Changes in the expression of miRNAs at the pericentral and periportal regions of the rat liver in response to hepatocellular injury: comparison with the changes in the expression of plasma miRNAs

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Changes in the expression of miRNAs at the pericentral and periportal regions of the rat liver in response to hepatocellular injury: comparison with the changes in the expression of plasma miRNAs

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Abstract

MicroRNAs (miRNAs) in body fluids have received attention as potential biomarkers of organ damage because miRNAs that are highly or specifically expressed in a given organ are likely released into body fluids as a result of damage to that organ. We previously determined that the plasma miRNA profile in rats was dramatically changed due to acetaminophen (APAP)-induced pericentral necrosis and methapyrilene (MP)-induced periportal necrosis in the liver. The purpose of this study was to examine whether the expression of hepatic miRNAs is differentially modulated at different zones due to injury and to examine the relationship of the hepatic miRNA profile with the changes in the plasma miRNA expression profile. Through the laser microdissection of the periportal and periportal regions of the liver and TaqMan microRNA Array analysis, we found that 49 miRNAs are differentially expressed between the pericentral and periportal regions of control rats. In both APAP- and MP-treated rats, the miRNAs that presented decreased expression dominated in both the injured and non-injured areas compared with the miRNAs that exhibited increased expression. The changes in miRNA expression in each region of the liver were compared with those observed in the plasma. Of the 301 plasma miRNAs with expression that was changed as a result of APAP administration, only 21% were changed in the injured area of the liver. Of the 263 plasma miRNAs with expression that was changed due to MP administration, only 24% were changed in the injured area of the liver. Thus, the miRNA expression profiles in the plasma do not merely reflect the release of miRNAs from the damaged cells in the liver. This report provides the first demonstration of zonal miRNA expression in the liver and of the relationship of the miRNA expression profile in a tissue with the plasma miRNA profile.

Keywords

microRNA; liver injury; zonal expression; biomarker

Introduction

miRNAs are small non-coding RNAs that control gene expression through translational repression or mRNA degradation. miRNAs regulate various biological processes, such as lipid metabolism (Esau et al., 2006), apoptosis (Cimmino et al., 2005), and carcinogenesis (Lu et al., 2005). To date, more than 2500, 1900, and 700 miRNAs have been identified in human, mouse, and rat, respectively. miRNA expression varies from highly specific to ubiquitous. It has been demonstrated that the aberrant expression of miRNAs is associated with a variety of diseases, including cancer (Dillhoff et al., 2009), viral hepatitis (Ura et al., 2009), and heart disease (Ikeda et al., 2007). In 2008, it was reported that miRNAs stably exist in plasma (Chim et al., 2008, Mitchell et al., 2008) and serum (Lawrie et al., 2008, Mitchell et al., 2008). Subsequent studies reported the presence of miRNAs in various body fluids, including saliva (Park et al., 2009), urine (Hanke et al., 2010), breast milk (Kosaka et al., 2010), and tears (Weber et al., 2010). The miRNAs in body fluids, which may reflect the changes in the miRNA expression levels in tissues, have received considerable attention as biomarkers of disease (Cortez and Calin, 2009).

We previously investigated the plasma miRNA profile in rats with acute liver injury as a result of a single administration of APAP or MP (Yamaura et al., 2012) and demonstrated that approximately 300 plasma miRNAs are altered due to APAP- or MP-induced liver injury. Most of the altered miRNAs (> 85%) were common to the two models, and the remaining miRNAs were specifically changed in one of the models. APAP and MP induce hepatocellular injury at the pericentral and periportal regions, respectively. It would be reasonable to speculate that the specifically altered miRNAs in the plasma may reflect the change in the miRNA expression profile at the pericentral and periportal regions that exhibit hepatocellular necrosis. Although a previous study using a mouse model with liver injury caused by the administration of APAP reported that the miRNAs that exhibited an increased expression level in the plasma were decreased in the liver (Wang et al., 2009), the relationship of the changes in miRNA expression between plasma and tissues remains to be fully elucidated. In the

present study, we examined the miRNA expression profiles at the pericentral and periportal regions of the liver in two rat models of liver injury to clarify whether the miRNAs in the liver are uniformly or variably expressed through the region and to understand the relationship between the plasma and tissue miRNA expression profiles.

1. Materials and methods

1.1. Chemicals and reagents

APAP and MP were purchased from Wako Pure Chemicals (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO), respectively. The RNAqueous Kit, *mir*Vana PARIS Kit, RNAqueous Kit, Megaplex Pools, TaqMan MicroRNA Reverse Transcription Kit, TaqMan MicroRNA Assays, TaqMan 2x Universal PCR Master Mix (No AmpErase UNG), and TaqMan Rodent MicroRNA Array v2.0 were obtained from Applied Biosystems (Foster City, CA). All of the other chemicals and solvents were of the highest grade commercially available.

1.2. Animal models

The animal maintenance and treatments were conducted in accordance with the National Institutes of Health Guide for Animal Welfare of Japan and were approved by the Institutional Animal Care and Use Committee of Kanazawa University, Japan. The study was approved by the Animal Ethics Committee of Kanazawa University (No. 31203). Male five-week-old Sprague-Dawley rats were purchased from Japan SLC (Hamamatsu, Japan). The rats were housed in a controlled environment (temperature 25 ± 1°C, humidity 50 ± 10%, and 12-h light/12-h dark cycle) in the institutional animal facility with access to food and water *ad libitum*. Each set of six rats was orally administered 1,000 mg/kg APAP suspended in 0.5% carboxymethylcellulose (CMC) after fasting for 48 h, 300 mg/kg MP dissolved in 0.5% CMC, or vehicle. Twenty-four hours after administration, their blood and liver were collected. Using EDTA as an anticoagulant, the plasma was separated by centrifugation and maintained at -80°C until use. Part of the liver was fixed in buffered neutral 10% formalin, and the

remaining part was stored at -80°C.

1.3. Biochemical assay and pathological examination

The plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (T-Bil) levels were determined using Dri-Chem 4000 (FUJIFILM Saitama, Japan) according to the manufacturer's instructions. The formalin-fixed samples were embedded in paraffin, sectioned, and then stained with hematoxylin and eosin (Wako Pure Chemicals, Osaka, Japan) for microscopic examination.

1.4. Laser microdissection and RNA isolation

Part of the frozen livers were embedded in O.C.T. compound (Sakura Finetek Japan, Tokyo, Japan), cut into 20-μm-thick sections, and stained with 10% hematoxylin and eosin. The pericentral and periportal regions of the livers from rats administered APAP, MP, or vehicle were collected by laser microdissection using an Application Solutions Laser Microdissection System (Leica Microsystems, Wetzler, Germany). The total RNA from the microdissected sections was extracted using the RNAqueous Kit according to the manufacturer's protocol, and the total RNA from the same group was pooled.

