**Effects of a 28-day dietary co-exposure to melamine and cyanuric acid on the levels of
serum microRNAs in male and female Fisher 344 rats**

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Running Title: Serum miRNAs are altered by nephrotoxic doses of melamine and cyanuric acid
in rats

Abstract

We showed previously that a 28-day combined dietary exposure to melamine and cyanuric acid (MEL&CYA) induced kidney lesions in NCTR Fisher 344 (F344) rats. Histopathological changes were significant in females dosed with ≥ 240 ppm MEL&CYA and in males dosed with ≥ 180 ppm MEL&CYA; however, the nephrotoxicity biomarkers blood urea nitrogen (BUN) and serum creatinine (SCr) were increased only by ≥ 240 ppm MEL&CYA. The serum miRNome has been reported to reflect toxicity of several organs, including the kidney. Here, we compared the dose-response of alterations in serum miRNAs to those of BUN, SCr, and kidney histopathology in rats co-exposed to MEL&CYA. The serum miRNome of male F344 rats dosed with 0, 180, or 240ppm MEL&CYA was screened using quantitative real-time RT-PCR (qRT-PCR) and the levels of selected serum miRNAs were analyzed further in both sexes over the full dose-response. The levels of several miRNAs were significantly reduced in rats treated with 240ppm MEL&CYA versus control. In addition, miR-128-3p and miR-210-3p were decreased in males treated with 180ppm MEL&CYA, a dose at which the levels of BUN and SCr were not yet affected by treatment. These data suggest that the serum miRNome is affected by nephrotoxic doses of MEL&CYA in male and female rats.

Keywords: Serum miRNA; Kidney; BUN, SCr; Melamine; Cyanuric Acid.

1. Introduction

Melamine and cyanuric acid (MEL&CYA) are high production volume industrial chemicals with various applications. Melamine is commonly used in the manufacture of laminates, houseware items, and flame retardants (Crews *et al.*, 2006), while cyanuric acid is used as a chlorine stabilizer in swimming pool water and to prepare herbicides and dyes (Huthmacher and Most, 2003). Individually, MEL&CYA exhibit low toxicity (Choi *et al.*, 2010; Jacob *et al.*, 2011); however, the combined exposure to MEL&CYA results in the formation of melamine cyanurate crystals that can obstruct the nephron tubules and result in acute kidney injury (Dobson *et al.*, 2008; Puschner *et al.*, 2007). We reported previously that a 28-day daily dietary co-exposure to MEL&CYA induces dose-dependent nephrotoxicity in F344 rats, with males more sensitive than females (Gamboa da Costa *et al.*, 2012). Consistent with the kidney histopathological data, the levels of BUN and SCr were increased in a dose-dependent manner in both sexes; however, these biomarkers failed to reflect the nephrotoxic effects induced by 180ppm MEL&CYA in males (Gamboa da Costa *et al.*, 2012).

Changes in the serum miRNome have been suggested as sensitive biomarkers of nephrotoxicity (Nassirpour *et al.*, 2016; Pavkovic and Vaidya, 2016). MicroRNAs (miRNAs) are small non-coding RNAs that regulate protein-coding genes and play important regulatory roles in cellular processes (Bartel, 2004; Esau *et al.*, 2006; Shcherbata *et al.*, 2006; Shivdasani, 2006). These biomolecules are essential in kidney development and homeostasis, and are modulated in the pathogenesis of various renal diseases (Bhatt *et al.*, 2011; Chandrasekaran *et al.*, 2012). miRNAs can be released, actively or passively, from cells into blood and other biofluids, and cell-free miRNAs are stable and detectable in biofluids (Etheridge *et al.*, 2011; Schwarzenbach *et al.*, 2014). Because the levels of miRNAs in biofluids may reflect physiological changes

and/or specific pathological conditions, including of the kidney, they have the potential to serve as sensitive, minimally invasive biomarkers of toxicity and disease (Aguado-Fraile *et al.*, 2015; Bonventre *et al.*, 2010; Pavkovic *et al.*, 2015).

