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Circulating microRNAs and sudden cardiac arrest outcomes

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Abstract

Aim—MicroRNAs (miRNAs) have regulatory functions in organs critical in resuscitation from sudden cardiac arrest due to ventricular fibrillation (VF-SCA); therefore, circulating miRNAs may be markers of VF-SCA outcome.

Methods—We measured candidate miRNAs (N=45) in plasma using qRT-PCR among participants of a population-based VF-SCA study. Participants were randomly selected cases who died in the field (DF, n=15), died in hospital (DH, n=15), or survived to discharge (DC, n=15), and, age-, sex-, and race-matched controls (n=15). MiRNA levels were compared using ANOVA, t-tests, and fold-changes.

Results—Mean age of groups ranged from 66.9 to 69.7. Most participants were male (53%–67%) and white (67%). Comparing cases to controls, plasma levels of 17 miRNAs expressed in heart, brain, liver, and other tissues (including miR-29c, -34a, -122, -145, -200a, -210, -499-5p, and -663b) were higher and three non-specific miRNAs lower (miR-221, -330-3p, and -9-5p). Among DH or DC compared with DF cases, levels of two miRNAs (liver-specific miR-122 and non-specific miR-205) were higher and two heart-specific miRNAs (miR-208b and -499-5p) lower. Among DC vs. DF cases, levels of three miRNAs (miR-122, and non-specific miR-200a

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and -205) were higher and four heart-specific miRNAs (miR-133a, -133b, -208b, and -499-5p) lower. Among DC vs. DH cases, levels of two non-specific miRNAs (miR-135a and -9-3p) were lower.

Conclusions—Circulating miRNAs expressed in heart, brain, and other tissues differ between VF-SCA cases and controls and are related to resuscitation outcomes. Measurement of miRNAs may clarify mechanisms underlying resuscitation, improve prognostication, and guide development of therapies. Results require replication.

Keywords

cardiac arrest; outcomes; epigenetics; microRNAs; epidemiology

INTRODUCTION

In sudden cardiac arrest due to ventricular fibrillation (VF-SCA), a minority of VF-SCA victims survive to hospital discharge¹. Trials of rapid defibrillation² and targeted temperature management³ have demonstrated that improvement in outcomes is possible when treatment is informed by understanding of disease pathophysiology. VF-SCA involves electrical, circulatory and metabolic changes in many cells and tissues, particularly of the heart and brain; however, risk factors (genetic or environmental) and molecular mechanisms underlying successful resuscitation are poorly understood.

Epigenetic regulation of genes involved in cellular remodeling, fibrosis, and cardiac conduction may play key roles in determining VF-SCA outcome⁴. One component of the epigenetic regulatory mechanism is microRNAs (miRNAs), an evolutionarily conserved class of non-coding RNAs that direct degradation of messenger RNAs or inhibit their translation to proteins⁵. Tissue and cell-specificity (including specificity for endothelial cells, neurons, cardiomyocytes, and hepatocytes) of miRNA expression has been well documented^{6, 7}. Additionally, miRNAs have been measured in the circulation, potentially serving as footprints of tissue changes or systemic alterations. For example, after myocardial infarction and stroke, higher levels of cardiomyocyte- and neuron-specific miRNAs are present in plasma, respectively^{8, 9}.

Previous studies have identified higher levels of three miRNAs 48 hours after cardiac arrest in the plasma of individuals with poor neurologic outcome (liver-specific miR-122, brain-specific miR-124, and a non-specific miRNA, miR-21)^{10, 11}. These studies did not measure miRNAs immediately following cardiac arrest, a critical period for therapy and prevention of complications. For this analysis, we measured circulating miRNAs regulating neurologic, circulatory, metabolic, or vascular processes and expressed in organs relevant to these systems, in peripheral blood of VF-SCA cases collected at the time of arrest and in healthy controls. We hypothesized that miRNA levels would differ between VF-SCA cases and population controls, as well as between VF-SCA cases with different outcomes: death without hospital admission, cardiac resuscitation alone, or both cardiac and brain resuscitation.

