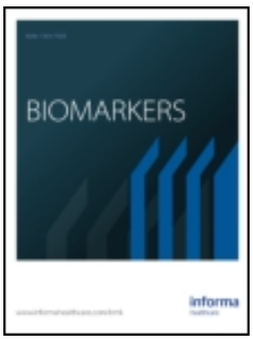

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RESEARCH ARTICLE

Short- and long-term changes in blood miRNA levels after nanogold injection in rats—potential biomarkers of nanoparticle exposure

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Abstract

Context: Increased use of engineered nanoparticles may result in exposure of workers and consumers, making them a health concern.

Objective: To identify potential blood miRNA biomarkers after intravenous gold nanoparticle (AuNP) exposure.

Materials and methods: miRNA microarray analysis was carried out on blood of rats at 1 week and 2 months after injection.

Results: Many up- and downregulated miRNAs were detected. Of these, rno-miR-298 was confirmed to be increased at 1 week postinjection by reverse transcription-PCR (RT-PCR).

Discussion and conclusion: Blood miRNAs could be useful as biomarkers for exposure to nanoparticles. miR-298 regulates β-amyloid (Aβ) precursor protein-converting enzyme-1 (BACE1) in Alzheimer's disease.

Keywords: Gold nanoparticles, intravenous injection, miRNA microarrays, blood biomarkers, health effects

Introduction

Increased industrial use of engineered nanoparticles can result in frequent exposure through inhalation, ingestion, or dermal contact during manufacture, use and disposal. Engineered nanoparticles possess greater surface-to-volume ratio and functionalities on their surfaces compared to fine particles, and could result in greater biological activity if taken into the body, making them a potential health concern (Gwinn & Vallyathan, 2006; Hoet et al., 2004). This suggests the need to monitor health effects in individuals at risk.

Gold nanoparticles (AuNPs) are some of the most widely used engineered nanoparticles, being employed in a wide range of products including cosmetics, hair tonics, conductive ink and lubricant oil (Balasubramanian et al., 2010a). In addition, their strong-binding affinity to thiols, disulfides, and amines facilitate their conjugation with biomolecules such as DNA and antibodies, and use in biomedical applications (Brown et al., 2007; El-Sayed et al., 2006; Ferrari, 2005; Katz & Willner, 2004). Previous studies have demonstrated the presence of AuNPs in various organs after intratracheal instillation (Lipka et al., 2010; Semmler-Behnke et al., 2008)/inhalation exposure (Yu et al., 2007) or intravenous injection (Balasubramanian et al., 2010a; De Jong et al., 2008; Hirn et al., 2011; Lipka et al., 2010; Semmler-Behnke et al., 2008). Studies using inductively coupled plasma mass spectroscopy (ICP-MS), show accumulation of

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AuNPs in the liver and spleen of rats at different time intervals after a single intravenous injection of the AuNP (Balasubramanian et al., 2010a). Moreover, AuNPs are detected in the blood of rats, 2 months after a single intravenous injection. Gene expression microarray analyses of the liver and spleen show changes in mRNAs related to "detoxification," "lipid metabolism," "cell cycle," "defense response," and "circadian rhythm," after a single intravenous injection of AuNPs (Balasubramanian et al., 2010a). In comparison, little is known about changes in blood after AuNPs exposure.

The discovery of microRNAs (miRNAs) has broadened an overall understanding of the mechanisms that regulate gene expression, with the addition of a new level of regulatory control (Bartel, 2004). These small, noncoding, single-stranded segments of RNA containing 19–24 nucleotides are posttranscriptional regulators of gene expression by reducing the transcription and/or translation of target mRNAs, thereby downregulating gene expression (Lee et al., 1993). Serum levels of miRNA have been proposed as biomarkers of diseases. miRNAs are well-protected from RNases and remain stable even after being subjected to harsh conditions (Chen et al., 2008; Mitchell et al., 2008). Therefore, a simple and relatively non-invasive method of obtaining serum samples, and their stability, make miRNA well-suited as blood biomarkers in patient samples (Chin & Slack, 2008; Wittmann & Jack, 2010). Recent studies have suggested the potential use of blood miRNAs as serum biomarkers for detection of cancer (Chin & Slack, 2008; Mitchell et al., 2008), acute myocardial infarction (Wang et al., 2010), diabetes (Zampetaki et al., 2010), or ischemic stroke (Tan et al., 2009). Till date, however, little is known about possible changes in blood/serum miRNAs after exposure to nanoparticles. The present study was carried out to elucidate miRNA changes in the blood, and to identify possible miRNA blood biomarkers in response to AuNP exposure in rats.