1.5. TaqMan microRNA array analysis

The cDNAs were synthesized from the pooled total RNA using Megaplex Pools according to the manufacturer's protocol. Preamplification was performed by the addition of 2x TaqMan PreAmp Master Mix and 10x Megaplex PreAmp Primers to the cDNA sample. TaqMan 2x Universal PCR Master Mix (No AmpErase UNG) was then added to the preamplification products. The mixture was applied to individual ports of TaqMan Array Rodent MicroRNA A+B Cards Sets v2.0 384-well microfluidics cards containing 375 (A array) or 210 (B array) primer-probe sets for individual miRNAs. Quantitative real-time PCR was performed using a 7900HT Fast Real-Time PCR system (Applied Biosystems) with the SDS software v.2.4. The expression levels were evaluated using the comparative cycle

threshold (Ct) method. The Ct values ranged from 0 to 40. The miRNAs that gave Ct values greater than 32 in all of the groups were omitted from the data analysis because this 32 is the cut-off value recommended by the manufacturer. The data are presented as (40 - Ct). The ΔCt values [ΔCt = (40 - Ct control) - (40 - Ct model)] were calculated as fold changes. The miRNAs showing expression changes of at least two-fold were analyzed. A heat map was generated with Microsoft Excel 2010.

1.6. Statistical analysis

The data are expressed as the means ± SD. The comparisons of two groups were made using Mann-Whitney's U-test. A value of $P < 0.05$ was considered statistically significant.

2. Results

2.1. ALT, AST, and histopathology of the liver

After the administration of APAP and MP, the plasma ALT and AST levels were significantly elevated (Fig. 1A). The histopathological analysis revealed that APAP caused hepatocellular necrosis at the pericentral regions, whereas MP caused hepatocellular necrosis at the periportal region (Fig. 1B). These two models were used for the subsequent studies.

2.2. miRNA expression at the pericentral and periportal regions of the liver

The miRNA expression at pericentral and periportal regions of the liver was determined by TaqMan microRNA array analysis. The numbers of the detected miRNAs and the miRNAs whose expression exceeded the cutoff (Ct < 32) are shown in Table 1. No large difference was observed in these numbers between the pericentral and periportal regions within a group or between the treated and control groups. Among the miRNAs with Ct values less than 32 in all of the groups, 125 miRNAs were common to all of the groups; therefore, these 125 miRNAs were used for the subsequent analyses. Fig. 2 shows the heat maps of the expression of the 125 miRNAs in each sample; note that the miRNAs are ordered based on descending expression level in the pericentral region of the control rats. The top three miRNAs that

exhibited high expression in both the pericentral and periportal regions in all of the samples are miR-709, miR-122, and miR-720. The comparison of the miRNA levels between the control rats and fasted-control rats revealed that 36 miRNAs presented higher levels and eight miRNAs exhibited lower levels at the pericentral region of the fasted-control rats (Tables 1 and 2). At the periportal region, 52 miRNAs and two miRNAs presented higher and lower levels, respectively, in the fasted-control rats compared with the control rats. Among these miRNAs, 17 miRNAs, which are shown in bold, were found in both the pericentral and periportal regions. Thus, the results demonstrate that fasting affects the expression of some miRNAs in the liver. The comparison of the miRNA levels between the pericentral and periportal regions in control rats showed that 27 miRNAs exhibited higher expression levels in the pericentral region and that 22 miRNAs presented higher expression levels in the periportal region compared with the other region (Tables 1 and 3). This study provides the first demonstration that some miRNAs are differentially expressed at the periportal and periportal regions.

After the administration of APAP, the levels of 18 miRNAs were increased and the levels of 27 miRNAs were decreased at the pericentral region (Table 1). At the periportal region, seven miRNAs and 21 miRNAs were increased and decreased, respectively. As a result of the administration of MP, 10 miRNAs presented increased expression and 62 miRNAs exhibited decreased expression at the pericentral region. At the periportal region, four miRNAs and 56 miRNAs were increased and decreased, respectively. Thus, although the miRNAs that exhibited decreased expression levels were dominant, particularly in the MP-treated rats, there were certainly a number of miRNAs that presented increased expression. These miRNAs were further classified based on the fluctuation in their expression at another hepatic region (Table 4). Table 4 includes the numbers of the unchanged miRNAs as well as the altered 125 miRNAs. Of the 18 and 27 miRNAs that exhibited increased and decreased expression at the pericentral region in APAP-treated rats, respectively, 14 (78%) and 20 (74%) miRNAs were not changed at the periportal region, respectively. Thus, most of the altered miRNAs at the pericentral region, where the hepatocytes were injured by APAP,

were not affected at the periportal region. It is interesting that four (57%) and 13 (62%) miRNAs were specifically changed at the periportal region, where hepatocytes were not injured. In MP-treated rats, of the four and 56 miRNAs that exhibited increased and decreased expression at the periportal region, respectively, three miRNAs (75%) and 42 miRNAs (75%) also presented changed expression levels at the pericentral region. Thus, the results demonstrate that a large number of miRNAs were decreased at both the periportal and pericentral regions.

2.3. Comparison of the miRNA expression changes in the liver and plasma

We then sought to examine the relationship between the miRNA expression changes in the liver and those in the plasma because we previously reported the plasma miRNA profiles in these model rats (Yamaura et al., 2012). The miRNAs that were classified into nine groups (groups A to I) in Table 4 are specified in Table 5. In the groups that include more than 10 miRNAs, the ten most abundant miRNAs are shown. The changes in the levels of these miRNAs in the plasma are shown with arrows in Table 5. Interestingly, in the APAP-treated rats, 122 of the 125 miRNAs were increased in the plasma, regardless of the changes observed in the liver. The remaining three miRNAs were not changed in the plasma. In the MP-treated rats, 117 of 125 miRNAs were increased in the plasma, six miRNAs were unchanged, and two miRNAs were decreased in the plasma. The fold changes of these miRNAs in the liver (pericentral and periportal regions) and plasma are shown in Figs. 3 and 4. In the groups with more than five miRNAs, the five most abundant miRNAs are shown. Reciprocal changes in the miRNAs between the liver and plasma (decrease in the liver and increase in plasma) were observed in some cases, but this relationship was not general. Of special interest is that the miRNAs that were not affected in the liver at all were markedly increased in the plasma, as shown in group E. Thus, the relationship between the changes in the miRNA levels in the plasma and tissue is not simple.

We then examined the expression changes in the liver of the miRNAs that were changed in the plasma (Fig. 5). We previously reported that 317 and 295 miRNAs are

increased and that six and 10 miRNAs are decreased in the rat plasma as a result of APAP- and MP-induced liver injury, respectively (Yamaura et al., 2012). In our previous study, several miRNAs for which two probes were included on the array were counted individually. Excluding the overlapped miRNAs, the numbers of plasma miRNAs that were changed by APAP and MP administration were recalculated to 301 and 263 miRNAs, respectively. Among the 301 miRNAs that exhibited a change in their expression level in the APAP-treated rats, 48 miRNAs (16%) were changed only at the pericentral region, 14 miRNAs (5%) were changed at both the pericentral and periportal regions, and 27 miRNAs (9%) were changed only at the periportal region. Among the 263 miRNAs that presented a changed expression level in the MP-treated rats, 32 miRNAs (12%) were changed only in the pericentral region, 44 miRNAs (17%) were changed at both the pericentral and periportal region, and 18 miRNAs (7%) were changed only at the periportal region. Collectively, most (~70%) of the miRNAs whose levels were changed in the plasma of rats with liver injury were not changed in the liver.