METHODS

Study setting and population

The study was conducted among participants of the Cardiac Arrest Blood Study (CABS). Details have been published previously¹². Briefly, CABS was a population-based case-control study of determinants of cardiac arrest in Seattle and King County, Washington. Cases in CABS were out-of-hospital cardiac arrest patients attended by paramedics between 1988 and 2002 with available blood samples. Cardiac arrest was defined as a sudden pulseless condition in the absence of evidence of a noncardiac cause. Emergency medical services incident reports, death certificates, and when available, medical examiner and autopsy reports, were reviewed to exclude patients with cardiac arrest attributable to a noncardiac cause. Cases were restricted to out-of-hospital arrests without clinically-diagnosed heart disease to minimize the possibility of bias from lifestyle changes as a result of the knowledge of presence of heart disease, were 25 to 74 years of age, and were not residents of a nursing home, to avoid misclassification as to cause of death. Race was ascertained from genotypes. Furthermore, cases were restricted to married individuals to obtain spousal information on risk factors and comorbidities. Controls were selected concurrently with cases from the community by random-digit dialing. For this study, fifteen healthy controls were selected at random from among the CABS controls and frequency matched by age, sex, and race to the combined case groups below. The University of Washington institutional review board approved the study, and participants or spouses provided written informed consent.

Cases belonged to one of three groups: died in the field (DF), died in the hospital (DH), or survived to hospital discharge (DC). We defined “successful cardiac resuscitation” as sustained return of spontaneous circulation (ROSC) following VF-SCA. This was defined as admission to hospital after arrest and corresponded to the combined groups DH and DC. We defined “brain recovery” as discharge from the hospital with favorable neurologic function (DC). Lastly, we defined “brain recovery given heart resuscitation” for cases who survived to discharge (DC) among cases who were admitted to the hospital (DH and DC).

Pre-processing and RNA extraction

For cases, samples were obtained at time of arrest. For controls, samples were obtained at time of interview. For both groups, specimens were collected at a single time point. Case and control samples were brought to the same lab, where they were kept at 4°C until processing, usually within 48 hours of the blood draw. Previous investigation has suggested that even in specimens classified as “cell free” (e.g., plasma), residual contamination by blood cells, which are reservoirs of miRNA, is a major problem^{13, 14}, requiring additional specimen processing steps. We therefore centrifuged thawed 1mL plasma aliquots at 1940g (3000 RPM) continuously for 10 minutes at 25°C in a Sorvall Legend RT centrifuge (Thermo Fisher Scientific, Waltham, MA). Supernatants were collected as platelet-poor plasma. Complete blood counts were performed on a Sysmex Automated Hematology Analyzer (Sysmex Corp., Kobe, Japan) and particle counts in the 0.4µM–10µM range measured using Beckman Multisizer™ 4 Coulter Counter® (Beckman Coulter Inc., Brea, CA). Levels of hemolysis were also visually assessed.

RNA was isolated and purified using the miRCURY™ RNA Isolation Kit (Exiqon, Woburn, MA) for miRNA expression measurements. We assessed the integrity, purity, and quantity of purified miRNA using spectrophotometry and an Agilent 2100 Bioanalyzer capillary electrophoresis system (Agilent Technologies Inc, Palo Alto, CA). To further assess quality of extracted RNA, we measured spike-in values of cel-miR-39.

