277	4.91935168	UP
novel_mir165	0	28	0.001	0.267333333	4.91935168	UP
novel_mir398	0	28	0.001	0.314	4.91935168	UP
novel_mir671	0	28	0.001	0.267333333	4.91935168	UP
novel_mir610	0	28	0.001	0.267333333	4.91935168	UP
novel_mir335	0	27	0.001	0.257333333	4.86688426	UP
novel_mir493	0	27	0.001	0.263666667	4.86688426	UP
novel_mir490	0	27	0.001	0.257333333	4.86688426	UP
novel_mir222	0	27	0.001	0.257333333	4.86688426	UP
novel_mir123	0	27	0.001	0.257333333	4.86688426	UP
novel_mir613	0	27	0.001	0.257333333	4.86688426	UP
novel_mir563	0	27	0.001	0.257333333	4.86688426	UP
novel_mir376	0	27	0.001	0.257333333	4.86688426	UP

novel_mir218	14	79	0.187333333	0.860333333	1.608422584	UP
novel_mir659	38	21	0.404	0.200666667	-1.743613333	DOWN
novel_mir777	14	0	0.150666667	0.001	-5.695358164	DOWN
novel_mir298	14	0	0.150666667	0.001	-5.695358164	DOWN
novel_mir739	14	0	0.150666667	0.001	-5.695358164	DOWN
novel_mir803	14	0	0.150666667	0.001	-5.695358164	DOWN
novel_mir254	14	0	0.150666667	0.001	-5.695358164	DOWN
novel_mir792	14	0	0.150666667	0.001	-5.695358164	DOWN
novel_mir546	14	0	0.150666667	0.001	-5.695358164	DOWN
novel_mir768	55	40	0.587333333	0.380666667	-1.347434861	DOWN
novel_mir323	0	26	0.001	0.247333333	4.812436476	UP
novel_mir26	0	26	0.001	0.247333333	4.812436476	UP
novel_mir429	0	26	0.001	0.247333333	4.812436476	UP
novel_mir728	0	26	0.001	0.247333333	4.812436476	UP
novel_mir223	0	26	0.001	0.290666667	4.812436476	UP
novel_mir443	0	26	0.001	0.267	4.812436476	UP
novel_mir565	15	80	0.160666667	0.853666667	1.527034257	UP
novel_mir159	0	25	0.001	0.240666667	4.755852948	UP
novel_mir307	0	25	0.001	0.240666667	4.755852948	UP
novel_mir79	0	25	0.001	0.240666667	4.755852948	UP
novel_mir705	0	25	0.001	0.240666667	4.755852948	UP
novel_mir91	0	25	0.001	0.240666667	4.755852948	UP
novel_mir810	13	0	0.140666667	0.001	-5.58844296	DOWN
novel_mir205	13	0	0.140666667	0.001	-5.58844296	DOWN
novel_mir801	13	0	0.140666667	0.001	-5.58844296	DOWN
novel_mir168	26	11	0.277333333	0.124	-2.129011341	DOWN
novel_mir303	0	24	0.001	0.230666667	4.696959259	UP
novel_mir110	0	24	0.001	0.230666667	4.696959259	UP
novel_mir776	0	24	0.001	0.230666667	4.696959259	UP
mmu-miR-125b-1-	70	62	0.873333333	0.636666667	-1.063089949	DOWN
novel_mir488	12	0	0.127333333	0.001	-5.472965743	DOWN
novel_mir315	12	0	0.127333333	0.001	-5.472965743	DOWN
mmu-miR-3058-3p	12	0	0.147	0.001	-5.472965743	DOWN
novel_mir140	12	0	0.127333333	0.001	-5.472965743	DOWN
novel_mir194	12	0	0.127333333	0.001	-5.472965743	DOWN
novel_mir219	0	23	0.001	0.220666667	4.635558714	UP
novel_mir699	0	23	0.001	0.220666667	4.635558714	UP
novel_mir385	0	23	0.001	0.220666667	4.635558714	UP

novel_mir115	0	22	0.001	0.210666667	4.571428377	UP
novel_mir64	0	22	0.001	0.210666667	4.571428377	UP
novel_mir150	0	22	0.001	0.210666667	4.571428377	UP
novel_mir507	0	22	0.001	0.210666667	4.571428377	UP
novel_mir76	0	22	0.001	0.210666667	4.571428377	UP
novel_mir781	0	22	0.001	0.210666667	4.571428377	UP
novel_mir245	11	0	0.147333333	0.001	-5.347434861	DOWN
novel_mir305	11	0	0.117333333	0.001	-5.347434861	DOWN
novel_mir324	11	0	0.117333333	0.001	-5.347434861	DOWN