3. Discussion

In this study, we examined the hepatic miRNA expression in rats with liver injury to evaluate the relationship between the changes in the plasma miRNA profile and to determine whether the miRNA expression is differently modulated at injured and not-injured regions. We also examined whether miRNA expression varies between different zones in the normal liver and whether hepatic miRNA expression is affected by fasting.

In the rat liver, we found that the three most abundant miRNAs are miR-709, miR-122, and miR-720. The high expression of miR-709 and miR-720 was unexpected because it is known that miR-122 constitutes 70% of the total hepatic miRNAs (Lagos-Quintana et al., 2002, Chang et al., 2004). We noticed that miR-720 had been removed from the database (http://www.mirbase.org/index.shtml) because Schopman et al. (2010) reported that the sequence annotated as miR-720 is likely to be a fragment of a tRNA. Although miR-709 remains to be registered, we found that the sequence of miR-709 completely matches with

that of a tRNA, indicating that it may also be misdetection. Supporting this assumption, the abundant miRNAs in the rat liver (other than miR-709 and miR-720), such as miR-122, miR-126-3p, miR-191, and miR-146a, were also found to be highly expressed in human hepatocytes in our previous study (Takahashi et al., 2014).

We found that fasting changes the expression of some miRNAs in the rat liver. Although we did not examine the causes of the changes in this study, the changes in glycolysis, glucogenesis, ketone body metabolism, fatty acid metabolism, and lipid metabolic processes due to fasting would be possible causes. It would be of interest to investigate whether the expression of the target genes of these miRNAs is modulated because it has been reported that fasting changes the expression of various genes in the liver (Zhang et al., 2011); in other words, it would be interesting to determine whether the changes in gene expression are due to direct effects of fasting or indirect effects due to changes in the miRNA expression profile.

We found that 27 and 22 miRNAs of the 125 miRNAs were dominantly expressed at the pericentral and periportal regions, respectively, in the normal rat liver. This finding is not surprising because it is well known that some genes or proteins show zonal expression. For example, drug-metabolizing enzymes are predominantly expressed at the pericentral region (Hailfinger et al., 2006), whereas cadherin and ATP-citrate lyase are predominantly expressed at the periportal region (Braeuning et al., 2006). It has been reported that such zonal expressions are due to differences in transcriptional regulation (Gebhardt, 1992, Jungermann, 1995). Because the expression of miRNAs is regulated at the transcriptional level to a greater or lesser extent, the zonal expression of miRNAs may be due to differences in their transcriptional regulation. Additionally, differences in post-transcriptional regulation, i.e., maturation step or degradation, may also contribute to the zonal expression. It would also be of interest to investigate whether such differences in miRNA expression between regions may cause differences in the expression of the target mRNAs between regions.

We evaluated the changes in miRNA expression in the liver caused by the administration of APAP or MP, which cause hepatocellular injury at the pericentral or

periportal regions, respectively. In APAP-treated rats, 45 and 30 miRNAs were changed at the pericentral or periportal regions, respectively, and in the MP-treated rats, 72 and 60 miRNAs were changed at the pericentral or periportal regions, respectively. It was interesting that miRNA expression was affected at the region where hepatocytes were not injured. Because we previously reported that 301 and 263 miRNAs were changed in the plasma of APAP- and MP-treated rats, respectively (Yamaura et al., 2012), this study demonstrated that the number of miRNAs changed in the liver is lower than that of miRNAs change in the plasma. In both groups, miRNAs that exhibit decreased expression were dominant in the liver compared with the miRNAs that presented increased expression. This finding is reminiscent of the fact that, in the plasma of rats with liver injury, the miRNAs that presented increased expression were dominant compared with the miRNAs that exhibited decreased expression (Yamaura et al., 2012), implying reciprocal changes in miRNAs between the liver and plasma. It is generally believed that miRNAs that are highly expressed in a tissue would be plasma biomarkers of tissue injury. However, an inverse association was observed for some miRNAs, but there were many exceptions (Table 5 and Fig. 5). For example, miR-155 was increased at the pericentral region and in the plasma in APAP-treated rats. miR-155 has been reported to be expressed in immune cells, including B cells (Rodriguez et al., 2007; Thai et al., 2007), T cells (Haasch et al., 2002), and macrophages (O'Connell et al., 2007; Taganov et al., 2006), and to be upregulated as a result of activation by immune stimuli, such as inflammatory cytokines. Thus, the increased level of miR-155 in the liver may be due to the activation of immune cells, and this may be reflected in the increased level of this miRNA observed in the plasma. As shown in Fig. 5, many of the changed miRNAs in the plasma, including miR-150 and miR-223, were not altered in the liver. Recently, Pritchard et al. (2012) reported that the circulating miRNAs are largely obtained from blood cells. It appears that miR-150 is the most abundant miRNA in lymphocytes, and miR-223 is the most abundant miRNA in platelets, neutrophils, eosinophils, and monocytes. Accordingly, the changes in the expression levels of these miRNAs in the plasma may reflect the increase of lymphocytes that migrated to the inflammation area.

In summary, the present study clarified that 1) certain miRNAs in the liver are differentially expressed between the pericentral and periportal regions, 2) fasting affects the expression of a set of miRNAs in the liver, 3) the miRNA expression in the liver is modulated not only in the injured region but also the non-injured region, and 4) a reciprocal relationship between the miRNAs that presented increased levels in the plasma and the miRNAs that exhibit decreased levels in the liver is not necessarily found.

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Figure legends

Fig. 1. Plasma ALT and AST levels and histopathological presentation of liver injury in rat models. (A) Plasma ALT and AST levels in rats after the administration of APAP or MP. (B) Histopathological changes in rats after the administration of APAP or MP. The data are the means ± SD (n = 4 to 8). **Significantly different from the control group ($P < 0.01$). The liver sections were stained with HE. Original magnification, ×200. CV: central vein, P: portal region.

Fig. 2. Heat map of the expression profiles of miRNAs in the pericentral and periportal regions of the liver from APAP- and MP-treated rats. The miRNAs were seriated in descending order of the expression level in the control rats.

Fig. 3. Fold change of the miRNAs whose expressions were upregulated at the CV and P (A), upregulated only at the CV (B), upregulated at the pericentral region and downregulated at the P (C), upregulated only at the P (D), not changed (E), downregulated only at the P (F), downregulated at the CV and upregulated at the P (G), downregulated only at the CV (H), and downregulated at the CV and P (I) in APAP-administered rats.

Fig. 4. Fold change of the miRNAs whose expressions were upregulated at the CV and P (A), upregulated only at the CV (B), upregulated at the pericentral region and downregulated at the P (C), upregulated only at the P (D), not changed (E), downregulated only at the P (F), downregulated at the CV and upregulated at the P (G), downregulated only at the CV (H), and downregulated at the CV and P (I) in MP-administered rats.

Fig. 5. Composition of miRNAs whose expression was changed as a result of hepatocellular injury in the rat plasma.

Table 1. The numbers of miRNAs whose expression was detected or changed (by at least two-fold) in the pericentral and periportal regions of the liver in rats.

