

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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1 **Abstract**

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5 MicroRNAs (miRNAs) in body fluids have received attention as potential biomarkers  
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7 of organ damage because miRNAs that are highly or specifically expressed in a given organ  
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9 are likely released into body fluids as a result of damage to that organ. We previously  
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11 determined that the plasma miRNA profile in rats was dramatically changed due to  
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13 acetaminophen (APAP)-induced pericentral necrosis and methapyrilene (MP)-induced  
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15 periportal necrosis in the liver. The purpose of this study was to examine whether the  
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17 expression of hepatic miRNAs is differentially modulated at different zones due to injury and  
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19 to examine the relationship of the hepatic miRNA profile with the changes in the plasma  
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21 miRNA expression profile. Through the laser microdissection of the periportal and periportal  
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23 regions of the liver and TaqMan microRNA Array analysis, we found that 49 miRNAs are  
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25 differentially expressed between the pericentral and periportal regions of control rats. In both  
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27 APAP- and MP-treated rats, the miRNAs that presented decreased expression dominated in  
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29 both the injured and non-injured areas compared with the miRNAs that exhibited increased  
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31 expression. The changes in miRNA expression in each region of the liver were compared with  
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33 those observed in the plasma. Of the 301 plasma miRNAs with expression that was changed  
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35 as a result of APAP administration, only 21% were changed in the injured area of the liver. Of  
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37 the 263 plasma miRNAs with expression that was changed due to MP administration, only  
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39 24% were changed in the injured area of the liver. Thus, the miRNA expression profiles in the  
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41 plasma do not merely reflect the release of miRNAs from the damaged cells in the liver. This  
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43 report provides the first demonstration of zonal miRNA expression in the liver and of the  
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45 relationship of the miRNA expression profile in a tissue with the plasma miRNA profile.  
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53 **Keywords**

54 microRNA; liver injury; zonal expression; biomarker  
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## 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

1 present study, we examined the miRNA expression profiles at the pericentral and periportal  
2 regions of the liver in two rat models of liver injury to clarify whether the miRNAs in the  
3 liver are uniformly or variably expressed through the region and to understand the  
4 relationship between the plasma and tissue miRNA expression profiles.  
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## 10 11 **1. Materials and methods**

### 12 *1.1. Chemicals and reagents*

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14 APAP and MP were purchased from Wako Pure Chemicals (Osaka, Japan) and  
15 Sigma-Aldrich (St. Louis, MO), respectively. The RNAqueous Kit, *mirVana* PARIS Kit,  
16 RNAqueous Kit, Megaplex Pools, TaqMan MicroRNA Reverse Transcription Kit, TaqMan  
17 MicroRNA Assays, TaqMan 2x Universal PCR Master Mix (No AmpErase UNG), and  
18 TaqMan Rodent MicroRNA Array v2.0 were obtained from Applied Biosystems (Foster City,  
19 CA). All of the other chemicals and solvents were of the highest grade commercially  
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### 31 *1.2. Animal models*

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33 The animal maintenance and treatments were conducted in accordance with the National  
34 Institutes of Health Guide for Animal Welfare of Japan and were approved by the Institutional  
35 Animal Care and Use Committee of Kanazawa University, Japan. The study was approved by  
36 the Animal Ethics Committee of Kanazawa University (No. 31203). Male five-week-old  
37 Sprague-Dawley rats were purchased from Japan SLC (Hamamatsu, Japan). The rats were  
38 housed in a controlled environment (temperature  $25 \pm 1^\circ\text{C}$ , humidity  $50 \pm 10\%$ , and 12-h  
39 light/12-h dark cycle) in the institutional animal facility with access to food and water *ad*  
40 *libitum*. Each set of six rats was orally administered 1,000 mg/kg APAP suspended in 0.5%  
41 carboxymethylcellulose (CMC) after fasting for 48 h, 300 mg/kg MP dissolved in 0.5% CMC,  
42 or vehicle. Twenty-four hours after administration, their blood and liver were collected. Using  
43 EDTA as an anticoagulant, the plasma was separated by centrifugation and maintained at  
44  $-80^\circ\text{C}$  until use. Part of the liver was fixed in buffered neutral 10% formalin, and the  
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1 remaining part was stored at -80°C.  
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### 5 *1.3. Biochemical assay and pathological examination* 6

7 The plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total  
8 bilirubin (T-Bil) levels were determined using Dri-Chem 4000 (FUJIFILM Saitama, Japan)  
9 according to the manufacturer's instructions. The formalin-fixed samples were embedded in  
10 paraffin, sectioned, and then stained with hematoxylin and eosin (Wako Pure Chemicals,  
11 Osaka, Japan) for microscopic examination.  
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### 20 *1.4. Laser microdissection and RNA isolation* 21

22 Part of the frozen livers were embedded in O.C.T. compound (Sakura Finetek Japan,  
23 Tokyo, Japan), cut into 20- $\mu$ m-thick sections, and stained with 10% hematoxylin and eosin.  
24 The pericentral and periportal regions of the livers from rats administered APAP, MP, or  
25 vehicle were collected by laser microdissection using an Application Solutions Laser  
26 Microdissection System (Leica Microsystems, Wetzlar, Germany). The total RNA from the  
27 microdissected sections was extracted using the RNAqueous Kit according to the  
28 manufacturer's protocol, and the total RNA from the same group was pooled.  
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### 40 *1.5. TaqMan microRNA array analysis* 41