novel_mir328	11	0	0.117333333	0.001	-5.347434861	DOWN
novel_mir127	11	0	0.117333333	0.001	-5.347434861	DOWN
novel_mir238	11	0	0.117333333	0.001	-5.347434861	DOWN
novel_mir344	11	0	0.117333333	0.001	-5.347434861	DOWN
mmu-miR-154-3p	6	45	0.07	0.466666667	2.018887354	UP
mmu-miR-190a-3p	15	74	0.173666667	0.74	1.414559528	UP
novel_mir628	0	21	0.001	0.200666667	4.504314181	UP
novel_mir248	0	21	0.001	0.200666667	4.504314181	UP
novel_mir84	0	21	0.001	0.214	4.504314181	UP
novel_mir797	0	21	0.001	0.200666667	4.504314181	UP
novel_mir73	0	21	0.001	0.200666667	4.504314181	UP
novel_mir417	0	21	0.001	0.200666667	4.504314181	UP
novel_mir57	0	21	0.001	0.200666667	4.504314181	UP
novel_mir724	0	21	0.001	0.200666667	4.504314181	UP
mmu-miR-297b-3p	0	20	0.001	0.203333333	4.433924853	UP
novel_mir300	0	20	0.001	0.190666667	4.433924853	UP
novel_mir104	0	20	0.001	0.190666667	4.433924853	UP
novel_mir701	0	20	0.001	0.190666667	4.433924853	UP
novel_mir686	0	20	0.001	0.190666667	4.433924853	UP
mmu-miR-5114	13	66	0.150333333	0.663333333	1.455951159	UP
mmu-miR-182-3p	31	19	0.37	0.193333333	-1.594272039	DOWN
novel_mir397	10	0	0.107333333	0.001	-5.209931337	DOWN
novel_mir276	10	0	0.107333333	0.001	-5.209931337	DOWN
novel_mir737	10	0	0.107333333	0.001	-5.209931337	DOWN
novel_mir395	10	0	0.107333333	0.001	-5.209931337	DOWN
novel_mir279	10	0	0.107333333	0.001	-5.209931337	DOWN
novel_mir553	10	0	0.107333333	0.001	-5.209931337	DOWN
novel_mir733	10	0	0.107333333	0.001	-5.209931337	DOWN
novel_mir263	10	0	0.107333333	0.001	-5.209931337	DOWN

novel_mir718	23	11	0.244	0.104	-1.952133579 DOWN
novel_mir590	10	56	0.107333333	0.587	1.597423585 UP
novel_mir201	0	19	0.001	0.180666667	4.359924271 UP
novel_mir538	0	19	0.001	0.180666667	4.359924271 UP
novel_mir651	0	19	0.001	0.180666667	4.359924271 UP
novel_mir780	0	19	0.001	0.180666667	4.359924271 UP
novel_mir195	0	19	0.001	0.180666667	4.359924271 UP
novel_mir518	0	19	0.001	0.180666667	4.359924271 UP
novel_mir710	0	19	0.001	0.180666667	4.359924271 UP
novel_mir431	0	19	0.001	0.180666667	4.359924271 UP
novel_mir632	0	19	0.001	0.180666667	4.359924271 UP
novel_mir430	0	19	0.001	0.210666667	4.359924271 UP
novel_mir642	0	19	0.001	0.180666667	4.359924271 UP
mmu-let-7g-3p	8	48	0.100333333	0.496666667	1.696959259 UP
mmu-miR-770-5p	13	3	0.163333333	0.030333333	-3.003480459 DOWN
mmu-miR-6969-3p	13	3	0.163333333	0.030666667	-3.003480459 DOWN
mmu-miR-299a-5p	10	1	0.123333333	0.010666667	-4.209931337 DOWN
novel_mir181	40	31	0.427333333	0.297333333	-1.255735026 DOWN
novel_mir808	0	18	0.001	0.170666667	4.281921759 UP
novel_mir147	0	18	0.001	0.170666667	4.281921759 UP
novel_mir641	0	18	0.001	0.170666667	4.281921759 UP
novel_mir365	0	18	0.001	0.170666667	4.281921759 UP
novel_mir336	0	18	0.001	0.170666667	4.281921759 UP
mmu-miR-3966	22	88	0.234	0.89	1.111996758 UP
mmu-miR-3105-3p	26	98	0.300333333	0.976666667	1.026266884 UP
mmu-miR-3970	7	43	0.074	0.433333333	1.730906591 UP
novel_mir485	34	25	0.364	0.240666667	-1.331609893 DOWN
novel_mir183	0	17	0.001	0.164	4.199459599 UP
novel_mir470	0	17	0.001	0.164	4.199459599 UP