Treatment	Region	Detected	Ct < 32	Fasted ctrl > Ctrl	Fasted ctrl < Ctrl	CV > P	CV < P	Treated > Ctrl*	Treated < Ctrl*
Fasted ctrl	CV	284	183	36	8	-	-		
	P	308	188	52	2	-	-		
APAP	CV	315	187	-	-	-	-	18	27
	P	311	187	-	-	-	-	7	21
Ctrl	CV	263	176			27	22		
	P	279	165						
MP	CV	239	144	-	-	-	-	10	62
	P	273	143	-	-	-	-	4	56

Ctrl: control, CV: central vein, P: portal vein, Treated: APAP or MP, -: not determined.

* Fasted ctrl or Ctrl.

Table 2. Effects of fasting on miRNA expression in the pericentral and periportal regions of the liver.

Pericentral region				Periportal region			
miRNAs	Fasted-ctrl (40 - Ct)	Ctrl (40 - Ct)	Fold	miRNAs	Fasted-ctrl (40 - Ct)	Ctrl (40 - Ct)	Fold
Fasted-ctrl > Ctrl (36 miRNAs)				Fasted-ctrl > Ctrl (52 miRNAs)			
mmu-miR-222	15.2	21.6	84.4	**mmu-miR-99a**	12.2	16.2	16.0
mmu-miR-706	8.6	13.2	24.3	**mmu-miR-100**	13.0	16.2	8.6
mmu-miR-690	14.8	19.0	18.4	**mmu-miR-467a***	12.5	15.4	8.0
mmu-miR-135a*	10.4	14.5	16.0	**mmu-miR-706**	9.6	12.4	7.0
mmu-miR-877*	8.6	12.0	10.6	mmu-miR-130a	9.6	12.2	6.1
mmu-miR-101b	12.9	16.3	10.6	mmu-let-7i	11.3	13.9	6.1
mmu-miR-193*	11.5	14.4	8.0	mmu-let-7b	14.4	16.8	5.3
mmu-miR-22	11.1	14.0	7.5	mmu-miR-99b	10.4	12.8	5.3
mmu-miR-467a*	12.3	15.2	7.5	**mmu-miR-135a***	11.7	14.0	4.9
mmu-miR-93*	9.7	12.5	7.0	mmu-let-7c	15.6	17.9	4.9
rno-miR-664	13.6	16.4	6.5	**mmu-miR-125b-5p**	13.2	15.3	4.6
rno-miR-28*	8.6	11.3	6.5	**mmu-miR-690**	15.2	17.4	4.3
mmu-miR-21*	8.9	11.6	6.1	mmu-miR-328	13.6	15.6	4.3
mmu-miR-378	13.1	15.5	5.3	mmu-miR-21	12.8	14.8	4.0
mmu-miR-31*	11.3	13.7	5.3	mmu-miR-497	10.1	12.1	4.0
mmu-miR-30e*	15.1	17.5	5.3	**mmu-miR-467b***	8.7	10.6	3.7
mmu-miR-30a*	15.1	17.4	4.6	mmu-miR-145	17.4	19.2	3.5
mmu-miR-709	21.7	23.9	4.3	mmu-miR-25	10.2	12.0	3.5
mmu-miR-674*	9.6	11.7	4.3	mmu-miR-200b	12.8	14.6	3.2
mmu-miR-678	9.7	11.8	4.3	mmu-miR-26a	16.1	17.8	3.2
mmu-miR-99a	13.0	15.0	4.0	mmu-miR-30a	14.3	16.0	3.2
rno-miR-125b*	9.0	10.9	4.0	mmu-miR-27a	9.5	11.2	3.2
mmu-miR-28*	12.4	14.3	3.7	**mmu-miR-375**	12.5	14.1	3.2
mmu-miR-872*	11.2	13.1	3.5	**mmu-miR-193***	11.9	13.5	3.0
mmu-miR-720	21.2	22.9	3.2	mmu-miR-301b	9.8	11.3	3.0
mmu-miR-455*	8.0	9.6	3.0	**mmu-miR-93***	12.0	13.5	2.8
mmu-miR-188-5p	9.5	11.0	3.0	mmu-miR-335-5p	10.1	11.6	2.8
mmu-miR-375	13.1	14.5	2.6	mmu-miR-29c	11.4	12.9	2.8
mmu-miR-467b*	9.0	10.4	2.6	**mmu-miR-222**	15.8	17.3	2.8
mmu-miR-29a	15.2	16.5	2.5	mmu-miR-26b	13.5	15.0	2.8
mmu-miR-125b*	9.3	10.6	2.3	mmu-miR-152	13.3	14.8	2.8
mmu-miR-425*	8.4	9.6	2.3	mmu-miR-532-3p	11.8	13.3	2.8
rno-miR-7a*	11.1	12.4	2.3	**mmu-miR-29a**	14.7	16.1	2.8
mmu-miR-100	13.8	14.9	2.1	mmu-miR-181a	9.5	11.0	2.8
mmu-miR-19a	13.1	14.2	2.1	mmu-miR-28	11.6	13.0	2.6
mmu-miR-877	8.5	9.6	2.1	mmu-miR-92a	17.0	18.4	2.5
				mmu-miR-138	13.4	14.7	2.5
Fasted-ctrl < Ctrl (8 miRNAs)				mmu-miR-93	12.1	13.5	1.3
mmu-miR-200a	12.8	9.8	0.1	mmu-miR-188-5p	9.2	10.5	2.5
mmu-miR-200c	13.3	11.4	0.3	**mmu-miR-877***	10.9	12.1	2.3
mmu-miR-151-3p	11.1	9.6	0.4	mmu-let-7e	16.8	18.0	2.3
mmu-miR-335-3p	9.8	8.3	0.4	**mmu-miR-19a**	12.2	13.4	2.3
mmu-miR-155	13.9	12.5	0.4	mmu-miR-17	15.9	17.1	2.3
mmu-miR-365	14.7	13.4	0.4	**mmu-miR-678**	9.8	11.0	2.3
mmu-miR-214	13.4	12.1	0.4	mmu-miR-143	13.3	14.4	2.1
mmu-miR-99b*	11.8	10.8	0.5	**mmu-miR-378**	14.3	15.4	2.1
				mmu-miR-126-5p	14.6	15.7	2.1
				mmu-miR-331-3p	13.1	14.2	2.1
				mmu-miR-140	13.9	15.0	2.1
				mmu-miR-199a-3p	12.8	13.8	2.0
				mmu-miR-139-5p	15.2	16.2	2.0
				Fasted-ctrl < Ctrl (2 miRNAs)			
				mmu-miR-322*	11.8	9.8	0.3
				mmu-miR-484	13.5	12.4	0.5

The miRNAs shown in bold are common to the pericentral and periportal regions.