Materials and methods

Synthesis of AuNPs

Synthesis of AuNPs of 20 nm was carried out following established procedures (Balasubramanian et al., 2010b). In brief, 95 mL of tetrachloroauric acid ($HAuCl_4$) (Sigma-Aldrich, St Louis, MO, USA) containing 5 mg of Au was boiled at 100°C with vigorous mechanical stirring for 20 min. Fifty micrograms of trisodium citrate dihydrate (5 mL of 1% solution in water) (Sigma-Aldrich) was then added and boiled for an additional 20 min before the solution was gradually cooled to room temperature. AuNPs are formed during this process, and continue to grow until all the $HAuCl_4$ was fully reduced. The synthesized 20-nm AuNP solution was washed by repeated centrifugation and resuspension in ultra pure water to remove all non-AuNP components in the synthesized suspensions (Balasubramanian et al., 2010b). Finally, an aliquot (1 mL) of the AuNP suspension was centrifuged at 9,000 rpm (7,000*g*) for 20 min, the supernatant was removed, and the remaining AuNPs re-suspended in 1 mL of ultra pure water and used for experiments. Samples of the treated AuNP solution were mounted on Formvar-coated grids and viewed using a transmission electron microscope (CM120 Bio Twin; FEI–Philips, Hillsboro, OR, USA). Washed AuNPs exhibited a plasmon-resonance peaking at 520 nm, consistent with the particle sizes observed. To conduct intravenous injections, washed AuNPs were further diluted (2.6 times) using ultra pure water resulting in a mass concentration of 15.1 ± 1.3 mg/mL ($n = 24$) based on measurements

Table 1. Differentially expressed miRNAs in the blood at 1 week after a single intravenous injection of AuNPs.

miRNA	Fold change	Regulation	*p* value	miRNA	Fold change	Regulation	*p* value
rno-miR-298	2.34	Up	0.0064	rno-miR-98	3.23	Down	0.0098
rno-miR-664	2.21	Up	0.012	rno-miR-188	2.95	Down	0.031
rno-miR-409-3p	1.94	Up	0.010	rno-let-7f	2.93	Down	0.0048
rno-miR-92b	1.87	Up	0.027	rno-let-7c	2.76	Down	9.81×10^{4}
rno-miR-483	1.78	Up	0.018	rno-let-7b	2.72	Down	1.09×10^{4}
				rno-let-7a	2.56	Down	0.0027
				rno-miR-26b	2.49	Down	0.0098
				rno-miR-352	2.23	Down	0.023
				rno-let-7i	2.22	Down	9.90×10^{4}
				rno-miR-195	2.10	Down	0.0026
				rno-let-7d	2.06	Down	0.0022
				rno-miR-15b	1.94	Down	0.0081
				rno-miR-16	1.82	Down	0.0064
				rno-miR-146b	1.74	Down	0.045
				rno-miR-532-3p	1.72	Down	0.042
				rno-miR-27b	1.56	Down	0.0081
				rno-miR-23b	1.55	Down	0.0098
				rno-miR-20a	1.54	Down	0.023

These are miRNAs that show up- or downregulation at 1 week after a single intravenous injection of AuNPs, compared to controls.
AuNP, gold nanoparticle; miRNA, microRNA.

using ICP-MS (Perkin Elmer, Waltham MA, USA). All intravenous injections were completed within 2 h from the time of availability of washed AuNPs.