42 The cDNAs were synthesized from the pooled total RNA using Megaplex Pools  
43 according to the manufacturer's protocol. Pre-amplification was performed by the addition of  
44 2x TaqMan PreAmp Master Mix and 10x Megaplex PreAmp Primers to the cDNA sample.  
45 TaqMan 2x Universal PCR Master Mix (No AmpErase UNG) was then added to the  
46 pre-amplification products. The mixture was applied to individual ports of TaqMan Array  
47 Rodent MicroRNA A+B Cards Sets v2.0 384-well microfluidics cards containing 375 (A  
48 array) or 210 (B array) primer-probe sets for individual miRNAs. Quantitative real-time PCR  
49 was performed using a 7900HT Fast Real-Time PCR system (Applied Biosystems) with the  
50 SDS software v.2.4. The expression levels were evaluated using the comparative cycle  
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1 threshold (Ct) method. The Ct values ranged from 0 to 40. The miRNAs that gave Ct values  
2 greater than 32 in all of the groups were omitted from the data analysis because this 32 is the  
3 cut-off value recommended by the manufacturer. The data are presented as (40 - Ct). The  $\Delta$ Ct  
4 values [ $\Delta$ Ct = (40 - Ct control) - (40 - Ct model)] were calculated as fold changes. The  
5 miRNAs showing expression changes of at least two-fold were analyzed. A heat map was  
6 generated with Microsoft Excel 2010.  
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### 13 14 15 16 *1.6. Statistical analysis*

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18 The data are expressed as the means  $\pm$  SD. The comparisons of two groups were made  
19 using Mann-Whitney's U-test. A value of  $P < 0.05$  was considered statistically significant.  
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## 24 25 **2. Results**

### 26 27 *2.1. ALT, AST, and histopathology of the liver*

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29 After the administration of APAP and MP, the plasma ALT and AST levels were  
30 significantly elevated (Fig. 1A). The histopathological analysis revealed that APAP caused  
31 hepatocellular necrosis at the pericentral regions, whereas MP caused hepatocellular necrosis  
32 at the periportal region (Fig. 1B). These two models were used for the subsequent studies.  
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### 40 41 *2.2. miRNA expression at the pericentral and periportal regions of the liver*

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43 The miRNA expression at pericentral and periportal regions of the liver was determined  
44 by TaqMan microRNA array analysis. The numbers of the detected miRNAs and the miRNAs  
45 whose expression exceeded the cutoff (Ct < 32) are shown in Table 1. No large difference was  
46 observed in these numbers between the pericentral and periportal regions within a group or  
47 between the treated and control groups. Among the miRNAs with Ct values less than 32 in all  
48 of the groups, 125 miRNAs were common to all of the groups; therefore, these 125 miRNAs  
49 were used for the subsequent analyses. Fig. 2 shows the heat maps of the expression of the  
50 125 miRNAs in each sample; note that the miRNAs are ordered based on descending  
51 expression level in the pericentral region of the control rats. The top three miRNAs that  
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1 exhibited high expression in both the pericentral and periportal regions in all of the samples  
2 are miR-709, miR-122, and miR-720. The comparison of the miRNA levels between the  
3 control rats and fasted-control rats revealed that 36 miRNAs presented higher levels and eight  
4 miRNAs exhibited lower levels at the pericentral region of the fasted-control rats (Tables 1  
5 and 2). At the periportal region, 52 miRNAs and two miRNAs presented higher and lower  
6 levels, respectively, in the fasted-control rats compared with the control rats. Among these  
7 miRNAs, 17 miRNAs, which are shown in bold, were found in both the pericentral and  
8 periportal regions. Thus, the results demonstrate that fasting affects the expression of some  
9 miRNAs in the liver. The comparison of the miRNA levels between the pericentral and  
10 periportal regions in control rats showed that 27 miRNAs exhibited higher expression levels  
11 in the pericentral region and that 22 miRNAs presented higher expression levels in the  
12 periportal region compared with the other region (Tables 1 and 3). This study provides the  
13 first demonstration that some miRNAs are differentially expressed at the periportal and  
14 periportal regions.

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



1 were not affected at the periportal region. It is interesting that four (57%) and 13 (62%)  
2 miRNAs were specifically changed at the periportal region, where hepatocytes were not  
3 injured. In MP-treated rats, of the four and 56 miRNAs that exhibited increased and decreased  
4 expression at the periportal region, respectively, three miRNAs (75%) and 42 miRNAs (75%)  
5 also presented changed expression levels at the pericentral region. Thus, the results  
6 demonstrate that a large number of miRNAs were decreased at both the periportal and  
7 pericentral regions.  
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### 18 2.3. *Comparison of the miRNA expression changes in the liver and plasma*