microRNA selection, profiling, data processing, and normalization

We chose miRNAs that participate in neurologic, circulatory, metabolic, or vascular processes (Supplementary Table 1). We performed real-time polymerase chain reaction (qRT-PCR) assays at the Pritchard Laboratory at the University of Washington, where technicians were blinded to case-control status. To minimize the impact of batch effects, samples were run in 15 batches of 4, each batch containing 1 sample from the control and each of the case groups. A custom targeted panel of 45 experimental miRNAs and 3 control (cel-miR39, UniSp3, and UniSp6) assays was constructed using ExiqonLNA™primers. qPCR was conducted in duplicate using 384-well qPCR plates. Each 384-well qPCR plate had 48 assays repeated 8 times, so 8 total samples could be run per plate. Reactions were run on an ABI PRISM 7000 Real Time PCR machine (Applied Biosystems, Foster City, CA), using default cycling conditions. We recorded threshold cycle (C_T) values on two measurements per sample. True replicates were done in the sense that the original plasma samples were split, completely independent RNA preps were done, each replicate had an independent RT reaction, and each replicate was run on a different qPCR 384-well plate. C_T values of the duplicates differing by greater than 0.2 times the standard deviation were re-tested, and replicates were averaged for analyses.

Data from miRNA qRT-PCR arrays were imported into SDS Enterprise Software (V2.2.2, Applied Biosystems), and C_T values were calculated using a consistent thresholding value for each assay across all plates. Raw measurements were normalized using results from three spike-in control assays. UniSp3 (IPC) and UniSp6 (CP) were used for inter-plate calibration attributable to PCR and the RT step, respectively. In addition, samples were assayed for spike-in cel-miR-39-3p, as above. Levels were used to normalize across samples to control for variation attributable to RNA prep efficiency.

Statistical methods

We examined distributions of age, sex, and race according to case or control status. We used parallel coordinates plots of miRNA levels to visualize patterns of variability (i.e., 2 standard deviations above mean values) that differ by case-control status, platelet counts, or hemoglobin concentrations. We compared mean-normalized C_T values across the four groups using Student's t-tests as follows: First, we compared all cardiac arrests (DF, DH, and DC) to controls. Second, to identify miRNAs differentially expressed in successful cardiac resuscitation, we compared cases that were admitted to the hospital (DH and DC) to cases that died in the field (DF). Third, to identify miRNAs differentially expressed in successful brain resuscitation, we compared cases that were admitted and survived to discharge (DC) to cases that died in the field (DF). Fourth, we compared cases that survived to discharge (DC) to cases that died in the hospital (DH), to identify miRNAs differentially

expressed in “brain given heart resuscitation.” For these tests, which are reported as our primary results, $p < 0.05$ was used to determine significance.

For t-test comparisons with p -values < 0.05 , we repeated the comparison of means using permutation tests ($N = 100,000$ permutations), to provide inference free from major modeling assumptions¹⁵. MiRNAs with p -values < 0.00028 in these permutation tests (the Bonferroni-corrected threshold for the 180 t-tests we performed) were deemed statistically significant. Additionally, we calculated $\log_2$ -fold changes and related 95% confidence intervals using the normalized expression (C_T value) to quantify expression differences between comparison groups. We also used analysis of variance (ANOVA), to assess differences in miRNA levels across the groups. In these analyses, miRNAs with a p -value $< 0.05/45 = 0.0011$ (the Bonferroni-corrected threshold for the 45 ANOVAs we examined) were deemed statistically significant. Lastly, to assess the effect of platelet and hemoglobin variability across samples on our findings, we performed sensitivity analyses in which we repeated ANOVAs and t-tests excluding samples with values of platelet count and hemoglobin concentration ≥ 2 standard deviations above mean values. We used Stata version 12.1 (College Station, TX) and R (www.R-project.org), including the MASS package¹⁶. All tests were two-sided and statistical significance was defined as specified above.

RESULTS

Mean age of participants ranged from 66.9 to 69.7 across groups (Table 1). Fifty-three percent of participants were male in each of the case groups, while 67% were male in the control group. Sixtyseven percent were white. The majority of arrests were witnessed (73%), and fewer than half received bystander cardiopulmonary resuscitation (44%). Distribution of quality-control variables, including plasma platelet counts and free hemoglobin, were similar across case groups, but differed from respective values in the control group. There were eight participants with values ≥ 2 standard deviations (SD) above mean platelet or hemoglobin values. Parallel coordinates plots did not reveal systematic patterns of variability in miRNA expression profiles among samples with high plasma platelet counts or free hemoglobin values (not shown); therefore, we present findings from analyses using all participants as our primary results.