Table 3. miRNAs whose expression was different between the pericentral and periportal regions of the liver.

miRNAs	Number of miRNAs CV	P	Fold	miRNAs	Number of miRNAs CV	P	Fold
CV > P (27 miRNAs)				CV < P (22 miRNAs)			
mmu-miR-130a	9.6	11.7	4.3	mmu-miR-322*	11.8	8.9	0.1
mmu-miR-148a	12.2	14.3	4.3	mmu-miR-200c	15.9	13.3	0.2
mmu-miR-335-5p	10.1	11.9	3.5	mmu-miR-877*	10.9	8.6	0.2
mmu-miR-21	12.8	14.4	3.0	mmu-miR-31*	13.6	11.3	0.2
mmu-miR-26b	13.5	15.1	3.0	mmu-miR-93*	12.0	9.7	0.2
mmu-miR-532-3p	11.8	13.3	2.8	rno-miR-28*	10.8	8.6	0.2
mmu-miR-335-3p	8.3	9.8	2.8	rno-miR-7a*	13.0	11.1	0.3
mmu-miR-27a	9.5	10.9	2.6	rno-miR-125b*	10.8	9.0	0.3
mmu-miR-194	14.5	15.9	2.6	mmu-miR-200a	14.3	12.8	0.3
mmu-miR-93	12.1	13.5	2.5	mmu-miR-674*	11.1	9.6	0.4
mmu-miR-126-5p	14.6	15.9	2.5	mmu-miR-101b	14.3	12.9	0.4
mmu-let-7i	11.3	12.6	2.5	mmu-miR-30e*	16.5	15.1	0.4
mmu-miR-200b	12.8	14.1	2.5	mmu-miR-184	13.2	11.9	0.4
mmu-miR-30d	11.9	13.1	2.5	rno-miR-664	14.9	13.6	0.4
mmu-miR-151-3p	9.9	11.1	2.3	mmu-miR-135a*	11.7	10.4	0.4
mmu-miR-26a	16.1	17.3	2.3	mmu-miR-22	12.3	11.1	0.4
mmu-miR-17	15.9	17.1	2.3	mmu-miR-378	14.3	13.1	0.4
mmu-miR-122	20.5	21.7	2.3	mmu-miR-30a*	16.3	15.1	0.4
mmu-miR-106a	15.9	17.0	2.1	mmu-miR-872*	12.4	11.2	0.4
mmu-miR-365	13.6	14.7	2.1	mmu-miR-125b*	10.4	9.3	0.5
mmu-let-7b	14.4	15.5	2.1	mmu-miR-28*	13.5	12.4	0.5
mmu-miR-497	10.1	11.2	2.1	mmu-miR-720	22.2	21.2	0.5
mmu-miR-192	17.2	18.3	2.1				
mmu-miR-92a	17.0	18.1	2.1				
mmu-miR-25	10.2	11.3	2.0				
mmu-let-7c	15.6	16.6	2.0				
mmu-miR-19b	17.2	18.3	2.0				

Table 4. Numbers of miRNAs whose expression in the rat liver was changed by the administration of APAP or MP.

APAP		Periportal			
		Up	±	Down	Sum
Pericentral	Up	2	14	2	18
	±	4	63	13	80
	Down	1	20	6	27
	Sum	7	97	21	125
MP		Periportal			
		Up	±	Down	Sum
Pericentral	Up	2	7	1	10
	±	1	38	14	53
	Down	1	20	41	62
	Sum	4	65	56	125

Table 5. miRNAs whose expression in the pericentral or periportal regions was changed due to APAP- or MP-induced liver injury.

APAP vs. Fasted-ctrl		Periportal		
		Up	±	Down
Pericentral	Up	Group A [a]mmu-miR-685 [a]mmu-miR-322* 2 miRNAs ↑	Group B [a]mmu-miR-223 [a]mmu-miR-155 [a]mmu-miR-27a [a]mmu-miR-34c* [a]mmu-miR-142-3p [a]mmu-miR-877* [a]mmu-miR-467b* [a]mmu-miR-21* [a]mmu-miR-214 [a]mmu-miR-130a 14 miRNAs ↑	Group C [a]mmu-miR-93* [a]mmu-miR-467a* 2 miRNAs ↑
	±	Group D [a]mmu-miR-484 [a]mmu-miR-872 [a]mmu-miR-30d [a]mmu-miR-192 (4 miRNAs ↑)	Group E [a]rno-miR-28* [a]mmu-miR-27b [a]mmu-miR-22 [a]mmu-miR-29a [a]rno-miR-7a* [a]mmu-miR-15b [a]mmu-miR-200c [a]mmu-miR-106b [a]mmu-miR-195 [a]mmu-miR-143 62 miRNAs ↑,1 miRNA →	Group F [a]mmu-miR-138 [a]mmu-miR-425* mmu-miR-99b [a]mmu-miR-328 mmu-miR-29b* [a]mmu-miR-494 [a]mmu-miR-150 [a]mmu-miR-181a [a]mmu-miR-24 [a]mmu-miR-99b* 11 miRNAs ↑, 2 miRNAs →
	Down	Group G [a]rno-miR-339-3p 1 miRNA ↑	Group H [a]mmu-miR-200b [a]mmu-miR-31 [a]mmu-miR-101b [a]mmu-miR-193* [a]mmu-let-7e [a]mmu-miR-122 [a]mmu-miR-148a [a]mmu-miR-194 [a]mmu-miR-31* [a]mmu-miR-29c 20 miRNAs ↑	Group I [a]mmu-miR-222 [a]mmu-miR-99a [a]mmu-miR-375 [a]mmu-miR-100 [a]mmu-miR-199a-3p [a]mmu-miR-188-5p 6 miRNAs ↑

Continued

MP vs. Ctrl		Periportal		
		Up	±	Down
Pericentral	Up	Group A mmu-miR-21* [a]mmu-miR-709 1 miRNA ↑,1 miRNA →	Group B [a]mmu-miR-877 [a]mmu-miR-322* [a]mmu-miR-93* [a]mmu-miR-674* [a]mmu-miR-101b [a]mmu-miR-135a* [b]mmu-miR-30a* 6 miRNAs ↑, 1 miRNA ↓	Group C [a]rno-miR-28* 1 miRNA ↑
	±	Group D [a]mmu-miR-34c* 1 miRNA ↑	Group E [a]mmu-miR-685 [a]mmu-miR-28* [a]mmu-miR-378 [a]mmu-miR-706 [a]mmu-miR-22 [a]mmu-miR-31* [a]rno-miR-7a* [a]mmu-miR-223 [a]mmu-miR-150 [b]mmu-miR-30e* 37 miRNAs ↑,1 miRNA ↓	Group F [a]mmu-let-7g [a]mmu-miR-375 [a]mmu-miR-101a [a]mmu-miR-19a [a]mmu-miR-192 [a]mmu-miR-28 [a]mmu-miR-193b [a]mmu-miR-19b [a]mmu-miR-125b* [a]mmu-miR-16 14 miRNAs ↑
	Down	Group G [a]mmu-miR-27a 1 miRNA ↑	Group H [a]mmu-miR-467a* [a]mmu-miR-99b* [a]mmu-miR-200b [a]mmu-miR-130a [a]mmu-miR-301b [a]mmu-miR-181a [a]mmu-miR-222 [a]mmu-miR-532-3p [a]mmu-miR-142-3p [a]mmu-miR-93 19 miRNAs ↑,1 miRNA →	Group I [a]mmu-let-7b mmu-miR-27b [a]mmu-miR-301a [a]mmu-miR-194 [a]mmu-miR-99a [a]mmu-miR-365 [a]mmu-miR-100 [a]mmu-miR-21 [a]mmu-miR-200c [a]mmu-miR-214 37 miRNAs ↑, 4 miRNAs →

[a] upregulated (↑), [b] downregulated (↑), and not changed (→) miRNAs in the plasma.