In the current study, we hypothesized that a 28-day dietary co-exposure to MEL&CYA alters the serum miRNome of F344 rats, and that these changes occur at nephrotoxic doses of MEL&CYA below those required to significantly increase the levels of BUN and SCr. Our data show that the serum miRNome is affected by MEL&CYA in a dose-dependent manner in both sexes and that two serum miRNAs (miR-128-3p and miR-210-3p) are reduced in males treated with 180ppm MEL&CYA, a dose that induced significant kidney histopathological changes, but did not increase the levels of BUN or SCr, in males. These serum miRNAs were also affected in males and females co-exposed to 240ppm MEL&CYA, but not in rats treated with 30, 60, or 120ppm MEL&CYA, doses below those needed to induce significant kidney lesions. These data suggest that a 28-day dietary co-exposure to MEL&CYA affects the serum miRNome in male and female rats and that serum miRNAs may serve as sensitive biomarkers of the nephrotoxicity induced by a co-exposure to MEL&CYA in rats.

2. Materials and Methods

2.1. Serum samples

The sera analyzed in the present study were obtained from the same animals used in Gamboa da Costa *et al.* (2012), where detailed information regarding the animal husbandry and treatment, kidney histopathology, and clinical chemistry analyses is described. Briefly, 10-week-old F344 rats (12 males and 12 females per dose group) were fed *ad libitum* for 28 days with NIH-41 irradiated meal containing 0, 30, 60, 120, 180, or 240ppm each of MEL&CYA. At the end of the

28-day exposure period, the animals were euthanized by carbon dioxide inhalation. Blood was collected by cardiac puncture into a serum separator tube, allowed to clot, and centrifuged at 1000 xg for 10 min to separate the serum. Serum was frozen at -80°C until further processing. All procedures involving care and handling of animals were reviewed and approved by the NCTR Institutional Animal Care and Use Committee (IACUC). All available serum samples were used in the current study; a few samples had insufficient volume of serum remaining after the clinical chemistry analyses (males: one 60ppm MEL&CYA and two 180ppm MEL&CYA; females: one 0ppm MEL&CYA, one 60ppm MEL&CYA, one 120ppm MEL&CYA, and one 240ppm MEL&CYA), resulting in a sample size of 10-12/sex/dose group.

2.2. Assessment of hemolysis

Given the potential confounding effect of hemolysis in the quantification of serum miRNome (Pritchard *et al.*, 2012), serum samples were assessed for the presence of free hemoglobin by measuring the absorbance at 414nm (A_{414}) using a Nanodrop 1000 Spectrophotometer (ThermoScientific, Waltham, MA). In addition, the serum levels of miR-451a, a miRNA abundant in erythrocytes (Kirschner *et al.*, 2011), and of miR-23a-3p, a miRNA not affected by hemolysis (Blondal *et al.*, 2013), were quantified by real-time qRT-PCR, as described below. Table 1 lists the Exiqon primer sets used to quantify these hemolysis controls and their respective Ct values (mean $\pm$ SD). Blondal *et al.* (2013) proposed that, in human serum/plasma, a ΔCt (miR-23a-3p minus miR-451a-5p) >5 indicates possible erythrocyte miRNA contamination, while a $\Delta\text{Ct} >7$ indicates a high risk of hemolysis affecting the data. Even though there are no indicator references reported for rodents, our data show $\Delta\text{Ct} <5$ for all the samples (Table 1).

2.3. RNA isolation, reverse-transcription, and quality control of the samples

Total RNA, including miRNAs, was extracted from 50 μ L serum using a miRCURYTM RNA Isolation Kit for Biofluids (Exiqon, Vedbaek, Denmark), according to the manufacturer's protocol. For each sample, 1 μ g MS2 RNA carrier (Roche Applied Science, IN, USA) and 1 μ L RNA Spike-in template mixture (miRCURY LNATM Universal RT microRNA PCR, RNA Spike-in kit, Exiqon) were added to 60 μ L Lysis Solution BF. RNA was eluted in 50 μ L of RNase-free water and stored in aliquots at -80°C. The carrier was used in order to enhance the RNA isolation efficiency. Three miRNA spike-ins (UniSp2, UniSp4, and UniSp5) were added for quality control of the RNA extraction efficiency. RNA was reverse-transcribed by using poly-A tailing (miRCURY LNATM Universal cDNA Synthesis Kit II, Exiqon), following the manufacturer's instructions. Two miRNA spike-ins (UniSp6 and cel-miR-39-3p) were added for quality control of the reverse transcription reaction. Table 2 lists the Exiqon primer sets used to quantify the miRNA spike-ins levels and their respective Ct values (mean $\pm$ SD); all miRNA spike-ins passed the quality control criteria recommended by the manufacturer.