In our primary analyses (Table 2), circulating levels of 17 cardiomyocyte- (miR-133b, -208b, and -499-5p^{17, 18}), skeletal muscle- (-206¹⁹), hepatocyte- (-122), endothelium- (-34a²⁰), and epithelium-specific miRNAs (-205), as well as others that are poorly characterized or not tissue-specific (miR-10a, -16, -183, -200a⁶, -210, -29c²¹, -451, and -663b²²) were higher in VF-SCA cases compared with age-, sex-, and race-matched controls at $p < 0.05$. Circulating levels of three non-specific miRNAs (miR-221, -330-3p, and -9-5p) were lower among cases than controls ($p < 0.05$). When we compared mean-normalized C_T levels between cases and controls using t-tests with 100,000 permutations, at the Bonferroni-corrected threshold of 0.00028, levels of circulating miR-29c, -34a, -122, -145, -200a, -210, -499-5p, and -663b were higher among cases than controls. No miRNA had levels that were lower among cases compared with controls at this more rigorous significance level.

In comparisons between outcome groups, comparing participants who were admitted to the hospital (DC+DH), i.e., “successful cardiac resuscitation,” to those who died in the field (DF), levels of mean circulating hepatocyte-specific miR-122 and non-specific -205 were higher, while levels of cardiomyocyte-specific miR-208b and -499-5p were lower among the successful cardiac resuscitation group ($p<0.05$). Compared to those participants who died in the field (DF), those who survived to hospital discharge (DC group), i.e., “brain recovery,” had higher levels of hepatocyte-specific miR-122, and -200a and -205, both of which are non-specific, and lower levels of cardiomyocyte-specific miR-133a, -133b, -208b, and -499-5p ($p<0.05$). Among those admitted to the hospital, comparing participants who were discharged alive (DC group) vs. those who died in the hospital (DH group), i.e., “brain recovery given cardiac resuscitation,” levels of non-specific miR-9-3p and -135 were lower among the DC group ($p<0.05$). There were no differences between VF-SCA case groups at the Bonferroni-corrected threshold of 0.00028. In ANOVA, there were significant differences in the following miRNAs among the four groups at the Bonferroni-corrected p -value <0.0011 : miR-29c, -34a, -122, -145, -200a, -205, and -499-5p (Supplementary Table 2). Log₂-fold changes and 95% CI are shown in Supplementary Table 3.

In sensitivity analyses restricted to participants with free hemoglobin and platelet counts no more than 2SD greater than the mean, results were similar overall (Supplementary Table 4). There were some differences in levels of statistical significance, although we did not observe major shifts in comparisons between the groups (that is, higher to lower or lower to higher).

DISCUSSION

In this study, we found significant differences in circulating levels of miRNAs, including miRNAs predominantly expressed in cardiomyocytes, skeletal muscle, and hepatocytes, among VF-SCA cases compared with controls. At $p<0.05$, among cases that had “successful cardiac resuscitation” or “brain recovery,” levels of the hepatocyte-specific miRNA, miR-122, and the epithelial-specific miRNA, miR-205, were higher and cardiomyocyte-specific miRNAs (including miR-208b and -499-5p) were lower compared with cases that died in the field. Among the hospital.

Two previous studies evaluated circulating miRNA profiles in cardiac arrest cases in relation to resuscitation. Both compared participants with favorable and poor neurologic outcomes and were restricted to individuals who survived to hospital admission, a comparison most similar to our DC vs. DH comparison. Stammet et al. used microarray techniques to compare levels of 115 miRNAs 48 hours after arrest among SCA cases with good and poor Cerebral Performance Scale (CPC) scores ($n=28$)¹⁰. They reported higher levels of miR-21 and -122 among participants with poor outcomes. Gilje et al. compared levels of 20 miRNAs in 65 individuals with SCA and low and high CPC scores who survived to admission¹¹, reporting higher levels of miR-124 at 48 hours in participants with poor outcomes. We saw no difference in levels of miR-21 or -124 between DC and DH groups, and, counter to Stammet’s findings, levels of miR-122 were lower among participants who survived to discharge. Additionally, we identified lower levels of miR-9-3p and -135a in the DC vs. DH groups. Gilje also compared miRNA expression levels between controls and individuals with

SCA who survived to admission¹¹, reporting higher levels of miR-124 among cases at admission, which we did not find in case-control comparisons.