Animals and nanogold intravenous injection

Eighteen adult male Wistar rats (200–300 g each) were purchased from the Centre for Animal Resources, Lim Chu Kang, Singapore. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the National University of Singapore. A single tail-vein injection of 0.2 mL AuNPs (15.1 mg/mL) was administered over 30 s to rats. This dose was based on the blood level of Au, after a 15-day inhalation exposure to "roadside" level (2×10^6 #/cm^3) of aerosolized 20 nm-AuNPs (Yu et al., 2007). The average level of Au in the blood in these animals (18.7 ng Au/g blood) was used as a basis for calculating the total mass of AuNPs that need to be delivered by intravenous injection into a 200 g rat, assuming even distribution of AuNPs throughout the entire volume of the body (Balasubramanian et al., 2010a). Rats were randomly divided into three groups (six in each group) and sacrificed at two time points: 1 week and 2 months after AuNP. They showed no obvious abnormal behaviors after AuNP injection. A group of six animals that were not exposed to AuNPs was used as untreated controls. An additional eight rats were injected with 0.2 mL ultra pure water over 30 s as vehicle-injected controls. These rats were subsequently divided randomly into two groups (four in each group) and sacrificed at 1 week and 2 months after vehicle injection.

miRNA microarray data collection and analysis

Rats were deeply anaesthetized with intraperitoneal injection of anesthesia (200 mg/kg of ketamine + 40 mg/kg of xylazil) and blood obtained from the heart. miRNA expression profiles of the blood were analyzed using a rat miRNA microarray, 8 × 15K (Agilent Technologies, San Jose, CA, USA). Total RNA including the miRNA fraction was extracted from the blood using the miRNeasy Protect Animal Blood Kit (Qiagen, Hilden, Germany). The amount and purity of the miRNA were examined using the Nanodrop and Agilent 2100 Bioanalyzer. Total RNA was dephosphorylated, labeled, and each sample was hybridized to a single array according to standard Agilent protocols. A total of 12 microarrays were used, 4 for each time point and 4 for the controls. The raw data was exported into GeneSpring v11 (Agilent Technologies) software for analysis, and unpaired *t*-test with Benjamini Hochberg False Discovery Rate correction was used to identify differentially expressed miRNAs.

Real-time RT-PCR

AuNP-treated rats were compared with matching vehicle controls for both 1 week and 2 months time points. RNA samples were reverse transcribed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Singapore). Reaction conditions were 16°C for 30 min, 42°C for 30 min, and 85°C for 5 min. Real-time PCR amplification was then carried out in the 7500 Real-time PCR system (Applied Biosystems) using TaqMan Universal PCR Master Mix (Applied Biosystems) and miRNA-specific primers and probes rno-miR-298 (002598; Applied Biosystems), rno-miR-409-3p (002679; Applied Biosystems) and rno-miR-483 (001291; Applied Biosystems) according to the manufacturer's protocols. U87 (001712; Applied Biosystems) was used as an internal control. Primers and probes were synthesized by Applied Biosystems. The PCR conditions were: an initial incubation of 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All reactions were carried out in triplicate. The threshold cycle, CT, which correlates inversely with the levels of target mRNA, was measured as the number of cycles at which the reporter fluorescence emission exceeds the preset threshold level. The amplified transcripts were quantified using the comparative CT method described previously (Livak & Schmittgen, 2001), with the formula for relative fold change = $2^{-\Delta\Delta CT}$. The mean was calculated and possible significant differences analyzed using Student's *t*-test. $p < 0.05$ was considered significant.

miRNA target prediction

Databases for miRNA target prediction including miRBase (http://www.mirbase.org/) (Griffiths-Jones, 2006; Griffiths-Jones et al., 2008), miRDB (http://mirdb.org/) (Wang, 2008), and TargetScan (integrated into GeneSpring v11) (Agilent Technologies) that uses miRanda, MirTarget2, and TargetScan algorithm, respectively were used to identify predicted targets of the miRNAs. Common mRNAs from the three databases were then identified.

Results

miRNA microarray analysis

The miRNA expression profile of blood from rats 1 week after intravenous injection of AuNPs, showed significant changes in 23 miRNAs, with fold change >1.5 (p value < 0.05) compared to controls. Of the 23 miRNAs, 5 miRNAs were upregulated, and 18 miRNAs were downregulated (Table 1). Similarly, miRNA expression profile of blood from rats 2 months after intravenous injection of AuNPs, showed significant changes in 45 miRNAs, with fold change >1.5 (p value < 0.05) compared to controls. Of the 45 miRNAs, 7 miRNAs were upregulated and 38 miRNAs were downregulated (Table 2).