19 We then sought to examine the relationship between the miRNA expression changes in the  
20 liver and those in the plasma because we previously reported the plasma miRNA profiles in  
21 these model rats (Yamaura et al., 2012). The miRNAs that were classified into nine groups  
22 (groups A to I) in Table 4 are specified in Table 5. In the groups that include more than 10  
23 miRNAs, the ten most abundant miRNAs are shown. The changes in the levels of these  
24 miRNAs in the plasma are shown with arrows in Table 5. Interestingly, in the APAP-treated rats,  
25 122 of the 125 miRNAs were increased in the plasma, regardless of the changes observed in the  
26 liver. The remaining three miRNAs were not changed in the plasma. In the MP-treated rats, 117  
27 of 125 miRNAs were increased in the plasma, six miRNAs were unchanged, and two miRNAs  
28 were decreased in the plasma. The fold changes of these miRNAs in the liver (pericentral and  
29 periportal regions) and plasma are shown in Figs. 3 and 4. In the groups with more than five  
30 miRNAs, the five most abundant miRNAs are shown. Reciprocal changes in the miRNAs  
31 between the liver and plasma (decrease in the liver and increase in plasma) were observed in  
32 some cases, but this relationship was not general. Of special interest is that the miRNAs that  
33 were not affected in the liver at all were markedly increased in the plasma, as shown in group E.  
34 Thus, the relationship between the changes in the miRNA levels in the plasma and tissue is not  
35 simple.  
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57 We then examined the expression changes in the liver of the miRNAs that were  
58 changed in the plasma (Fig. 5). We previously reported that 317 and 295 miRNAs are  
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1 increased and that six and 10 miRNAs are decreased in the rat plasma as a result of APAP-  
2 and MP-induced liver injury, respectively (Yamaura et al., 2012). In our previous study,  
3 several miRNAs for which two probes were included on the array were counted individually.  
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5 Excluding the overlapped miRNAs, the numbers of plasma miRNAs that were changed by  
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7 APAP and MP administration were recalculated to 301 and 263 miRNAs, respectively.  
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9 Among the 301 miRNAs that exhibited a change in their expression level in the APAP-treated  
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11 rats, 48 miRNAs (16%) were changed only at the pericentral region, 14 miRNAs (5%) were  
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13 changed at both the pericentral and periportal regions, and 27 miRNAs (9%) were changed  
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15 only at the periportal region. Among the 263 miRNAs that presented a changed expression  
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17 level in the MP-treated rats, 32 miRNAs (12%) were changed only in the pericentral region,  
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19 44 miRNAs (17%) were changed at both the pericentral and periportal region, and 18  
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21 miRNAs (7%) were changed only at the periportal region. Collectively, most (~70%) of the  
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23 miRNAs whose levels were changed in the plasma of rats with liver injury were not changed  
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25 in the liver.  
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### 31 **3. Discussion**