Several possibilities might account for these variable results. First, the groups studied are different. Previous studies included asystolic and pulseless electrical activity (PEA) arrests as well as VF-SCA. Second, the outcomes measured are different. Classification by CPC score includes both death and devastating neurologic injury as “poor outcomes,” while our method of classification restricted membership in the poor outcome group to participants who died. Additionally, the time between arrest and specimen collection was different. Previous studies presented 48-hour results, although Gilje et al. also compared samples obtained at admission and found no differences between the groups. Few studies have investigated time from event to presence of brain-specific miRNAs in the circulation; however, results consistently suggest that differences may be seen within hours, if not sooner. Redell et al.²³ and Balakathiresan et al.²⁴ measured differing levels of TBI-related miRNAs in human plasma at 24 hours and rat plasma at three hours after injury, respectively. To our knowledge, no studies have rigorously measured time to presence of miRNAs in the circulation.

In case-control comparisons, we detected differences in levels of miRNAs that are differentially expressed in a variety of disease states and cell types. Individually, they are not specific biomarkers for VF-SCA but together may present a distinctive pattern of systemic dysregulation subsequent to the disease condition. For example, miR-210 is widely expressed, mechanistically linked to several cellular targets, and thought to be sensitive to hypoxia in a variety of contexts²⁵. Similarly, changes in expression of the cardiomyocyte-specific miRNAs we report above have been demonstrated in a number of cardiac diseases, including myocardial infarction and heart failure^{26, 27}.

Among groups defined by resuscitation outcomes, differences in miRNA levels may reflect severity of injury. Among individuals who experienced “successful cardiac resuscitation” or “brain recovery,” lower levels of cardiomyocyte-specific miRNAs may reflect less injury to cardiac muscle. We were surprised, however, to see higher levels of the hepatocyte-specific miR-122, which could be interpreted to reflect a greater extent of liver injury in the setting of hypoxia and ischemia. One possibility, suggested by these results when considered in conjunction with findings by Stammett et al., is that there may be changes in levels of miRNAs across the time course after resuscitation. Clarification will require further studies and serial measurement of the miRNA after arrest. Among individuals who attained brain resuscitation given heart resuscitation, the absence of differences in expression of cardiomyocyte-specific miRNAs seen in the other comparisons may be due to the fact that the comparison group included only individuals who survived to admission. This is consistent with the findings of Stammett and Gilje, who also did not detect differences in levels of cardiac-specific miRNAs. These survivors also had lower levels of two other widely expressed miRNAs, not seen in other analyses, that warrant discussion. MiR-135a is widely expressed⁷ and is elevated in circulating monocytes of individuals with myocardial infarction compared to controls²⁸, suggesting it may be a marker of inflammation.

Our study has several strengths. First, we used a well-characterized population-based cohort, which allows broad generalizability. Second, we investigated both cardiac and neurologic outcomes. Third, we limited our population to VF cases. Previous studies have been more heterogeneous. Fourth, we obtained immediate post-arrest plasma, facilitating novel comparisons among cardiac arrest phenotypes. It also allowed comparisons with a previously unstudied population: individuals who died in the field. Fifth, rigorous quality-control methods reduced the impact of confounding by residual platelets or hemolysis, as demonstrated by our sensitivity analysis results, which are generally consistent.

Our study has limitations. First, we used a candidate-miRNA approach, which might exclude important miRNAs related to cardiac arrest or resuscitation outcomes. Our list was based on thorough literature review, however, and candidates were biologically plausible with appropriate cell-type specificity and mRNA targets. Second, length of resuscitation time was not available. Third, because of small sample sizes, we performed unadjusted analyses, therefore our findings are hypothesis-generating. Future studies should replicate the findings and assess effect modification by demographic or resuscitation-related factors. Lastly, we did not assess downstream effects or potential targets.