miRNAs that were altered in both the 1 week and 2 months time points were identified. miRNAs that were altered and unique to 1 week but not 2 months could represent miRNA that are important as acute biomarkers of nanoparticle exposure such as rno-miR-92b, and -664 (Figure 1 and Table 3). Likewise, miRNAs that were altered and unique to 2 months but not 1 week could represent miRNA that are important as long-term biomarkers of nanoparticle exposure such as rno-miR-214, -327, -466b, and -494 (Figure 1 and Table 3). Twenty-one

Table 2. Differentially expressed miRNAs in the blood at 2 months after a single intravenous injection of AuNPs.

miRNA	Fold change	Regulation	*p* value	miRNA	Fold change	Regulation	*p* value
rno-miR-327	3.70	Up	0.042	rno-miR-98	3.61	Down	0.031
rno-miR-214	3.47	Up	0.039	rno-miR-7a	3.12	Down	0.027
rno-miR-298	2.62	Up	0.0032	rno-miR-194	3.10	Down	0.035
rno-miR-466b	2.27	Up	0.0043	rno-let-7f	3.10	Down	0.0055
rno-miR-409-3p	2.04	Up	0.0044	rno-let-7b	3.04	Down	0.0022
rno-miR-494	2.02	Up	0.044	rno-let-7c	2.94	Down	0.0029
rno-miR-483	1.91	Up	0.015	rno-miR-352	2.92	Down	0.0044
				rno-let-7a	2.88	Down	0.0044
				rno-miR-26b	2.82	Down	0.0044
				rno-miR-195	2.72	Down	0.0043
				rno-miR-15b	2.45	Down	0.0029
				rno-miR-16	2.39	Down	0.0029
				rno-let-7d	2.37	Down	0.0055
				rno-let-7i	2.35	Down	0.0029
				rno-miR-144	2.29	Down	0.039
				rno-miR-19a	2.28	Down	0.029
				rno-miR-148b-3p	2.04	Down	0.0038
				rno-miR-320	2.04	Down	0.0043
				rno-miR-146b	2.00	Down	9.81×10^4
				rno-miR-27b	1.98	Down	9.81×10^4
				rno-miR-26a	1.98	Down	0.0029
				rno-miR-188	1.95	Down	0.022
				rno-miR-221	1.90	Down	0.029
				rno-miR-20a	1.82	Down	0.012
				rno-miR-23b	1.75	Down	0.0029
				rno-miR-222	1.71	Down	0.012
				rno-miR-532-3p	1.70	Down	0.0029
				rno-miR-451	1.69	Down	0.0089
				rno-miR-140	1.67	Down	0.039
				rno-miR-425	1.61	Down	0.0067
				rno-miR-181d	1.61	Down	0.019
				rno-miR-22	1.60	Down	0.030
				rno-miR-103	1.60	Down	0.022
				rno-miR-365	1.59	Down	0.012
				rno-miR-93	1.57	Down	0.021
				rno-miR-151	1.55	Down	0.0060
				rno-miR-92a	1.54	Down	0.0029
				rno-miR-30d	1.53	Down	0.039

These are miRNAs that show up- or downregulation at 2 months after a single intravenous injection of AuNPs, compared to controls.
AuNP, gold nanoparticle; miRNA, microRNA.

Figure 1. Venn diagram showing the number of common and different miRNAs that are expressed at the two different time points—1 week and 2 months.

differentially expressed miRNAs were in common between these two time points (Figure 1 and Table 4). These could represent miRNA that are important as both acute and chronic biomarkers of nanoparticle exposure.

miRNA validation using real-time RT-PCR

Common miRNAs that were upregulated with >1.5-fold at both 1 week and 2 months i.e. rno-miR-298, -409-3p, and -483, were selected for analysis using real-time reverse transcription-PCR (RT-PCR). There was a significant 3.46-fold increase in the expression of rno-miR-298 at 1 week after AuNP injection, whereas there was an insignificant 1.85-fold increase at 2 months after AuNP injection (Figure 2). Changes in rno-miR-409-3p and rno-miR-483 could not be verified by real-time RT-PCR.

miR-298 target prediction

Target prediction was carried out on the upregulated rno-miR-298 based on three independent miRNA target prediction databases—miRBase, miRDB, and TargetScan. A list of common targets predicted by all three algorithms was compiled to provide an indication of likely mRNA targets for the differentially expressed miRNAs. In this way, 17 predicted targets of rno-miR-298 were identified (Figure 3 and Table 5).