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36 In this study, we examined the hepatic miRNA expression in rats with liver injury to  
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38 evaluate the relationship between the changes in the plasma miRNA profile and to determine  
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40 whether the miRNA expression is differently modulated at injured and not-injured regions.  
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42 We also examined whether miRNA expression varies between different zones in the normal  
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44 liver and whether hepatic miRNA expression is affected by fasting.  
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48 In the rat liver, we found that the three most abundant miRNAs are miR-709, miR-122,  
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50 and miR-720. The high expression of miR-709 and miR-720 was unexpected because it is  
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52 known that miR-122 constitutes 70% of the total hepatic miRNAs (Lagos-Quintana et al.,  
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54 2002, Chang et al., 2004). We noticed that miR-720 had been removed from the database  
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56 (<http://www.mirbase.org/index.shtml>) because Schopman et al. (2010) reported that the  
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58 sequence annotated as miR-720 is likely to be a fragment of a tRNA. Although miR-709  
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60 remains to be registered, we found that the sequence of miR-709 completely matches with  
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1 that of a tRNA, indicating that it may also be misdetection. Supporting this assumption, the  
2 abundant miRNAs in the rat liver (other than miR-709 and miR-720), such as miR-122,  
3 miR-126-3p, miR-191, and miR-146a, were also found to be highly expressed in human  
4 hepatocytes in our previous study (Takahashi et al., 2014).  
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9 We found that fasting changes the expression of some miRNAs in the rat liver.  
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11 Although we did not examine the causes of the changes in this study, the changes in  
12 glycolysis, gluconeogenesis, ketone body metabolism, fatty acid metabolism, and lipid metabolic  
13 processes due to fasting would be possible causes. It would be of interest to investigate  
14 whether the expression of the target genes of these miRNAs is modulated because it has been  
15 reported that fasting changes the expression of various genes in the liver (Zhang et al., 2011);  
16 in other words, it would be interesting to determine whether the changes in gene expression  
17 are due to direct effects of fasting or indirect effects due to changes in the miRNA expression  
18 profile.  
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29 We found that 27 and 22 miRNAs of the 125 miRNAs were dominantly expressed at  
30 the pericentral and periportal regions, respectively, in the normal rat liver. This finding is not  
31 surprising because it is well known that some genes or proteins show zonal expression. For  
32 example, drug-metabolizing enzymes are predominantly expressed at the pericentral region  
33 (Hailfinger et al., 2006), whereas cadherin and ATP-citrate lyase are predominantly expressed  
34 at the periportal region (Braeuning et al., 2006). It has been reported that such zonal  
35 expressions are due to differences in transcriptional regulation (Gebhardt, 1992, Jungermann,  
36 1995). Because the expression of miRNAs is regulated at the transcriptional level to a greater  
37 or lesser extent, the zonal expression of miRNAs may be due to differences in their  
38 transcriptional regulation. Additionally, differences in post-transcriptional regulation, i.e.,  
39 maturation step or degradation, may also contribute to the zonal expression. It would also be  
40 of interest to investigate whether such differences in miRNA expression between regions may  
41 cause differences in the expression of the target mRNAs between regions.  
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58 We evaluated the changes in miRNA expression in the liver caused by the  
59 administration of APAP or MP, which cause hepatocellular injury at the pericentral or  
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1 periportal regions, respectively. In APAP-treated rats, 45 and 30 miRNAs were changed at the  
2 pericentral or periportal regions, respectively, and in the MP-treated rats, 72 and 60 miRNAs  
3 were changed at the pericentral or periportal regions, respectively. It was interesting that  
4 miRNA expression was affected at the region where hepatocytes were not injured. Because  
5 we previously reported that 301 and 263 miRNAs were changed in the plasma of APAP- and  
6 MP-treated rats, respectively (Yamaura et al., 2012), this study demonstrated that the number  
7 of miRNAs changed in the liver is lower than that of miRNAs change in the plasma. In both  
8 groups, miRNAs that exhibit decreased expression were dominant in the liver compared with  
9 the miRNAs that presented increased expression. This finding is reminiscent of the fact that,  
10 in the plasma of rats with liver injury, the miRNAs that presented increased expression were  
11 dominant compared with the miRNAs that exhibited decreased expression (Yamaura et al.,  
12 2012), implying reciprocal changes in miRNAs between the liver and plasma. It is generally  
13 believed that miRNAs that are highly expressed in a tissue would be plasma biomarkers of  
14 tissue injury. However, an inverse association was observed for some miRNAs, but there were  
15 many exceptions (Table 5 and Fig. 5). For example, miR-155 was increased at the pericentral  
16 region and in the plasma in APAP-treated rats. miR-155 has been reported to be expressed in  
17 immune cells, including B cells (Rodriguez et al., 2007; Thai et al., 2007), T cells (Haasch et  
18 al., 2002), and macrophages (O'Connell et al., 2007; Taganov et al., 2006), and to be  
19 upregulated as a result of activation by immune stimuli, such as inflammatory cytokines. Thus,  
20 the increased level of miR-155 in the liver may be due to the activation of immune cells, and  
21 this may be reflected in the increased level of this miRNA observed in the plasma. As shown  
22 in Fig. 5, many of the changed miRNAs in the plasma, including miR-150 and miR-223, were  
23 not altered in the liver. Recently, Pritchard et al. (2012) reported that the circulating miRNAs  
24 are largely obtained from blood cells. It appears that miR-150 is the most abundant miRNA in  
25 lymphocytes, and miR-223 is the most abundant miRNA in platelets, neutrophils, eosinophils,  
26 and monocytes. Accordingly, the changes in the expression levels of these miRNAs in the  
27 plasma may reflect the increase of lymphocytes that migrated to the inflammation area.

1 In summary, the present study clarified that 1) certain miRNAs in the liver are  
2 differentially expressed between the pericentral and periportal regions, 2) fasting affects the  
3 expression of a set of miRNAs in the liver, 3) the miRNA expression in the liver is modulated  
4 not only in the injured region but also the non-injured region, and 4) a reciprocal relationship  
5 between the miRNAs that presented increased levels in the plasma and the miRNAs that  
6 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  $\pm$  SD (n = 4 to 8). \*\*Significantly different from the control group ( $P < 0.01$ ). The liver sections were stained with HE. Original magnification,  $\times 200$ . 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.

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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
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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.