novel_mir203	0	17	0.001	0.164	4.199459599 UP
novel_mir761	0	17	0.001	0.164	4.199459599 UP
novel_mir812	0	17	0.001	0.164	4.199459599 UP
novel_mir171	0	17	0.001	0.164	4.199459599 UP
novel_mir764	0	17	0.001	0.164	4.199459599 UP
novel_mir244	10	52	0.107333333	0.503666667	1.490508381 UP
novel_mir551	14	63	0.150666667	0.613666667	1.281921759 UP
novel_mir302	0	16	0.001	0.154	4.111996758 UP
novel_mir227	0	16	0.001	0.154	4.111996758 UP

novel_mir592	0	16	0.001	0.177333333	4.111996758	UP
novel_mir255	0	16	0.001	0.154	4.111996758	UP
novel_mir594	0	16	0.001	0.154	4.111996758	UP
novel_mir461	0	16	0.001	0.154	4.111996758	UP
novel_mir402	0	16	0.001	0.154	4.111996758	UP
novel_mir208	0	16	0.001	0.154	4.111996758	UP
novel_mir74	0	16	0.001	0.154	4.111996758	UP
mmu-miR-1968-5p	48	44	0.59	0.443333333	-1.013534124	DOWN
mmu-miR-466k	17	8	0.220333333	0.077	-1.975466083	DOWN
mmu-miR-1306-3p	19	75	0.230333333	0.746666667	1.092887935	UP
novel_mir277	0	15	0.001	0.144	4.018887354	UP
novel_mir633	0	15	0.001	0.144	4.018887354	UP
novel_mir674	0	15	0.001	0.144	4.018887354	UP
novel_mir177	0	15	0.001	0.144	4.018887354	UP
novel_mir547	0	15	0.001	0.144	4.018887354	UP
novel_mir450	0	15	0.001	0.144	4.018887354	UP
mmu-miR-125b-2-	35	29	0.436666667	0.286666667	-1.159305264	DOWN
mmu-miR-341-5p	1	17	0.014	0.176666667	3.199459599	UP
mmu-miR-186-3p	2	21	0.023666667	0.206666667	2.504314181	UP
novel_mir693	0	14	0.001	0.134	3.91935168	UP
novel_mir561	0	14	0.001	0.134	3.91935168	UP
novel_mir160	0	14	0.001	0.134	3.91935168	UP
novel_mir437	0	14	0.001	0.134	3.91935168	UP
novel_mir58	0	14	0.001	0.134	3.91935168	UP
novel_mir322	0	14	0.001	0.134	3.91935168	UP
novel_mir156	0	14	0.001	0.134	3.91935168	UP
novel_mir268	0	14	0.001	0.134	3.91935168	UP
novel_mir320	0	14	0.001	0.134	3.91935168	UP
novel_mir389	0	14	0.001	0.134	3.91935168	UP
novel_mir342	0	14	0.001	0.134	3.91935168	UP
novel_mir120	0	14	0.001	0.134	3.91935168	UP
novel_mir250	0	14	0.001	0.134	3.91935168	UP
novel_mir235	0	14	0.001	0.134	3.91935168	UP
novel_mir616	0	14	0.001	0.134	3.91935168	UP
novel_mir529	35	30	0.417	0.287333333	-1.110395663	DOWN
mmu-miR-701-5p	16	64	0.186666667	0.633333333	1.111996758	UP
novel_mir131	11	50	0.117333333	0.477333333	1.296421329	UP

Supple Table 2: Differential miRNAs between IR and HRS+IR groups

miRNA id	Read count(IR)	Read count(HRS+IR)	Expression(IR)	Expression(HRS+IR)	log2Ratio(HRS+IR/IR)	Up down regulation
novel_mir506	740	10980	8.14	134.21	4.978910269	UP
mmu-miR-5126	6918	17453	71.11	209.0566667	2.42274942	UP
mmu-miR-199a-3p	21854	765	210.5633333	9.16	-3.74859251	DOWN
novel_mir676	757	8338	8.233333333	100.8133333	4.549037463	UP
novel_mir515	394	5926	4.150333333	61.76	4.998492388	UP
novel_mir607	344	5636	3.743333333	69.83333333	5.121892438	UP
novel_mir1	32918	31871	349.0333333	390.8033333	1.041068898	UP
mmu-miR-6538	1784	7216	17.96666667	85.01333333	3.103785019	UP
novel_mir119	1627	6888	16.89666667	87.26333333	3.169572188	UP
novel_mir755	24062	5398	233.72	68.05	-1.068558384	DOWN
novel_mir669	4141	79	39.70666667	1.023333333	-4.624281443	DOWN
novel_mir582	454	2081	4.563666667	24.42	3.284214059	UP