Table 3. Differentially expressed miRNAs in the blood that are unique to 1 week and 2 months, after a single intravenous injection of AuNPs.

1 Week			2 Months		
miRNA	Fold change	*p* value	miRNA	Fold change	*p* value
rno-miR-664	2.21	0.012	rno-miR-327	3.70	0.042
rno-miR-92b	1.87	0.027	rno-miR-214	3.47	0.039
			rno-miR-466b	2.27	0.0043
			rno-miR-494	2.02	0.044
			rno-miR-7a	–3.12	0.027
			rno-miR-194	–3.10	0.035
			rno-miR-144	–2.29	0.039
			rno-miR-19a	–2.28	0.029
			rno-miR-148b-3p	–2.04	0.0038
			rno-miR-320	–2.04	0.0043
			rno-miR-26a	–1.98	0.0029
			rno-miR-221	–1.90	0.029
			rno-miR-222	–1.71	0.012
			rno-miR-451	–1.69	0.0089
			rno-miR-140	–1.67	0.039
			rno-miR-425	–1.61	0.0067
			rno-miR-181d	–1.61	0.019
			rno-miR-22	–1.60	0.030
			rno-miR-103	–1.60	0.022
			rno-miR-365	–1.59	0.012
			rno-miR-93	–1.57	0.021
			rno-miR-151	–1.55	0.0060
			rno-miR-92a	–1.54	0.0029
			rno-miR-30d	–1.53	0.039

AuNP, gold nanoparticle; miRNA, microRNA

Discussion

The present study was carried out to determine miRNA changes in the blood, and to identify possible miRNA blood biomarkers in response to nanoparticle exposure, in a rat model of a single intravenous injection of AuNPs. Rats were intravenously injected with a single bolus dose of nanogold particles, and miRNA changes in the blood examined at 1 week and 2 months after injection. miRNA microarray analyses showed relatively large numbers of alteration in miRNA expression with fold change greater than 1.5 (p value < 0.05), at both time points after AuNPs injection compared to controls.

Changes in miRNAs were observed at 1 week after intravenous injection of AuNPs, with the majority of miRNAs showing downregulation. Moreover, miRNAs changes were still observed at 2 months after a single intravenous injection of AuNPs, indicating long-term effects of exposure to AuNPs. Changes in 23 miRNAs were detected in the blood of rats 1 week after a single intravenous injection of AuNPs, whereas changes in 45 miRNAs were detected in the blood of rats 2 months after a single intravenous injection of AuNPs. Twenty-one differentially expressed miRNAs were in common between these two time points. Two miRNAs, rno-miR-664, and -92b were unique to the 1 week time point and could be used as potential blood biomarkers for acute exposure to AuNPs. On the other hand, changes in 24 miRNAs were unique to the 2 months time point and could be long-term blood biomarkers for exposure to AuNPs. Decreased expression of miR-26a, -19a, and -22 in the

Table 4. Differentially expressed miRNAs in the blood that are in common at 1 week and 2 months, after a single intravenous injection of AuNPs.

miRNA	Fold change (1 week)	*p* value	Regulation	Fold change (2 months)	*p* value	Regulation
rno-miR-298	2.34	0.0064	Up	2.62	0.0032	Up
rno-miR-409-3p	1.94	0.010	Up	2.04	0.0044	Up
rno-miR-483	1.78	0.018	Up	1.91	0.015	Up
rno-miR-98	3.23	0.0098	Down	3.61	0.031	Down
rno-miR-188	2.95	0.031	Down	1.95	0.022	Down
rno-let-7f	2.93	0.0048	Down	3.10	0.0055	Down
rno-let-7c	2.76	9.80E-04	Down	2.94	0.0029	Down
rno-let-7b	2.72	1.10E-04	Down	3.04	0.0022	Down
rno-let-7a	2.56	0.0027	Down	2.88	0.0044	Down
rno-miR-26b	2.49	0.0098	Down	2.82	0.0044	Down
rno-miR-352	2.23	0.023	Down	2.92	0.0044	Down
rno-let-7i	2.22	9.90E-04	Down	2.35	0.0029	Down
rno-miR-195	2.10	0.0026	Down	2.72	0.0043	Down
rno-let-7d	2.06	0.0022	Down	2.37	0.0055	Down
rno-miR-15b	1.94	0.0081	Down	2.45	0.0029	Down
rno-miR-16	1.82	0.0064	Down	2.39	0.0029	Down
rno-miR-146b	1.74	0.045	Down	2.00	9.80E-04	Down
rno-miR-532-3p	1.72	0.042	Down	1.70	0.0029	Down
rno-miR-27b	1.56	0.0081	Down	1.98	9.80E-04	Down
rno-miR-23b	1.55	0.0098	Down	1.75	0.0029	Down
rno-miR-20a	1.54	0.023	Down	1.82	0.0070	Down