miRNAs	Pericentral region			miRNAs	Periportal region		
	Fasted-ctrl (40 - Ct)	Ctrl (40 - Ct)	Fold		Fasted-ctrl (40 - Ct)	Ctrl (40 - Ct)	Fold
Fasted-ctrl > Ctrl (36 miRNAs)				Fasted-ctrl > Ctrl (52 miRNAs)			
<b>mmu-miR-222</b>	15.2	21.6	84.4	<b>mmu-miR-99a</b>	12.2	16.2	16.0
<b>mmu-miR-706</b>	8.6	13.2	24.3	<b>mmu-miR-100</b>	13.0	16.2	8.6
<b>mmu-miR-690</b>	14.8	19.0	18.4	<b>mmu-miR-467a*</b>	12.5	15.4	8.0
<b>mmu-miR-135a*</b>	10.4	14.5	16.0	<b>mmu-miR-706</b>	9.6	12.4	7.0
<b>mmu-miR-877*</b>	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
<b>mmu-miR-193*</b>	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
<b>mmu-miR-467a*</b>	12.3	15.2	7.5	<b>mmu-miR-135a*</b>	11.7	14.0	4.9
<b>mmu-miR-93*</b>	9.7	12.5	7.0	mmu-let-7c	15.6	17.9	4.9
rno-miR-664	13.6	16.4	6.5	<b>mmu-miR-125b-5p</b>	13.2	15.3	4.6
rno-miR-28*	8.6	11.3	6.5	<b>mmu-miR-690</b>	15.2	17.4	4.3
mmu-miR-21*	8.9	11.6	6.1	mmu-miR-328	13.6	15.6	4.3
<b>mmu-miR-378</b>	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	<b>mmu-miR-467b*</b>	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
<b>mmu-miR-678</b>	9.7	11.8	4.3	mmu-miR-26a	16.1	17.8	3.2
<b>mmu-miR-99a</b>	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	<b>mmu-miR-375</b>	12.5	14.1	3.2
mmu-miR-872*	11.2	13.1	3.5	<b>mmu-miR-193*</b>	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	<b>mmu-miR-93*</b>	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
<b>mmu-miR-375</b>	13.1	14.5	2.6	mmu-miR-29c	11.4	12.9	2.8
<b>mmu-miR-467b*</b>	9.0	10.4	2.6	<b>mmu-miR-222</b>	15.8	17.3	2.8
<b>mmu-miR-29a</b>	15.2	16.5	2.5	mmu-miR-26b	13.5	15.0	2.8
<b>mmu-miR-125b*</b>	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	<b>mmu-miR-29a</b>	14.7	16.1	2.8
<b>mmu-miR-100</b>	13.8	14.9	2.1	mmu-miR-181a	9.5	11.0	2.8
<b>mmu-miR-19a</b>	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
				mmu-miR-93	12.1	13.5	1.3
Fasted-ctrl < Ctrl (8 miRNAs)				Fasted-ctrl < Ctrl (2 miRNAs)			
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	<b>mmu-miR-877*</b>	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	<b>mmu-miR-19a</b>	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	<b>mmu-miR-678</b>	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	<b>mmu-miR-378</b>	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
				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			miRNAs	Number of miRNAs		
	CV	P	Fold		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	<u>Group A</u> <sup>a</sup> mmu-miR-685 <sup>a</sup> mmu-miR-322*  2 miRNAs ↑	<u>Group B</u> <sup>a</sup> mmu-miR-223 <sup>a</sup> mmu-miR-155 <sup>a</sup> mmu-miR-27a <sup>a</sup> mmu-miR-34c* <sup>a</sup> mmu-miR-142-3p <sup>a</sup> mmu-miR-877* <sup>a</sup> mmu-miR-467b* <sup>a</sup> mmu-miR-21* <sup>a</sup> mmu-miR-214 <sup>a</sup> mmu-miR-130a  14 miRNAs ↑	<u>Group C</u> <sup>a</sup> mmu-miR-93* <sup>a</sup> mmu-miR-467a*  2 miRNAs ↑
	±	<u>Group D</u> <sup>a</sup> mmu-miR-484 <sup>a</sup> mmu-miR-872 <sup>a</sup> mmu-miR-30d <sup>a</sup> mmu-miR-192  (4 miRNAs ↑)	<u>Group E</u> <sup>a</sup> rno-miR-28* <sup>a</sup> mmu-miR-27b <sup>a</sup> mmu-miR-22 <sup>a</sup> mmu-miR-29a <sup>a</sup> rno-miR-7a* <sup>a</sup> mmu-miR-15b <sup>a</sup> mmu-miR-200c <sup>a</sup> mmu-miR-106b <sup>a</sup> mmu-miR-195 <sup>a</sup> mmu-miR-143  62 miRNAs ↑, 1 miRNA →	<u>Group F</u> <sup>a</sup> mmu-miR-138 <sup>a</sup> mmu-miR-425* <sup>a</sup> mmu-miR-99b <sup>a</sup> mmu-miR-328 <sup>a</sup> mmu-miR-29b* <sup>a</sup> mmu-miR-494 <sup>a</sup> mmu-miR-150 <sup>a</sup> mmu-miR-181a <sup>a</sup> mmu-miR-24 <sup>a</sup> mmu-miR-99b*  11 miRNAs ↑, 2 miRNAs →
	Down	<u>Group G</u> <sup>a</sup> rno-miR-339-3p  1 miRNA ↑	<u>Group H</u> <sup>a</sup> mmu-miR-200b <sup>a</sup> mmu-miR-31 <sup>a</sup> mmu-miR-101b <sup>a</sup> mmu-miR-193* <sup>a</sup> mmu-let-7e <sup>a</sup> mmu-miR-122 <sup>a</sup> mmu-miR-148a <sup>a</sup> mmu-miR-194 <sup>a</sup> mmu-miR-31* <sup>a</sup> mmu-miR-29c  20 miRNAs ↑	<u>Group I</u> <sup>a</sup> mmu-miR-222 <sup>a</sup> mmu-miR-99a <sup>a</sup> mmu-miR-375 <sup>a</sup> mmu-miR-100 <sup>a</sup> mmu-miR-199a-3p <sup>a</sup> mmu-miR-188-5p  6 miRNAs ↑

Continued

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

<sup>a</sup> upregulated (↑), <sup>b</sup> downregulated (↓), and not changed (→) miRNAs in the plasma.