novel_mir5	467	1738	4.93	21.47	2.983634923	UP
novel_mir520	558	1747	5.716666667	21.69	2.734243877	UP
novel_mir508	149	1228	1.546666667	14.66666667	4.130627621	UP
mmu-let-7c-2-3p	4722	700	48.01333333	9.593666667	-1.666269918	DOWN
mmu-miR-342-3p	9281	2178	93.40666667	26.45	-1.00357501	DOWN
novel_mir785	44	586	0.443666667	6.556666667	4.823026532	UP
novel_mir496	289	819	3.12	10.22	2.590495255	UP
mmu-miR-296-5p	1749	1936	18.07666667	23.56666667	1.234249959	UP
mmu-miR-677-5p	2936	520	29.76333333	6.303333333	-1.409567144	DOWN
novel_mir473	28	364	0.300333333	4.276666667	4.788141014	UP
novel_mir259	826	29	7.917	0.397333333	-3.74431568	DOWN
novel_mir15	487	783	5.160333333	10.36333333	1.772791831	UP
novel_mir468	175	471	1.8	5.85	2.516073434	UP
mmu-miR-574-5p	2767	602	27.70666667	7.363333333	-1.112785956	DOWN
novel_mir465	55	256	0.597	3.173333333	3.306341583	UP
novel_mir6	1867	377	18.94666667	4.803333333	-1.220384203	DOWN
mmu-let-7a-1-3p	1004	1017	10.25666667	11.67	1.106261706	UP
novel_mir495	1872	392	19.73	4.516666667	-1.167953579	DOWN
novel_mir297	696	66	6.663666667	0.840333333	-2.31084808	DOWN
novel_mir585	338	504	3.463333333	6.186666667	1.664101783	UP
novel_mir8	494	626	5.066666667	8.046666667	1.429352911	UP
novel_mir491	41	211	0.457333333	2.667	3.45124848	UP
mmu-miR-2137	473	583	4.933333333	6.32	1.389356996	UP
mmu-miR-3470b	268	408	2.676666667	5.023333333	1.694037448	UP

mmu-miR-3470a	289	421	2.896666667	5.14	1.630452037	UP
mmu-miR-532-3p	45	180	0.466666667	2.263333333	3.087701296	UP
novel_mir525	0	173	0.001	2.083333333	9.522329524	UP
novel_mir14	900	148	9.536666667	1.893333333	-1.516626529	DOWN
novel_mir610	28	143	0.267333333	1.857	3.440217711	UP
novel_mir240	96	214	1.003333333	2.543333333	2.244205782	UP
mmu-miR-714	98	212	1.006666667	2.58	2.200911907	UP
mmu-miR-5121	808	142	8.053333333	1.8	-1.420763067	DOWN
novel_mir19	456	49	4.433333333	0.674	-2.130478874	DOWN
novel_mir228	228	0	2.263666667	0.001	-7.745188718	DOWN
novel_mir22	440	51	4.197333333	0.700666667	-2.021233075	DOWN
novel_mir471	283	349	2.95	4.326666667	1.390126279	UP
novel_mir72	57	153	0.6	1.803333333	2.512199125	UP
novel_mir689	199	0	1.897333333	0.001	-7.548923324	DOWN
mmu-miR-338-3p	45	132	0.493333333	1.62	2.640242319	UP
novel_mir656	183	0	1.744	0.001	-7.427998542	DOWN
novel_mir87	362	40	4.010333333	0.523666667	-2.090216496	DOWN
novel_mir448	0	91	0.001	1.090333333	8.595495936	UP
novel_mir657	220	277	2.416666667	3.396666667	1.420083749	UP
novel_mir722	111	183	1.16	2.163333333	1.808985268	UP
novel_mir354	83	157	0.867	1.993333333	2.007282614	UP
novel_mir659	21	90	0.200666667	1.113333333	3.18723697	UP
novel_mir18	136	194	1.4	2.52	1.600151297	UP
novel_mir13	53	118	0.507333333	1.553666667	2.242423891	UP
novel_mir163	11	70	0.124	0.873666667	3.757552694	UP
mmu-miR-3065-5p	423	70	4.243333333	0.88	-1.50752954	DOWN
novel_mir89	0	66	0.001	0.777333333	8.132095415	UP
novel_mir680	46	101	0.483666667	1.33	2.222350823	UP
novel_mir452	100	0	0.954	0.001	-6.556154894	DOWN
novel_mir141	262	259	2.676666667	3.113333333	1.071086582	UP
novel_mir619	96	0	0.930333333	0.001	-6.497261205	DOWN