AuNP, gold nanoparticle; miRNA, microRNA.

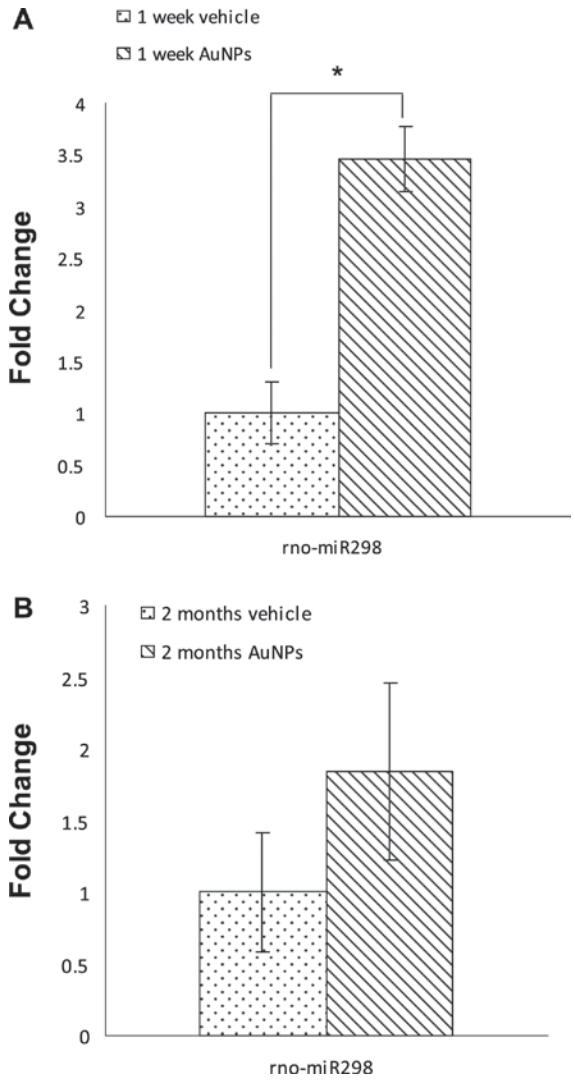

Figure 2. Real-time reverse transcription-PCR (RT-PCR) analysis of rno-miR-298 in the blood of rats at both (A) 1 week and (B) 2 months after a single intravenous injection of gold nanoparticles (AuNPs). One week vehicle: 1 week after a single intravenous injection of vehicle solution. One week AuNPs: 1 week after a single intravenous injection of AuNPs. Two months vehicle: 2 months after a single intravenous injection of vehicle solution. Two months AuNPs: 2 months after a single intravenous injection of AuNPs. Analyzed by Student's *t*-test. *Significant difference at p value of <0.05 (*).

blood at 2 months after nanogold injection are consistent with findings in the rat heart after exposure to particulate matter (Farraj et al., 2010). Downregulation of miR-26b in the blood at both 1 week and 2 months, and downregulation of miR-140, and -222 in the blood at 2 months after nanogold injection are consistent with results in the rat lung after exposure to cigarette smoke (Izzotti et al., 2009). Increased expression of miR-494 in the blood at 2 months after nanogold injection is consistent with findings that showed increased expression of miR-494 in human airway epithelial cells after exposure to diesel exhaust particles (Jardim et al., 2009). There appears to be a lack of data on blood miRNAs after exposure to the above particles, for comparison with this study.