Continued

MP vs. Ctrl		Periportal		
		Up	±	Down
Pericentral	Up	Group A mmu-miR-21* [a]mmu-miR-709 1 miRNA ↑,1 miRNA →	Group B [a]mmu-miR-877 [a]mmu-miR-322* [a]mmu-miR-93* [a]mmu-miR-674* [a]mmu-miR-101b [a]mmu-miR-135a* [b]mmu-miR-30a* 6 miRNAs ↑, 1 miRNA ↓	Group C [a]rno-miR-28* 1 miRNA ↑
	±	Group D [a]mmu-miR-34c* 1 miRNA ↑	Group E [a]mmu-miR-685 [a]mmu-miR-28* [a]mmu-miR-378 [a]mmu-miR-706 [a]mmu-miR-22 [a]mmu-miR-31* [a]rno-miR-7a* [a]mmu-miR-223 [a]mmu-miR-150 [b]mmu-miR-30e* 37 miRNAs ↑,1 miRNA ↓	Group F [a]mmu-let-7g [a]mmu-miR-375 [a]mmu-miR-101a [a]mmu-miR-19a [a]mmu-miR-192 [a]mmu-miR-28 [a]mmu-miR-193b [a]mmu-miR-19b [a]mmu-miR-125b* [a]mmu-miR-16 14 miRNAs ↑
	Down	Group G [a]mmu-miR-27a 1 miRNA ↑	Group H [a]mmu-miR-467a* [a]mmu-miR-99b* [a]mmu-miR-200b [a]mmu-miR-130a [a]mmu-miR-301b [a]mmu-miR-181a [a]mmu-miR-222 [a]mmu-miR-532-3p [a]mmu-miR-142-3p [a]mmu-miR-93 19 miRNAs ↑,1 miRNA →	Group I [a]mmu-let-7b mmu-miR-27b [a]mmu-miR-301a [a]mmu-miR-194 [a]mmu-miR-99a [a]mmu-miR-365 [a]mmu-miR-100 [a]mmu-miR-21 [a]mmu-miR-200c [a]mmu-miR-214 37 miRNAs ↑, 4 miRNAs →

[a] upregulated (↑), [b] downregulated (↑), and not changed (→) miRNAs in the plasma.

Figure

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Fig. 1

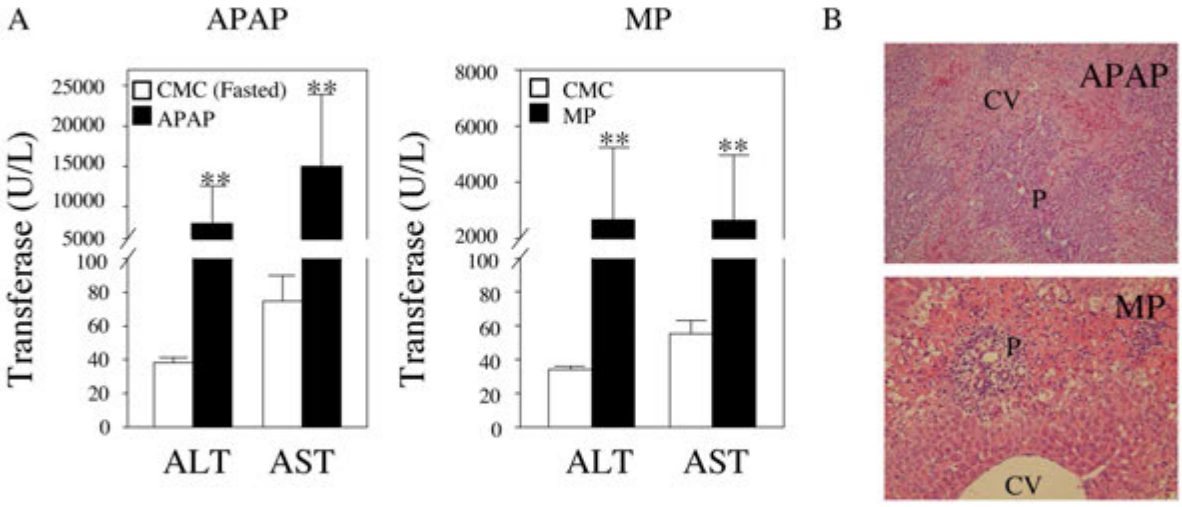

Figure 2

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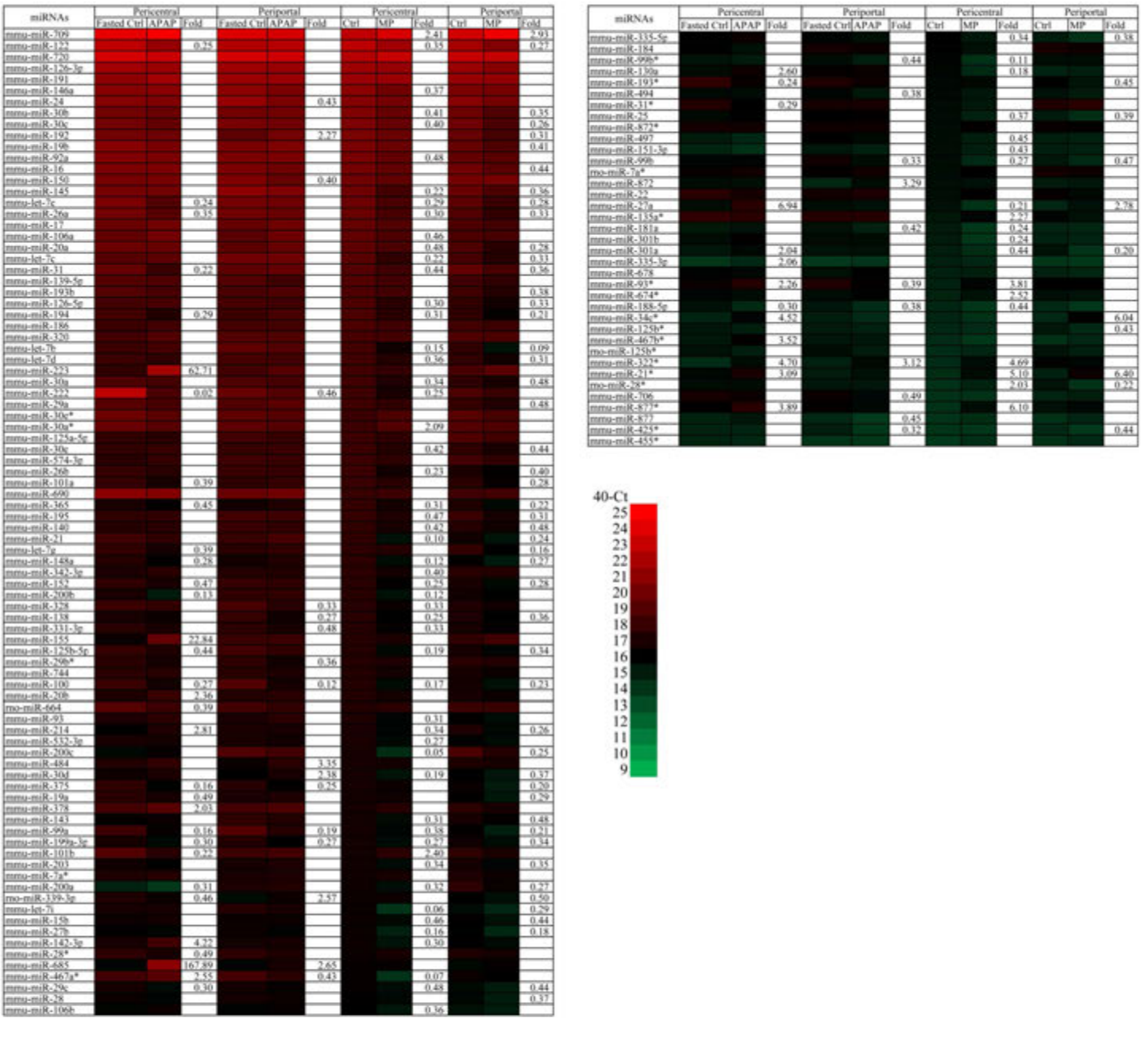
Fig. 2