novel_mir24	338	59	3.25	0.810666667	-1.430535091	DOWN
novel_mir600	94	0	0.897333333	0.001	-6.466887556	DOWN
novel_mir202	0	47	0.001	0.573666667	7.642290148	UP
mmu-miR-6240	501	111	4.993333333	1.306666667	-1.086549631	DOWN
novel_mir729	85	0	0.810666667	0.001	-6.32168964	DOWN
novel_mir766	82	0	0.784	0.001	-6.269850709	DOWN
mmu-miR-18b-5p	217	215	2.193333333	2.493333333	1.074342913	UP

novel_mir38	81	0	0.774	0.001	-6.252148707	DOWN
novel_mir580	80	0	0.773666667	0.001	-6.234226799	DOWN
novel_mir599	80	0	0.777	0.001	-6.234226799	DOWN
mmu-miR-6236	7	44	0.077	0.540333333	3.739777993	UP
novel_mir70	10	46	0.097333333	0.543333333	3.289335157	UP
novel_mir124	0	36	0.001	0.494	7.257626298	UP
novel_mir155	161	19	1.537333333	0.260666667	-1.995288069	DOWN
novel_mir386	126	11	1.200666667	0.130666667	-2.430147009	DOWN
novel_mir802	0	35	0.001	0.387	7.216984313	UP
novel_mir587	69	0	0.667	0.001	-6.020823161	DOWN
novel_mir784	68	0	0.657	0.001	-5.999761545	DOWN
novel_mir783	230	40	2.227	0.550666667	-1.43586066	DOWN
novel_mir511	63	0	0.600666667	0.001	-5.889578627	DOWN
novel_mir551	63	0	0.613666667	0.001	-5.889578627	DOWN
novel_mir588	0	30	0.001	0.410666667	6.994591892	UP
novel_mir126	60	0	0.590333333	0.001	-5.8191893	DOWN
novel_mir571	60	0	0.574	0.001	-5.8191893	DOWN
novel_mir410	60	0	0.574	0.001	-5.8191893	DOWN
novel_mir700	0	29	0.001	0.340666667	6.945682291	UP
novel_mir482	59	0	0.600666667	0.001	-5.794941753	DOWN
novel_mir444	57	0	0.544	0.001	-5.745188718	DOWN
novel_mir278	57	0	0.544	0.001	-5.745188718	DOWN
novel_mir611	12	40	0.114	0.550666667	2.82466689	UP
novel_mir590	56	0	0.587	0.001	-5.719653626	DOWN
novel_mir514	200	35	1.910333333	0.480666667	-1.426871877	DOWN
novel_mir541	54	0	0.546666667	0.001	-5.667186206	DOWN
novel_mir626	54	0	0.514	0.001	-5.667186206	DOWN
novel_mir244	52	0	0.503666667	0.001	-5.612738422	DOWN
novel_mir801	0	25	0.001	0.344	6.731557486	UP
novel_mir405	50	0	0.490333333	0.001	-5.556154894	DOWN
novel_mir190	50	0	0.477333333	0.001	-5.556154894	DOWN
novel_mir53	49	0	0.467333333	0.001	-5.527008548	DOWN
mmu-miR-351-5p	282	64	2.866666667	0.766666667	-1.051850056	DOWN
novel_mir359	0	23	0.001	0.317333333	6.611263252	UP
novel_mir114	48	0	0.457333333	0.001	-5.497261205	DOWN
novel_mir39	121	16	1.154	0.220666667	-1.831161941	DOWN
novel_mir180	134	20	1.283666667	0.274	-1.656459799	DOWN
novel_mir59	46	0	0.440666667	0.001	-5.43586066	DOWN

novel_mir576	45	0	0.480333333	0.001	-5.4041518	DOWN
novel_mir213	106	109	1.046666667	1.497333333	1.127965166	UP
novel_mir527	0	21	0.001	0.287333333	6.480018719	UP
novel_mir182	44	0	0.420666667	0.001	-5.371730323	DOWN
mmu-miR-3084-3p	18	40	0.18	0.476666667	2.23970439	UP
novel_mir267	43	0	0.410666667	0.001	-5.338563459	DOWN
novel_mir441	43	0	0.410666667	0.001	-5.338563459	DOWN
novel_mir258	43	0	0.410666667	0.001	-5.338563459	DOWN
mmu-miR-322-3p	235	52	2.363333333	0.636666667	-1.088375932	DOWN
novel_mir570	0	20	0.001	0.237333333	6.409629391	UP
novel_mir337	0	20	0.001	0.253666667	6.409629391	UP
novel_mir34	150	26	1.430666667	0.357333333	-1.440677676	DOWN