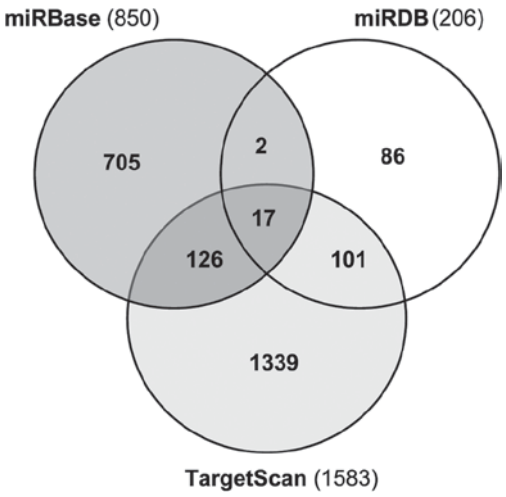

Figure 3. Predicted targets of rno-miR-298 were identified from miRBase, miRDB, and TargetScan databases. Seventeen predicted targets of rno-miR-298 were in common among these three databases.

Table 5. Targets of rno-miR-298.

miRNAs	Target gene symbol	Target gene name
rno-miR-298	*Abcg4*	ATP-binding cassette, subfamily G (WHITE), member 4
	Arhgdia	Rho GDP dissociation inhibitor (GDI) α
	Arl3	ADP-ribosylation factor-like 3
	Atp6v0e1	ATPase, H+ transporting, lysosomal 9 kDa, V0 subunit e1
	Btrc	β-Transducin repeat containing
	Cd14	CD14 molecule
	Eral1	Era (G-protein)-like 1
	Freq	Frequenin homolog
	Galnt2	UDP-*N*-acetyl-α-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase 2
	Ky	Kyphoscoliosis peptidase
	Mustn1	Musculoskeletal, embryonic nuclear protein 1
	Neo1	Neogenin homolog 1
	Ppp1r12b	Protein phosphatase 1, regulatory (inhibitor) subunit 12B
	Ssrp1	Structure specific recognition protein 1
	Stmn4	Stathmin-like 4
	Vdr	Vitamin D (1,25-dihydroxyvitamin D3) receptor
	Vpreb3	Pre-B lymphocyte 3

Common targets predicted by three independent miRNA target prediction databases—miRBase, miRDB, and TargetScan that uses miRanda, MirTarget2, and TargetScan algorithm, respectively.
miRNA, microRNA.

Common upregulated miRNAs were selected for validation as they could be more robust biomarkers compared to downregulated miRNAs, since any changes are unlikely due to miRNA degradation. Based on the real-time RT-PCR results, there were no significant differences in the

relative expression of rno-miR-409-3p and rno-miR-483 at both 1 week and 2 months time point (data not shown). In contrast, a significant increase in the expression for rno-miR-298 was found at 1 week after AuNP administration, compared to vehicle-injected controls. There was also a trend to an increase in rno-miR-298 expression at 2 months after AuNP injection, but this increase was not statistically significant. Thus, it is likely that rno-miR-298 represents a semi-acute blood biomarker of AuNP exposure.

One miRNA could potentially control the expression of a few to several thousand genes. Conversely, each mRNA could be affected by multiple miRNAs (Pillai, 2005). Target prediction was carried out on rno-miR-298 and 17 predicted targets of rno-miR-298 were identified. miR-298 was found to regulate the expression of β-amyloid (Aβ) precursor protein-converting enzyme-1 (BACE1) (Boissonneault et al., 2009; Provost, 2010), an enzyme involved in initiating Aβ generation leading to Alzheimer's disease (Vassar et al., 2009). Recently, miR-298 was shown to target specific binding sites in the 30-UTR of BACE1 mRNA and to regulate BACE1 protein expression in cultured neuronal cells (Boissonneault et al., 2009; Liu et al., 2010). It is possible that exposure to AuNPs may upregulate the expression of miR-298, which inhibits BACE1, thus reducing the formation of Aβ. This possibility should be explored in future studies.

Conclusions

In conclusion, this study suggests several possible potential biomarkers for both acute and chronic nanoparticle exposure. Further study is necessary to determine whether the effects of exposure to AuNPs are similar to that in humans, whether different routes of exposure result in changes to different miRNAs, and whether similar changes are found after exposure to other types of nanoparticles.

Declaration of interest

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