miRNAs	Pericentral			Periportal			Pericentral			Periportal		
	Fasted Ctrl	APAP	Fold	Fasted Ctrl	APAP	Fold	Ctrl	MP	Fold	Ctrl	MP	Fold
mmu-miR-709									2.41			2.93
mmu-miR-123			0.35						0.35			0.27
mmu-miR-720												
mmu-miR-126-3p												
mmu-miR-191												
mmu-miR-146a									0.37			
mmu-miR-24						0.43						
mmu-miR-30b									0.41			0.35
mmu-miR-30c									0.40			0.26
mmu-miR-192						2.27						0.31
mmu-miR-19b												0.41
mmu-miR-92a									0.48			
mmu-miR-16												0.44
mmu-miR-150						0.40						
mmu-miR-145									0.22			0.36
mmu-let-7c			0.34						0.29			0.28
mmu-miR-26a			0.35						0.30			0.33
mmu-miR-17												
mmu-miR-106a									0.46			
mmu-miR-20a									0.48			0.28
mmu-let-7c									0.22			0.33
mmu-miR-31			0.22						0.44			0.36
mmu-miR-139-5p												
mmu-miR-193b												0.38
mmu-miR-126-5p									0.30			0.33
mmu-miR-194			0.29						0.31			0.21
mmu-miR-186												
mmu-miR-320												
mmu-let-7b									0.15			0.09
mmu-let-7d									0.36			0.31
mmu-miR-223			62.71									
mmu-miR-30a									0.34			0.48
mmu-miR-222			0.02			0.46			0.25			
mmu-miR-29a												0.48
mmu-miR-30e*												
mmu-miR-30a*									2.09			
mmu-miR-125a-5p												
mmu-miR-30c									0.42			0.44
mmu-miR-574-3p												
mmu-miR-26b									0.23			0.40
mmu-miR-101a			0.39									0.28
mmu-miR-690												
mmu-miR-365			0.45						0.31			0.22
mmu-miR-195									0.47			0.31
mmu-miR-140									0.42			0.48
mmu-miR-21									0.10			0.24
mmu-let-7g			0.39									0.16
mmu-miR-148a			0.28						0.12			0.27
mmu-miR-342-3p									0.40			
mmu-miR-152			0.47						0.25			0.28
mmu-miR-200b			0.13						0.12			
mmu-miR-328						0.33			0.33			
mmu-miR-138						0.27			0.25			0.36
mmu-miR-331-3p						0.48			0.33			
mmu-miR-155			22.84									
mmu-miR-125b-5p			0.44						0.19			0.34
mmu-miR-296*						0.36						
mmu-miR-744												
mmu-miR-100			0.27			0.12			0.17			0.23
mmu-miR-20b			2.36									
rno-miR-664			0.39									
mmu-miR-93									0.31			
mmu-miR-214			2.81						0.34			0.26
mmu-miR-532-3p									0.27			
mmu-miR-200c									0.05			0.25
mmu-miR-484						3.35						
mmu-miR-30d						2.38			0.19			0.37
mmu-miR-375			0.16			0.25						0.20
mmu-miR-19a			0.49									0.29
mmu-miR-378			2.03									
mmu-miR-143									0.31			0.48
mmu-miR-99a			0.16			0.19			0.38			0.21
mmu-miR-199a-3p			0.30			0.27			0.27			0.34
mmu-miR-101b			0.22						2.40			
mmu-miR-203									0.34			0.35
mmu-miR-7a*												
mmu-miR-200a			0.31						0.32			0.27
rno-miR-339-3p			0.46			2.57						0.50
mmu-let-7i									0.06			0.29
mmu-miR-15b									0.46			0.44
mmu-miR-27b									0.16			0.18
mmu-miR-142-3p			4.22						0.30			
mmu-miR-28*			0.49									
mmu-miR-685			167.89			2.65						
mmu-miR-467a*			2.55			0.43			0.07			
mmu-miR-29c			0.30						0.48			0.44
mmu-miR-28												0.37
mmu-miR-106b									0.36			

miRNAs	Pericentral			Periportal			Pericentral			Periportal		
	Fasted Ctrl	APAP	Fold	Fasted Ctrl	APAP	Fold	Ctrl	MP	Fold	Ctrl	MP	Fold
mmu-miR-335-5p									0.34			0.38
mmu-miR-184												
mmu-miR-99b*						0.44			0.11			
mmu-miR-130a			2.60						0.18			
mmu-miR-191*			0.24									0.45
mmu-miR-494						0.38						
mmu-miR-31*			0.29									
mmu-miR-25									0.37			0.39
mmu-miR-877*												
mmu-miR-497									0.45			
mmu-miR-151-3p									0.43			
mmu-miR-99b						0.33			0.27			0.47
rno-miR-7a*												
mmu-miR-872						3.29						
mmu-miR-23a												
mmu-miR-27a			6.94						0.21			2.78
mmu-miR-133a*									2.57			
mmu-miR-181a						0.42			0.24			
mmu-miR-301b									0.24			
mmu-miR-301a			2.04						0.44			0.20
mmu-miR-335-3p			2.06									
mmu-miR-678												
mmu-miR-93*			2.26			0.39			3.81			
mmu-miR-674*									2.52			
mmu-miR-188-5p			0.30			0.38			0.44			
mmu-miR-34c*			4.52									6.04
mmu-miR-125b*												0.43
mmu-miR-467b*			3.52									
rno-miR-125b*												
mmu-miR-337*			4.70			3.12			4.69			
mmu-miR-21*			3.09						5.10			6.40
rno-miR-29*									2.03			0.22
mmu-miR-706						0.49						
mmu-miR-877*			3.89						6.10			
mmu-miR-877						0.45						
mmu-miR-425*						0.32						0.44
mmu-miR-455*												