2.4. Screening of serum miRNAs using Exiqon qPCR arrays

The levels of 752 miRNAs were quantified in the serum of male rats treated with 0, 180, or 240ppm MEL&CYA (n = 4/dose group) using MicroRNA Ready-to-use PCR panels (Mouse&Rat panels I + II, V3.M, Exiqon). Rox (Invitrogen by Life Technologies, NY, USA) was used as the passive reference dye. The qPCRs were run in an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems by Life Technologies, NY, USA) in a 384-well plate format. The amplification curves were analyzed using Sequence Detection Systems (SDS) software, version 2.4.1 (Applied Biosystems). A melting curve was generated for each template and

yielded a single peak per miRNA (data not shown). miR-342-3p was identified as a stable miRNA (minimum coefficient of variation and non-significant ANOVA *p*-value across dose groups), with a medium serum level (Ct ~ 30); its suitability as an endogenous miRNA normalizer both in male and female serum was confirmed further in the follow-up qRT-PCRs (n = 69-70/sex).

2.5. Quantification of serum miRNAs using Exiqon miRNA-specific assays

MicroRNA-specific and Locked Nucleic Acid (LNA)-enhanced forward and reverse primers (Exiqon, Table 1) were used to quantify the levels of selected serum miRNAs (n = 10-12/sex/dose group). Nuclease-free water (Life Technologies) was used as a no template control and a minimum of three technical replicates was run for each sample. All procedures were performed according to the protocol for the miRCURYTM LNATM Universal RT microRNA PCR (Exiqon).

2.6. Quantification of serum miRNAs using TaqMan miRNA-specific assays

TaqMan miRNA assays (Life Technologies, Table 1) were used to quantify the levels of selected serum miRNAs (n = 10-12/sex/dose group). cDNA was synthesized using a TaqMan[®] MicroRNA Reverse Transcription Kit (Life Technologies), according to the manufacturer's instructions. The cDNA was immediately used as a template in real-time qPCR using TaqMan[®] Universal PCR Master Mix II (2X), no AmpErase[®] UNG and TaqMan[®] Small RNA Assay (20x) (Life Technologies), following the manufacturer's protocol.

2.7. Data analysis

The relative levels of serum miRNAs (MEL&CYA-treated versus control) were calculated using the comparative $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001). One-way ANOVA followed by a posthoc Dunnett's test was used to compare differences between MEL&CYA-treated and control groups within sex. A p -value <0.05 was considered statistically significant. Data are presented as mean $\pm$ standard error.

3. Results

3.1. Characterization of the serum miRNome of male rats treated with nephrotoxic doses of MEL&CYA

Table 3 summarizes the findings previously reported by us for the same set of animals used in the current study (Gamboa da Costa *et al.*, 2012). A 28-day combined exposure to MEL&CYA induced significant, dose-dependent decreases in body weight, increases in kidney weight, increases in the levels of BUN and SCr, increases in the incidence of kidney histopathological lesions, and formation of melamine cyanurate crystals in the nephrons. These effects were observed in both sexes, but male rats were more sensitive to treatment, since 180ppm MEL&CYA was sufficient to induce significant kidney lesions in males, but not in females. In contrast, the levels of BUN and SCr were only significantly increased in males and females co-exposed to 240ppm MEL&CYA (Table 3). To assess if the levels of serum miRNAs were altered by MEL&CYA treatment in these rats, we first screened the serum miRNome of male rats co-exposed to 0, 180, or 240ppm MEL&CYA. The levels of several of the 752 serum miRNAs analyzed were significantly reduced in male rats treated with 240ppm MEL&CYA versus control (Supplementary Table 1). The levels of three of these miRNAs (miR-22-3p, miR-191a-5p, and miR-210-3p) were also >2 -fold less abundant in the 180ppm MEL&CYA dose

groups than in the controls, although these differences did not reach statistical significance (Supplementary Table 1). In addition, the levels of serum miR-128-3p were significantly lower (-3.22 ± 0.97 versus control) in the 180, but not 240, ppm MEL&CYA dose group (Supplementary Table 1). Of note, the levels of serum miRNAs that have been reported previously as biomarkers of toxicity for liver, such as miR-122-5p (Church *et al.*, 2016), heart, such as miR-208a (Chistiakov *et al.*, 2016; van Rooij *et al.*, 2007), or pancreas, such as miR-216a (Kong *et al.*, 2010), were similar across dose groups, consistent with the previously reported lack of toxicity of MEL&CYA in these organs (Supplementary Table 1).