novel_mir149	42	0	0.400666667	0.001	-5.304616127	DOWN
novel_mir231	49	65	0.477	0.870333333	1.495359265	UP
novel_mir504	41	0	0.390666667	0.001	-5.269850709	DOWN
novel_mir138	0	19	0.001	0.260666667	6.33562881	UP
novel_mir768	40	0	0.380666667	0.001	-5.234226799	DOWN
novel_mir523	0	18	0.001	0.247333333	6.257626298	UP
novel_mir770	38	0	0.364	0.001	-5.160226217	DOWN
novel_mir321	38	0	0.364	0.001	-5.160226217	DOWN
novel_mir117	37	0	0.354	0.001	-5.12175207	DOWN
novel_mir608	37	0	0.367	0.001	-5.12175207	DOWN
mmu-miR-17-3p	221	51	2.223333333	0.61	-1.027775921	DOWN
novel_mir645	0	17	0.001	0.234	6.175164137	UP
novel_mir746	0	17	0.001	0.234	6.175164137	UP
novel_mir579	117	19	1.117333333	0.260666667	-1.53473591	DOWN
mmu-miR-669c-5p	144	27	1.463333333	0.340333333	-1.327336203	DOWN
mmu-miR-331-3p	34	49	0.37	0.613333333	1.614948299	UP
novel_mir252	35	0	0.360333333	0.001	-5.041581721	DOWN
novel_mir101	35	0	0.334	0.001	-5.041581721	DOWN
novel_mir652	35	0	0.334	0.001	-5.041581721	DOWN
novel_mir557	35	0	0.356666667	0.001	-5.041581721	DOWN
novel_mir403	35	0	0.334	0.001	-5.041581721	DOWN
novel_mir457	0	16	0.001	0.187333333	6.087701296	UP
novel_mir100	0	16	0.001	0.220666667	6.087701296	UP
novel_mir366	0	16	0.001	0.220666667	6.087701296	UP
novel_mir130	0	16	0.001	0.220666667	6.087701296	UP
novel_mir400	34	0	0.324	0.001	-4.999761545	DOWN

novel_mir148	34	0	0.324	0.001	-4.999761545	DOWN
novel_mir49	34	0	0.324	0.001	-4.999761545	DOWN
novel_mir218	79	10	0.860333333	0.137333333	-1.894151357	DOWN
mmu-miR-3068-3p	6	22	0.063333333	0.26	2.962170414	UP
novel_mir198	33	0	0.314	0.001	-4.956692823	DOWN
novel_mir60	33	0	0.314	0.001	-4.956692823	DOWN
novel_mir61	33	0	0.314	0.001	-4.956692823	DOWN
novel_mir346	33	0	0.314	0.001	-4.956692823	DOWN
novel_mir562	33	0	0.314	0.001	-4.956692823	DOWN
novel_mir107	0	15	0.001	0.207333333	5.994591892	UP
novel_mir666	31	0	0.314	0.001	-4.866495014	DOWN
novel_mir476	31	0	0.297333333	0.001	-4.866495014	DOWN
novel_mir52	31	0	0.297333333	0.001	-4.866495014	DOWN
mmu-miR-23b-5p	182	42	1.803333333	0.53	-1.027775921	DOWN
novel_mir762	0	14	0.001	0.194	5.895056218	UP
novel_mir113	0	14	0.001	0.194	5.895056218	UP
novel_mir800	0	14	0.001	0.194	5.895056218	UP
novel_mir731	30	0	0.287333333	0.001	-4.8191893	DOWN
novel_mir715	30	0	0.287333333	0.001	-4.8191893	DOWN
novel_mir782	30	0	0.287333333	0.001	-4.8191893	DOWN
novel_mir568	30	0	0.287333333	0.001	-4.8191893	DOWN
novel_mir167	86	13	0.833666667	0.177333333	-1.63812374	DOWN
novel_mir421	29	0	0.277333333	0.001	-4.770279699	DOWN
novel_mir95	29	0	0.297	0.001	-4.770279699	DOWN
novel_mir629	29	0	0.277333333	0.001	-4.770279699	DOWN
novel_mir230	29	0	0.277333333	0.001	-4.770279699	DOWN
novel_mir188	29	0	0.277333333	0.001	-4.770279699	DOWN
novel_mir477	29	0	0.324	0.001	-4.770279699	DOWN
novel_mir425	29	0	0.277333333	0.001	-4.770279699	DOWN
novel_mir170	60	64	0.596666667	0.807	1.1808107	UP
novel_mir542	0	13	0.001	0.177333333	5.788141014	UP
novel_mir153	0	13	0.001	0.177333333	5.788141014	UP
novel_mir733	0	13	0.001	0.177333333	5.788141014	UP
novel_mir77	0	13	0.001	0.154	5.788141014	UP
novel_mir251	0	13	0.001	0.154	5.788141014	UP