Continued

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

<sup>a</sup> upregulated (↑), <sup>b</sup> downregulated (↓), and not changed (→) miRNAs in the plasma.

Fig. 1

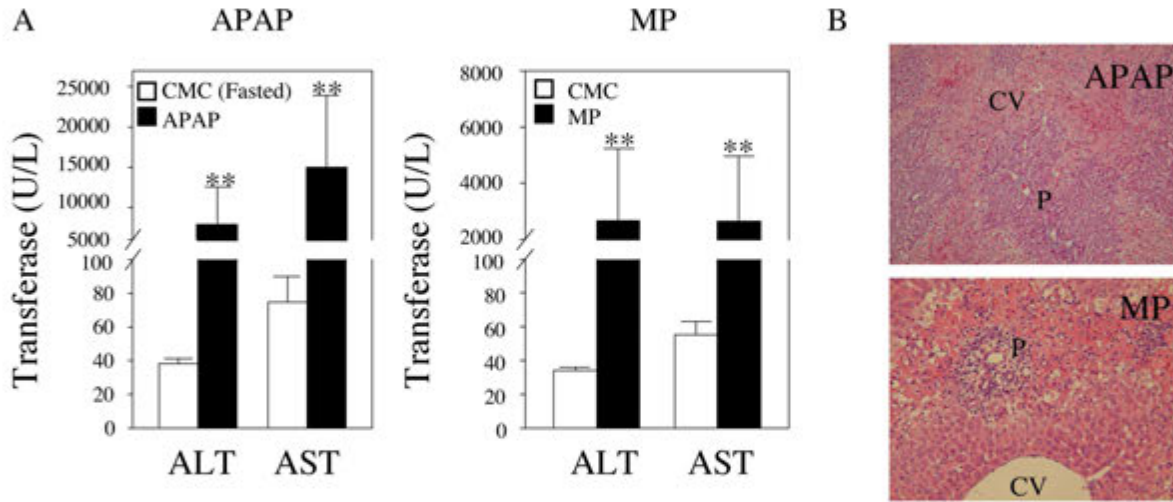


Figure 2

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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
mirna-miR-109									2.41			2.91
mirna-miR-122			0.75						0.35			0.47
mirna-miR-720												
mirna-miR-126-3p												
mirna-miR-191									0.37			
mirna-miR-146a						0.43						
mirna-miR-24									0.41			0.35
mirna-miR-10b									0.40			0.26
mirna-miR-192						2.27			0.31			0.31
mirna-miR-19b												0.41
mirna-miR-92a									0.48			
mirna-miR-16												0.44
mirna-miR-150						0.40						
mirna-miR-145									0.27			0.36
mirna-let-7c			0.24						0.29			0.58
mirna-miR-209			0.35						0.30			0.53
mirna-miR-17												0.46
mirna-miR-105a									0.46			
mirna-miR-20a									0.48			0.28
mirna-let-7c									0.27			0.51
mirna-miR-21			0.22						0.44			0.36
mirna-miR-139-5p												0.38
mirna-miR-193b												0.30
mirna-miR-126-5p									0.30			0.51
mirna-miR-194						0.29			0.51			0.21
mirna-miR-186												
mirna-miR-130												
mirna-let-7f									0.15			0.09
mirna-miR-203									0.36			0.31
mirna-miR-10a			62.71						0.34			0.48
mirna-miR-272						0.46			0.25			
mirna-miR-279												0.48
mirna-miR-10b*									2.09			
mirna-miR-10a*												0.42
mirna-miR-125a-5p												0.44
mirna-miR-10c												0.21
mirna-miR-574-3p												0.40
mirna-miR-208												0.28
mirna-miR-101a			0.39									
mirna-miR-690									0.51			0.22
mirna-miR-165									0.47			0.31
mirna-miR-195									0.43			0.48
mirna-miR-140									0.10			0.24
mirna-miR-21												0.16
mirna-let-7e			0.39									0.28
mirna-miR-145b									0.12			0.27
mirna-miR-133-3p									0.49			0.58
mirna-miR-152												0.13
mirna-miR-200b									0.33			0.36
mirna-miR-128									0.27			0.33
mirna-miR-138									0.33			0.36
mirna-miR-131-3p						0.48						0.19
mirna-miR-155			27.84									0.34
mirna-miR-125b-5p			0.44						0.36			0.17
mirna-miR-209*						0.36						0.17
mirna-miR-744									0.17			0.23
mirna-miR-100			0.27			0.17						0.36
mirna-miR-209			2.36									0.26
mirna-miR-664			0.39						0.31			0.27
mirna-miR-93									0.34			0.26
mirna-miR-214			2.81						0.27			0.25
mirna-miR-512-3p									0.05			0.37
mirna-miR-200c						3.35						0.20
mirna-miR-484						2.38			0.19			0.29
mirna-miR-30d												0.30
mirna-miR-375			0.16			0.25						0.20
mirna-miR-19a			0.49									0.29
mirna-miR-178			2.03						0.31			0.48
mirna-miR-143									0.19			0.38
mirna-miR-99a			0.16			0.19			0.35			0.27
mirna-miR-199a-3p			0.30			0.27			0.27			0.34
mirna-miR-101b			0.22						2.49			0.35
mirna-miR-203									0.24			
mirna-miR-73a*												0.32
mirna-miR-200a			0.31						0.32			0.27
mirna-miR-139-3p			0.46			2.57						0.50
mirna-let-7i									0.06			0.29
mirna-miR-15b									0.46			0.44
mirna-miR-27b									0.16			0.18
mirna-miR-142-3p			4.72						0.20			
mirna-miR-23*			0.49									
mirna-miR-685			167.89			2.65						
mirna-miR-467a*			2.55			0.43						0.07
mirna-miR-29c			0.30						0.48			0.44
mirna-miR-28												0.37
mirna-miR-109b									0.36			