To validate the screening data, the effect of 180 and 240ppm MEL&CYA on the levels of several serum miRNAs was analyzed independently using individual miRNA-specific assays and a larger sample size (Supplementary Figure 1). The subset of miRNAs analyzed in the confirmatory phase was selected among those identified as being significantly affected by treatment in the screening phase in order to encompass a range of fold-changes in their levels in the treated versus control groups. The levels of four selected miRNAs of interest (miR-22-3p, miR-128-3p, miR-191a-5p, and miR-210-3p; the serum miRNAs that showed >2-fold lower levels in the 180ppm MEL&CYA dose group versus control) were quantified further using a different qPCR chemistry (Figure 1A). Both qRT-PCR strategies confirmed the significantly reduced levels of miR-22-3p, miR-191a-5p, and miR-210-3p in males treated with 240ppm MEL&CYA versus controls (Figure 1A and Supplementary Figure 1). In addition, the decrease in the levels of miR-210-3p in the 180ppm MEL&CYA dose group versus control reached statistical significance, irrespective of the qPCR chemistry used. Unlike what was observed in the screening phase, the confirmatory qRT-PCR data showed also that miR-128-3p was

significantly reduced in a dose-dependent manner by both 180 and 240ppm MEL&CYA (Figure 1A and Supplementary Figure 1).

3.2. Assessment of the sex-specificity and dose-response of selected serum miRNAs of interest

In order to investigate whether the serum miRNA changes observed were male-specific, the levels of the miRNAs validated in males were quantified in the serum of females co-exposed to 0, 180, and 240ppm MEL&CYA (Figure 1B and Supplementary Figure 2). Similar to what was observed in males, the levels of serum miR-22-3p, miR-128-3p, miR-191a-5p, and miR-210-3p were decreased in a MEL&CYA dose-dependent manner in females, reaching statistical significance in the 240ppm MEL&CYA dose group. These data confirmed that the observed treatment dose-dependent decrease of these serum miRNAs and showed that these changes are not sex-dependent.

To verify further that the serum miRNA effects were observed only upon exposure to a nephrotoxic dose of MEL&CYA, the levels of the selected serum miRNAs of interest were analyzed in male and female rats co-exposed to three additional doses of MEL&CYA (30, 60, and 120ppm MEL&CYA), all below the dose needed to induce significant kidney lesions. Consistent with the absence of MEL&CYA-induced histopathological changes in the kidneys of these animals, no significant changes were observed in the levels of the serum miRNAs in either sex compared to the controls (Table 4). The only exception was a significant increase in the levels of miR-210-3p in males treated with 120ppm MEL&CYA; however, this observation was not confirmed when using TaqMan assays (fold-change in 120ppm MEL&CYA versus control, 1.17 ± 0.23 , $p > 0.05$; data not shown).

4. Discussion

In the current study, we assessed the levels of circulating miRNAs in the serum of male and female NCTR F344 rats fed for 28 days a diet containing different doses of MEL&CYA (0, 30, 60, 120, 180, or 240ppm each). We showed that the levels of several serum miRNAs were significantly reduced in animals co-exposed to nephrotoxic doses of MEL&CYA and that these decreases were dose-dependent, observed in both sexes, and consistent with the dose-response of the MEL&CYA-induced histopathological changes observed in the kidneys of these animals. Of note, the decrease of the serum miR-128-3p and miR-210-3p in males was observed at a dose of MEL&CYA that induced significant kidney lesions that were not diagnosed by BUN or SCr.

All serum miRNAs identified as of interest in our study have been reported previously as being modulated, sometimes decreased, in the context of kidney disease. miR-22 was found down-regulated in clear cell renal cell carcinoma (RCR) samples when compared to adjacent non-tumorous samples (He *et al.*, 2015). The expression of miR-128 was reported also as down-regulated in the kidneys of patients with progressive *versus* stable chronic kidney disease (Rudnicki *et al.*, 2016). In vitro studies in human embryonic kidney cells (HEK293T) suggest an anti-proliferative role for miR-128 through apoptosis regulation (Adlakha and Saini, 2011). The levels of miR-191 were found elevated in the serum of patients with RCR (Hauser *et al.*, 2012) and in the serum and kidneys of children with idiopathic childhood nephrotic syndrome (Lou *et al.*, 2013; Lu *et al.*, 2015). Decreased urinary levels of miR-210 have been proposed as a predictive marker for renal allograft rejection (Lorenzen *et al.*, 2011a). Additionally, serum miR-210 levels were reported to be decreased in chronic kidney disease (Neal *et al.*, 2011) and acute kidney injury (Aguado-Fraile *et al.*, 2015), whereas increased levels have been found in plasma