novel_mir165	28	0	0.267333333	0.001	-4.719653626	DOWN
novel_mir398	28	0	0.314	0.001	-4.719653626	DOWN
novel_mir672	28	0	0.267333333	0.001	-4.719653626	DOWN

novel_mir671	28	0	0.267333333	0.001	-4.719653626	DOWN
novel_mir544	27	0	0.257333333	0.001	-4.667186206	DOWN
novel_mir335	27	0	0.257333333	0.001	-4.667186206	DOWN
novel_mir493	27	0	0.263666667	0.001	-4.667186206	DOWN
novel_mir490	27	0	0.257333333	0.001	-4.667186206	DOWN
novel_mir222	27	0	0.257333333	0.001	-4.667186206	DOWN
novel_mir123	27	0	0.257333333	0.001	-4.667186206	DOWN
novel_mir613	27	0	0.257333333	0.001	-4.667186206	DOWN
novel_mir563	27	0	0.257333333	0.001	-4.667186206	DOWN
novel_mir376	27	0	0.257333333	0.001	-4.667186206	DOWN
novel_mir419	0	12	0.001	0.164	5.672663797	UP
novel_mir566	0	12	0.001	0.164	5.672663797	UP
novel_mir581	0	12	0.001	0.164	5.672663797	UP
novel_mir618	0	12	0.001	0.164	5.672663797	UP
novel_mir685	0	12	0.001	0.164	5.672663797	UP
novel_mir212	0	12	0.001	0.164	5.672663797	UP
novel_mir732	0	12	0.001	0.140666667	5.672663797	UP
novel_mir573	0	12	0.001	0.164	5.672663797	UP
novel_mir162	0	12	0.001	0.164	5.672663797	UP
novel_mir772	0	12	0.001	0.164	5.672663797	UP
novel_mir344	0	12	0.001	0.140666667	5.672663797	UP
novel_mir323	26	0	0.247333333	0.001	-4.612738422	DOWN
novel_mir429	26	0	0.247333333	0.001	-4.612738422	DOWN
novel_mir728	26	0	0.247333333	0.001	-4.612738422	DOWN
novel_mir443	26	0	0.267	0.001	-4.612738422	DOWN
novel_mir237	76	12	0.753333333	0.164	-1.575263717	DOWN
novel_mir159	25	0	0.240666667	0.001	-4.556154894	DOWN
novel_mir485	25	0	0.240666667	0.001	-4.556154894	DOWN
novel_mir79	25	0	0.240666667	0.001	-4.556154894	DOWN
novel_mir705	25	0	0.240666667	0.001	-4.556154894	DOWN
novel_mir91	25	0	0.240666667	0.001	-4.556154894	DOWN
novel_mir754	0	11	0.001	0.150666667	5.547132915	UP
novel_mir454	0	11	0.001	0.150666667	5.547132915	UP
novel_mir614	0	11	0.001	0.130666667	5.547132915	UP
novel_mir392	0	11	0.001	0.130666667	5.547132915	UP
novel_mir727	0	11	0.001	0.150666667	5.547132915	UP
novel_mir795	16	27	0.154	0.370666667	1.842588798	UP
novel_mir303	24	0	0.230666667	0.001	-4.497261205	DOWN

novel_mir110	24	0	0.230666667	0.001	-4.497261205	DOWN
novel_mir776	24	0	0.230666667	0.001	-4.497261205	DOWN
mmu-miR-3071-5p	15	26	0.157	0.31	1.881250419	UP
novel_mir574	65	63	0.647	0.864	1.042613407	UP
novel_mir99	30	38	0.287333333	0.520666667	1.428738214	UP
novel_mir638	73	12	0.737	0.140666667	-1.517160762	DOWN
novel_mir219	23	0	0.220666667	0.001	-4.43586066	DOWN
novel_mir699	23	0	0.220666667	0.001	-4.43586066	DOWN
novel_mir385	23	0	0.220666667	0.001	-4.43586066	DOWN
novel_mir549	0	10	0.001	0.117333333	5.409629391	UP
novel_mir233	0	10	0.001	0.117333333	5.409629391	UP
novel_mir445	0	10	0.001	0.117333333	5.409629391	UP
novel_mir30	0	10	0.001	0.137333333	5.409629391	UP
novel_mir753	0	10	0.001	0.137333333	5.409629391	UP
novel_mir439	0	10	0.001	0.137333333	5.409629391	UP
novel_mir735	0	10	0.001	0.137333333	5.409629391	UP
novel_mir94	0	10	0.001	0.137333333	5.409629391	UP
novel_mir115	22	0	0.210666667	0.001	-4.371730323	DOWN