miRNAs	Pericentral			Periportal			Pericentral			Periportal		
	Fasted Ctrl	APAP	Fold	Fasted Ctrl	APAP	Fold	Ctrl	MP	Fold	Ctrl	MP	Fold
mirna-miR-135-5p												0.34
mirna-miR-184												0.38
mirna-miR-99b*									0.44			0.11
mirna-miR-139a									2.60			0.18
mirna-miR-191*									0.24			0.45
mirna-miR-494									0.38			
mirna-miR-21*									0.29			0.39
mirna-miR-73												0.37
mirna-miR-872*												0.45
mirna-miR-497												0.43
mirna-miR-151-3p												0.43
mirna-miR-99b									0.33			0.47
mirna-miR-7a*												0.37
mirna-miR-872*									3.29			
mirna-miR-22												0.21
mirna-miR-202a						6.94						2.28
mirna-miR-135a*												0.21
mirna-miR-181a									0.42			0.24
mirna-miR-301b												0.24
mirna-miR-301a						2.04						0.44
mirna-miR-135-3p						2.06						0.20
mirna-miR-678												
mirna-miR-91*									2.26	0.39		5.81
mirna-miR-674*									0.30			2.52
mirna-miR-138-5p									4.57			6.04
mirna-miR-134*												0.44
mirna-miR-125b*												0.41
mirna-miR-467b*									3.52			
mirna-miR-125b*												4.69
mirna-miR-122*						4.70	3.12					5.10
mirna-miR-21*						3.09						2.01
mirna-miR-28*												6.40
mirna-miR-106												2.01
mirna-miR-206									0.49			0.22
mirna-miR-307*						3.89						6.10
mirna-miR-877									0.45			
mirna-miR-425*												0.44
mirna-miR-455*									0.32			