of acute kidney injury patients (Lorenzen *et al.*, 2011b). miR-210 has been suggested to affect the growth, migration, survival, and apoptosis of cells (Fasanaro *et al.*, 2008; Redova *et al.*, 2013; Yang *et al.*, 2015). *In silico* analysis for these miRNAs using the Ingenuity Pathway Analysis (QIAGEN, Redwood City, CA) identified target genes associated with renal inflammation and renal nephritis (data not shown).

In the current study, the levels of all miRNAs found to be affected by the MEL&CYA co-exposure were reduced in the serum of animals with kidney lesions. The mechanism for this decrease is currently unknown, but it may be due to down-regulation of the expression of these miRNAs in the kidney. Pavkovic and Vaidya (2016) reported that drug-induced kidney injury and acute nephron necrosis resulted in down-regulation in the expression of many kidney miRNAs. Another possibility could be the enhanced uptake of these circulating miRNAs by other cell types, given the presence of miRNAs in extracellular vesicles (Church *et al.*, 2016). Even though there was no kidney tissue available from the current study to investigate the miRNA expression levels in the MEL&CYA-treated rats versus controls, we found that all miRNAs of interest are expressed in the kidney of male and female NCTR F344 rats of similar age (data not shown). These data are consistent with those previously reported by Kwekel *et al.* (2015), who assessed the age and sex differences in the kidney miRNome of NCTR F344 rats.

In addition, the serum miRNome was analyzed at a single time point, when significant MEL&CYA-induced nephrotoxicity was already present. Hence, although our data support that these serum miRNAs may be better biomarkers of MEL&CYA-induced nephrotoxicity in rats than BUN or SCr, it remains to be explored if these serum miRNAs are able to diagnose nephrotoxicity at earlier stages. Future studies should assess the temporal dynamics of the serum miRNAs identified in this study and compare their dose- and time-response to that of BUN, SCr,

and kidney histopathology. These data would inform if the modulation of the serum miRNA levels by MEL&CYA precedes the induction of severe nephrotoxicity and would contribute to our understanding of the potential of these serum miRNAs to be used as early and/or sensitive biomarkers of MEL&CYA-induced nephrotoxicity.

5. Conclusions

In the current study, we assessed the levels of circulating miRNAs in the serum of male and female NCTR F344 rats fed for 28 days a diet containing different doses of MEL&CYA (0, 30, 60, 120, 180, or 240ppm each). We showed dose-dependent changes in the levels of serum miRNAs in male and female rats exposed to nephrotoxic doses of MEL&CYA. The reduced levels of serum miR-128-3p and miR-210-3p in males were observed at a dose of MEL&CYA that induced significant kidney lesions that were not diagnosed by BUN or SCr. Altogether, these data suggest that these serum miRNAs reflect, in a minimally invasive manner, the nephrotoxicity induced by co-exposure to MEL&CYA in rats.

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Figure 1. Quantification of the levels of selected serum miRNAs using TaqMan miRNA assays in (A) male and (B) female F344 rats (n = 10-12/dose group). Data were normalized to miR-342-3p and are presented as mean fold change $\pm$ standard error between MEL&CYA-treated and control groups. Statistically significant differences are indicated by *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (ANOVA, Dunnett's test).

Table 1. miRCURY LNA PCR primer sets and TaqMan miRNA assays used in the study. Ex, miRCURY LNA™ PCR primer set; Tq, TaqMan miRNA assay.