Figure 3

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Fig. 3

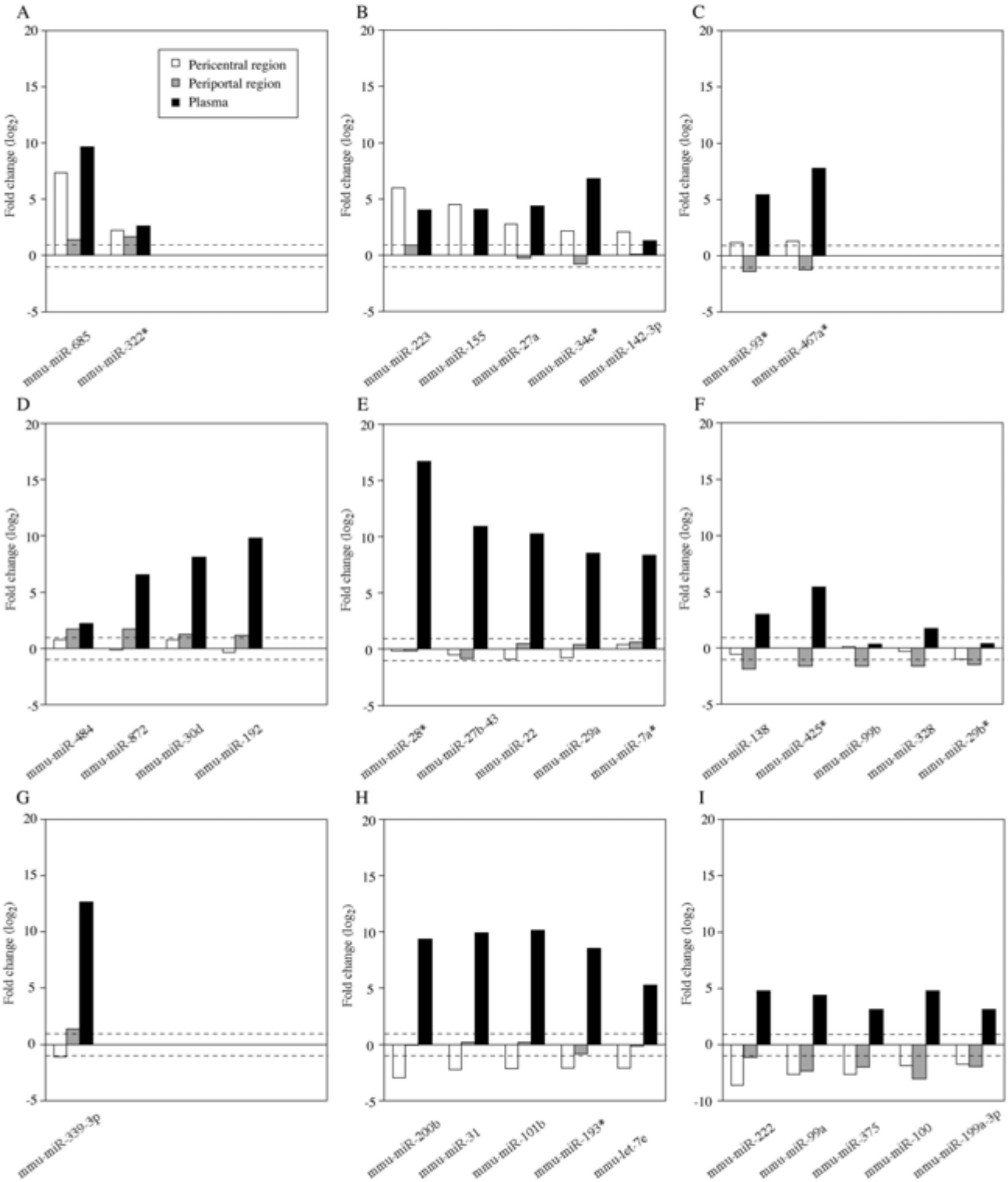

Figure 4

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Fig. 4

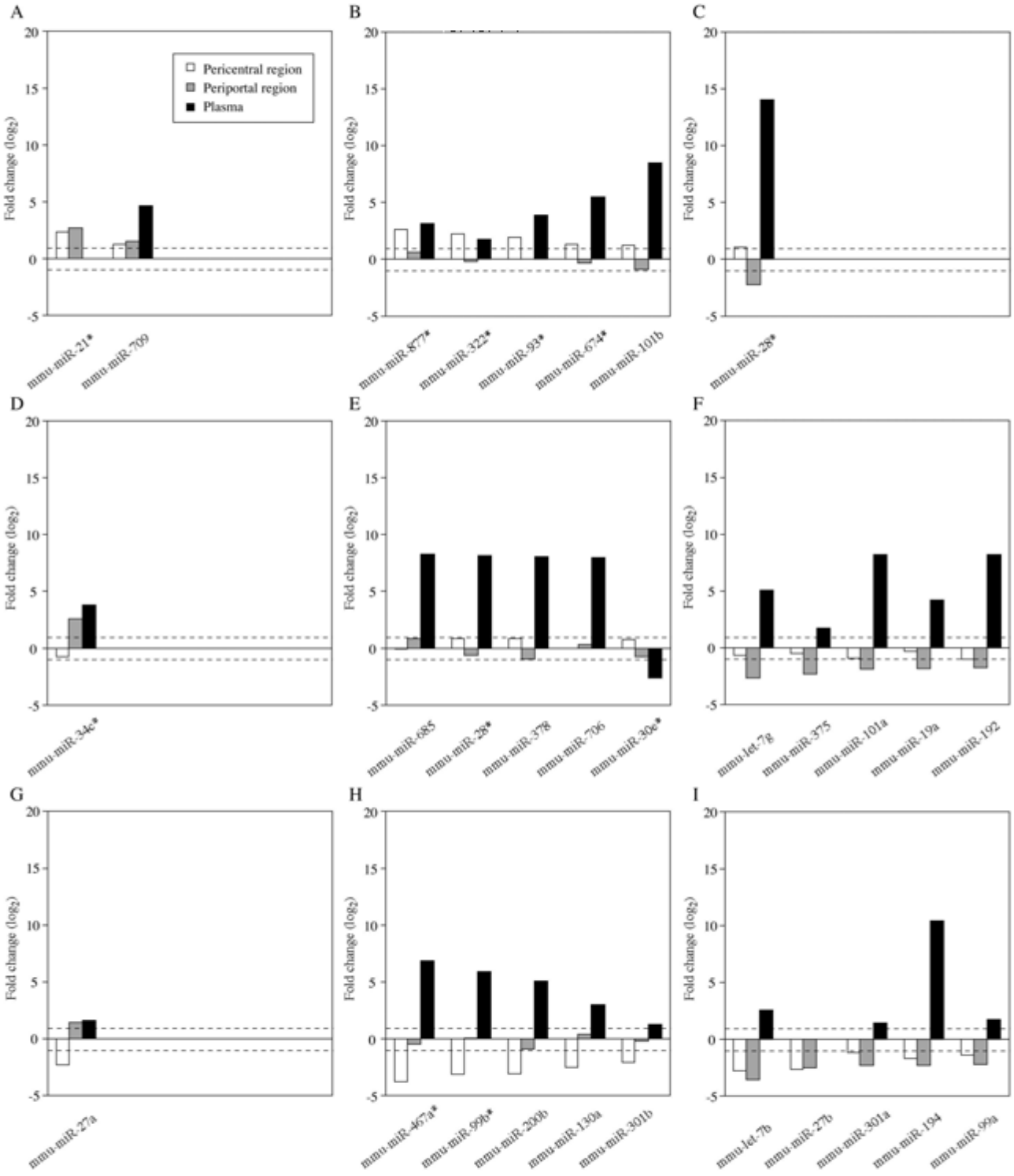

Figure 5
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Fig. 5