40-Ct



**Figure 3**  
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**Fig. 3**

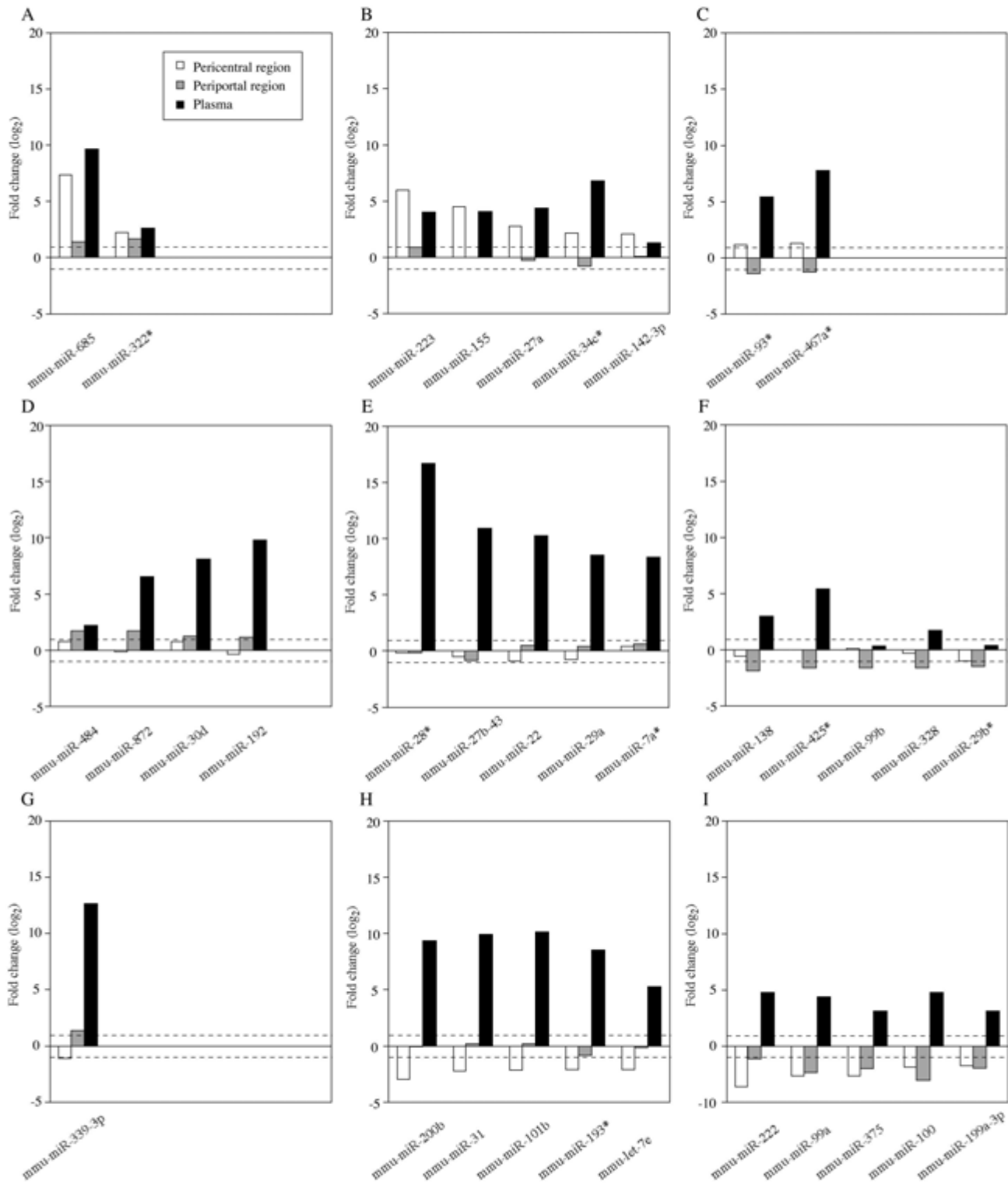


Fig. 4

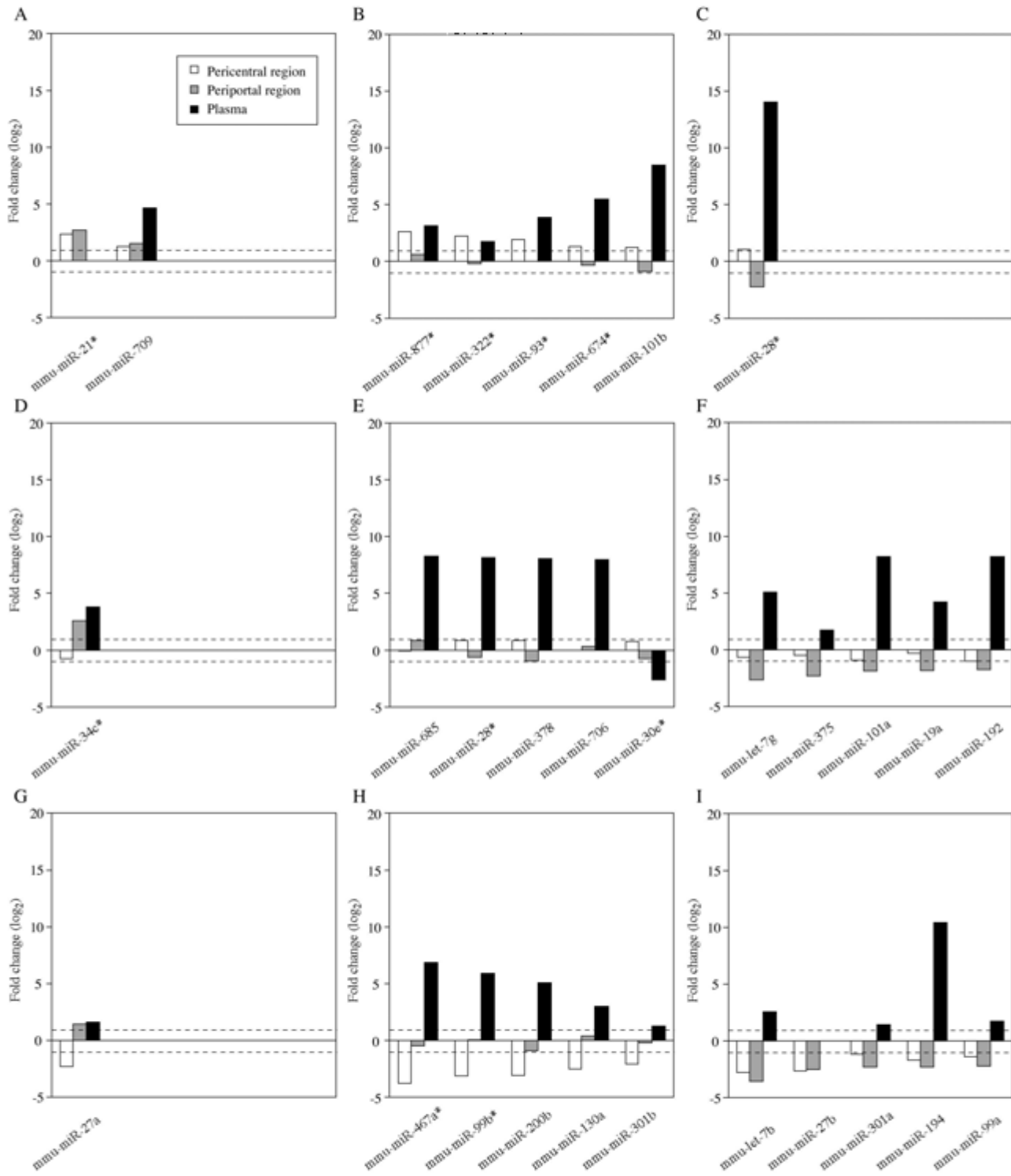


Fig. 5