miRNA Name	miRBase Accession Number	Product Number	Ct value (Mean $\pm$ SD)
miR-22-3p	MIMAT0000077	Ex: 204606	30.7 $\pm$ 1.0
		Tq: 00398	28.1 $\pm$ 0.9
miR-23a-3p	MIMAT0000078	Ex: 204772	28.6 $\pm$ 1.1
miR-128-3p	MIMAT0000424	Ex: 205995	29.8 $\pm$ 1.3
		Tq:002216	24.5 $\pm$ 1.2
miR-142-5p	MIMAT0000433	Ex: 204722	31 $\pm$ 0.8
miR-144-3p	MIMAT0000436	Ex: 204754	29.1 $\pm$ 0.9
miR-191a-5p	MIMAT0000440	Ex: 204306	28.5 $\pm$ 1.3
		Tq:002299	22.3 $\pm$ 1.3
miR-210-3p	MIMAT0000267	Ex: 204333	31.2 $\pm$ 1.1
		Tq:000512	27.4 $\pm$ 0.9
miR-342-3p	MIMAT0000753	Ex: 205625	30.1 $\pm$ 0.5
		Tq:002260	25.8 $\pm$ 0.5
miR-451-5p	MIMAT0001631	Ex: 204734	28.1 $\pm$ 1.3

Table 2. miRCURY LNA™ PCR miRNA spike-in control primer sets used in the study.

miRNA Name	Product Number	Ct value (Mean ± SD)
cel-miR-39-3p, LNA control primer set, UniRT	203952	26.2 ± 0.5
UniSp2, LNA control primer set, UniRT	203950	19.7 ± 0.8
UniSp4, LNA control primer set, UniRT	203953	26.3 ± 0.8
UniSp5 LNA™ PCR primer set v2, UniRT	203955	33.6 ± 1.3
UniSp6 LNA™ PCR primer set v2, UniRT	203956	18.8 ± 0.4

Table 3. Summary of the findings previously reported in male and female F344 rats co-exposed to dietary MEL&CYA for 28 days (Gamboa da Costa *et al.*, 2012). H&E, hematoxylin and eosin stained slides; ✓, significant effect versus control ($p < 0.05$; ANOVA, Dunnett's test); -, no significant effect versus control.

Endpoint	MEL&CYA (ppm in feed)									
	Male					Female				
	30	60	120	180	240	30	60	120	180	240
Terminal body weight	-	-	-	-	✓	-	-	-	-	✓
Kidney weight	-	-	-	-	✓	-	-	-	-	✓
Kidney crystals, H&E	-	-	✓	✓	✓	-	-	✓	✓	✓
Kidney histopathology lesions	-	-	-	✓	✓	-	-	-	-	✓
BUN and SCr	-	-	-	-	✓	-	-	-	-	✓

Table 4. Dose-response of the changes in serum miRNAs levels in male and female F344 rats co-exposed to dietary MEL&CYA for 28 days (n = 10-12/dose group/sex). Data are presented as mean fold-change $\pm$ standard error; *, $p < 0.05$, # $p = 0.058$ (ANOVA, Dunnett's test) versus control.

MEL&CYA (ppm in feed)										
miRNA	MALE					FEMALE				
	30	60	120	180	240	30	60	120	180	240
22-3p	1.39 $\pm$ 0.34	-1.26 $\pm$ 0.31	1.64 $\pm$ 0.36	-1.81 $\pm$ 0.56	-4.20 $\pm$ 0.97*	-1.32 $\pm$ 0.38	1.37 $\pm$ 0.34	-1.03 $\pm$ 0.31	1.24 $\pm$ 0.33	-1.98 $\pm$ 0.53 [#]
128-3p	1.07 $\pm$ 0.38	-1.64 $\pm$ 0.46	-1.48 $\pm$ 0.51	-2.47 $\pm$ 0.74*	-5.96 $\pm$ 1.92*	-1.86 $\pm$ 0.50	-1.42 $\pm$ 0.45	-1.65 $\pm$ 0.54	-1.62 $\pm$ 0.53	-3.11 $\pm$ 0.91*
191a-5p	1.06 $\pm$ 0.40	-1.59 $\pm$ 0.36	-1.36 $\pm$ 0.53	-1.56 $\pm$ 0.48	-2.80 $\pm$ 0.65*	-1.48 $\pm$ 0.47	-1.38 $\pm$ 0.47	-1.64 $\pm$ 0.59	-1.37 $\pm$ 0.46	-2.56 $\pm$ 0.72*
210-3p	1.43 $\pm$ 0.22	-1.18 $\pm$ 0.15	1.67 $\pm$ 0.24*	-1.67 $\pm$ 0.38*	-2.79 $\pm$ 0.38*	-1.43 $\pm$ 0.35	-1.17 $\pm$ 0.28	-1.39 $\pm$ 0.37	-1.12 $\pm$ 0.25	-2.45 $\pm$ 0.65*

Figure 1