CONCLUSIONS

Our findings suggest a signature of circulating miRNAs in VF-SCA that may relate to resuscitation outcomes. Studies in larger and diverse study populations are warranted. Follow-up experiments investigating targets of differentially expressed miRNAs may clarify mechanisms underlying resuscitation. To explore potential prognostic value, future studies should include formal prediction analyses with validation in external cohorts. Better mechanistic understanding may improve prognostication and guide development of targeted therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographic and quality-control characteristics of age, sex, and race frequency-matched Cardiac Arrest Blood Study participants, stratified by case-control status

	Controln = 15	Died in the field n = 15	Died in the hospital n = 15	Survived to discharge n = 15
<i>Demographic variables</i>				
Age, years (SD)	67.7 (4.2)	68.1 (3.7)	69.7 (3.7)	66.9 (4.6)
Sex, % male (n)	67 (10)	53 (8)	53 (8)	53 (8)
Race, % white (n)	67 (10)	67 (10)	67 (10)	67 (10)
Arrest witnessed, % yes (n)	—	53 (8)	80 (12)	87 (13)
Bystander CPR, % yes (n)	—	33 (5)	53 (8)	47 (7)
Arrest after EMS arrival, % yes (n)	—	7 (1)	0	13 (2)
Time to EMS response, minutes (SD)	—	4.9 (1.9)	5.3 (1.5)	4.7 (2.2)
ROSC by end of EMS care, % yes (n)	—	0	87 (13)	100 (15)
<i>Quality-control variables</i>				
Plasma platelet count, 10 ³ /μL (SD) *	31.0 (48.0)	12.0 (13.0)	8.7 (7.7)	16.0 (13.2)
Plasma free hemoglobin, g/dL (SD) **	12.1 (11.4)	92.9 (144.4)	77.7 (76.7)	77.1 (102.9)
cel-miR-39-3p (spike-in)	13.8 (1.0)	13.8 (1.0)	13.8 (1.0)	13.8 (1.0)
UniSp3 (spike-in)	19.8 (0.8)	19.8 (1.2)	19.7 (1.0)	19.5 (1.0)
UniSp6 (spike-in)	19.4 (0.5)	19.5 (0.5)	19.5	19.6

Abbreviations: CPR (cardiopulmonary resuscitation), ROSC (return of spontaneous circulation). Values are expressed as mean (SD) or as totals (percent).

* Values missing for two participants (one control and one who survived to discharge)

** Values missing for one participant (survived to discharge)

Table 2

ANOVA, t-tests, and multiple permutation test results for differentially expressed microRNAs in cases and controls from the Cardiac Arrest Blood Study