novel_mir64	22	0	0.210666667	0.001	-4.371730323	DOWN
novel_mir150	22	0	0.210666667	0.001	-4.371730323	DOWN
novel_mir507	22	0	0.210666667	0.001	-4.371730323	DOWN
novel_mir76	22	0	0.210666667	0.001	-4.371730323	DOWN
novel_mir781	22	0	0.210666667	0.001	-4.371730323	DOWN
novel_mir78	28	35	0.277	0.437	1.409629391	UP
novel_mir628	21	0	0.200666667	0.001	-4.304616127	DOWN
novel_mir248	21	0	0.200666667	0.001	-4.304616127	DOWN
novel_mir84	21	0	0.214	0.001	-4.304616127	DOWN
novel_mir797	21	0	0.200666667	0.001	-4.304616127	DOWN
novel_mir73	21	0	0.200666667	0.001	-4.304616127	DOWN
novel_mir417	21	0	0.200666667	0.001	-4.304616127	DOWN
novel_mir57	21	0	0.200666667	0.001	-4.304616127	DOWN
novel_mir528	21	0	0.200666667	0.001	-4.304616127	DOWN
mmu-miR-935	111	25	1.1	0.316666667	-1.06285838	DOWN
novel_mir46	47	48	0.447333333	0.620333333	1.118074945	UP
novel_mir35	101	22	0.977	0.304	-1.111078568	DOWN
novel_mir505	113	26	1.090333333	0.330333333	-1.032037948	DOWN
novel_mir102	61	10	0.593666667	0.137333333	-1.521107947	DOWN
novel_mir467	20	0	0.190666667	0.001	-4.234226799	DOWN

novel_mir54	20	0	0.207	0.001	-4.234226799	DOWN
novel_mir261	20	0	0.190666667	0.001	-4.234226799	DOWN
novel_mir104	20	0	0.190666667	0.001	-4.234226799	DOWN
novel_mir176	20	0	0.190666667	0.001	-4.234226799	DOWN
novel_mir701	20	0	0.190666667	0.001	-4.234226799	DOWN
novel_mir686	20	0	0.190666667	0.001	-4.234226799	DOWN
novel_mir201	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir538	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir651	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir780	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir195	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir518	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir710	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir431	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir632	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir430	19	0	0.210666667	0.001	-4.160226217	DOWN
novel_mir642	19	0	0.180666667	0.001	-4.160226217	DOWN
novel_mir409	14	22	0.134	0.304	1.739777993	UP
mmu-miR-34b-3p	98	22	0.993333333	0.273333333	-1.067576929	DOWN
mmu-miR-3473d	58	10	0.573333333	0.126666667	-1.448351604	DOWN
novel_mir641	18	0	0.170666667	0.001	-4.082223705	DOWN
novel_mir365	18	0	0.170666667	0.001	-4.082223705	DOWN
novel_mir336	18	0	0.170666667	0.001	-4.082223705	DOWN
mmu-miR-669a-3p	8	16	0.090666667	0.196666667	2.087701296	UP
mmu-miR-190a-3p	74	15	0.74	0.183333333	-1.214861474	DOWN
mmu-miR-877-5p	39	5	0.396666667	0.06	-1.875772828	DOWN
novel_mir805	28	32	0.267333333	0.440666667	1.280346374	UP
novel_mir223	26	30	0.290666667	0.410666667	1.294152174	UP
novel_mir183	17	0	0.164	0.001	-3.999761545	DOWN
novel_mir203	17	0	0.164	0.001	-3.999761545	DOWN
novel_mir761	17	0	0.164	0.001	-3.999761545	DOWN
novel_mir812	17	0	0.164	0.001	-3.999761545	DOWN
novel_mir171	17	0	0.164	0.001	-3.999761545	DOWN
novel_mir283	17	0	0.164	0.001	-3.999761545	DOWN
novel_mir798	17	0	0.164	0.001	-3.999761545	DOWN
novel_mir764	17	0	0.164	0.001	-3.999761545	DOWN
novel_mir179	75	16	0.717333333	0.187333333	-1.141117394	DOWN