Associated organ	miRNA	Cell type [‡]	All VF-SCA vs. controls				“Cardiac resuscitation” (DC+DH vs. DF)				“Brain recovery” (DC vs. DF)				“Brain given heart” (DC vs. DH)				ANOVA p-value
			Difference in means	t-test p-value	permutation test ** p-value		Difference in means	t-test p-value	permutation test ** p-value		Difference in means	t-test p-value	permutation test ** p-value		Difference in means	t-test p-value	permutation test ** p-value		
Brain	miR-135a	Not cell-type specific	-0.6	0.136			0.0	0.999			-0.4	0.370			-0.9	0.026	7.14E-02		0.114
	miR-183	Not cell-type specific	1.5	0.0004	4.48E-03		-0.5	0.452			-1.0	0.101			-1.1	0.101			0.007
	miR-330-3p	Not cell-type specific	-1.1	0.010	3.34E-01		0.0	0.972			-0.2	0.703			-0.4	0.340			0.068
	miR-9-5p	Not cell-type specific	-1.1	0.038	5.91E-02		-1.0	0.118			-1.4	0.059			-0.9	0.228			0.047
	miR-9-3p	Not cell-type specific	0.0	0.922			-0.6	0.255			-1.1	0.067			-1.0	0.029	3.42E-02		0.106
Cardiac	miR-133a	Myocyte	1.2	0.075			-1.6	0.088			-2.1	0.034	1.05E-02		-1.0	0.051			0.022
	miR-133b	Myocyte	1.4	0.037	3.35E-02		-1.5	0.099			-1.9	0.044	1.80E-02		-0.8	0.079			0.016
	miR-145	Ductal epithelial cell+/- adjacent endothelium	2.3	<0.0001	<1.00E-05		0.5	0.376			0.5	0.409			0.0	0.963			<0.0001
	miR-208b *	Cardiomyocyte	2.9	0.005	1.65E-03		-2.0	0.035	4.14E-02		-2.5	0.019	2.94E-02		-1.0	0.209			0.002
	miR-499-5p *	Cardiomyocyte	3.2	<0.0001	<1.00E-05		-1.9	0.016	1.39E-02		-1.9	0.017	2.81E-02		-0.2	0.760			<0.0001
	miR-663b	Brain?	2.4	0.0001	2.30E-04		-0.1	0.938			-0.3	0.757			-0.4	0.501			0.004
Fatty acid	miR-200a	Airway epithelial cell, neutrophil	2.8	<0.0001	<1.00E-05		1.2	0.056			1.7	0.023	1.89E-02		1.0	0.071			<0.0001
	miR-25	B-cell lineage	1.3	0.001	1.33E-02		-0.3	0.711			-0.6	0.459			-0.6	0.269			0.061
	miR-29c	Not cell-type specific	1.7	<0.0001	6.00E-05		0.1	0.915			-0.2	0.781			-0.5	0.178			0.001
	miR-34a	Endothelial cell, ductal cell, hepatocyte	2.9	<0.0001	<1.00E-05		0.2	0.762			0.2	0.696			0.1	0.789			<0.0001
Liver	miR-122	Hepatocyte	4.3	<0.0001	<1.00E-05		2.0	0.049	4.83E-02		2.5	0.026	2.97E-02		1.1	0.237			<0.0001
Skeletal muscle	miR-206	Myocyte	1.7	0.001	2.12E-02		-2.1	0.064			-1.8	0.115			0.6	0.371			0.005
Heme	miR-16	Neutrophil, red blood cell, B-cell lineage	1.3	0.0003	8.31E-03		-0.3	0.617			-0.7	0.374			-0.7	0.253			0.037
	miR-210	Natural killer cell	1.6	0.0006	1.90E-04		0.0	0.994			-0.2	0.738			-0.4	0.350			0.003
	miR-451	Red blood cell, monocyte, ductal epithelial cell	1.6	0.0001	2.33E-03		-0.4	0.579			-0.8	0.323			-0.7	0.203			0.012
Unspecified	miR-10a	Ductal epithelial cell+/- adjacent endothelium	1.1	0.026	3.93E-03		-0.2	0.623			-0.3	0.486			-0.2	0.391			0.037
	miR-205	Airway epithelial cell	1.2	0.0005	1.56E-02		1.2	0.032	1.88E-02		1.8	0.006	1.98E-03		1.2	0.054			0.001

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			Difference in means	t-test p-value	permutation test ** p-value	Difference in means	t-test p-value	permutation test ** p-value	Difference in means	t-test p-value	permutation test ** p-value	Difference in means	t-test p-value	permutation test ** p-value	
			-1.0	0.019	4.31E-02	0.0	0.960	-0.3	0.680	-0.7	0.162			0.155	
	miR-221	Adipocyte, fibroblast, endothelial cell, smooth muscle, lymphatic endothelial cell, myocyte													

* 7 controls have data on this miRNA

** p-value from 100,000 permutations

[‡]from Haider, et al., or Liang et al.

Bold formatting denotes p<0.05

Italic formatting denotes p<0.0011 (Bonferroni-corrected for 45 tests)