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Extracellular vesicle-enriched microRNAs interact in the association between long-term particulate matter and blood pressure in elderly men

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Abstract

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COMPETING INTERESTS

Authors declare no financial conflicts of interest.

Background—Several studies have shown that exposure to particulate matter (PM) may lead to increased systemic blood pressure, but the underlying biological mechanisms remain unknown. Emerging evidence shows that extracellular vesicle-enriched miRNAs (evmiRNAs) are associated with PM exposure and cardiovascular risk. In this study, we investigated the role of evmiRNAs in the association between PM and blood pressure, as well as their epigenetic regulation by DNA methylation.

Methods—Participants (n=22, men) were randomly selected from the Veterans Affairs Normative Aging Study (NAS).
Long-term (1-year and 6-month average) $PM_{2.5}$ exposure was estimated at 1×1-km resolution using spatio-temporal prediction models and BC was estimated
using validated time varying land use regression models. We analyzed 31 evmiRNAs detected in ≥90% of all individuals and for statistical analysis, we used mixed effects models with random intercept adjusted for age, body mass index, smoking, C-reactive protein, platelets, and white blood cells.

Results—We found that per each 2-standard deviations increase in 6-month $PM_{2.5}$ ambient levels, there was an increase in 0.19 mm Hg (95% Confidence Interval [95%CI]: 0.11, 0.28 mmHg; p<0.001) in systolic blood pressure (SBP). Per each 2-standard deviations increase in 1-year $PM_{2.5}$ levels, there was an increase in 0.11 mm Hg (95% Confidence Interval [95% CI]: 0.03, 0.19 mmHg; p=0.012) in SBP in older male individuals. We also found that both miR-199a/b (β=6.13 mmHg; 95% CI: 0.87, 11.39; $p_{interaction}$=0.07) and miR-223–3p (β=30.17 mmHg; 95% CI: 11.96, 48.39 mmHg; $p_{interaction}$=0.01) modified the association between 1-year $PM_{2.5}$ and SBP. When exploring DNA methylation as a potential mechanism that could epigenetically regulate expression of evmiRNAs, we found that $PM_{2.5}$ ambient levels were negatively associated with DNA methylation levels at CpG (cg23972892) near the enhancer region of miR-199a/b (β= −13.11; 95% CI: −17.70, −8.52; $p_{Bonferroni}$<0.01), but not miR-223–3p.

Conclusions—Our findings suggest that expression of evmiRNAs may be regulated by DNA methylation in response to long-term $PM_{2.5}$ ambient levels and modify the magnitude of association between $PM_{2.5}$ and systolic blood pressure in older individuals.

Keywords

Exosomes; miRNAs; DNA methylation; air pollution; blood pressure

1. Introduction

High blood pressure (BP) affects more than 25% of adults and causes ~9.5 million premature deaths each year worldwide.(Kearney et al., 2005) Among all cardiovascular risk factors, high BP has the highest attributable mortality(Lim et al., 2012) and is linked to preventable lifestyle and environmental factors, including air pollution.(Yamamoto et al., 2014)

Ambient particulate matter (PM) with an aerodynamic diameter of <2.5 μm ($PM_{2.5}$) are tiny air-suspended particles with well established toxic health effects.(Zanobetti et al., 2011) Black carbon (BC), a constituent of $PM_{2.5}$ and a surrogate for traffic-related pollution, has also been linked to human diseases in epidemiologic studies.(Chen et al., 2015; Highwood

and Kinnersley, 2006) Multiple studies have shown that exposure to PM leads to increases in systemic blood pressure (SBP).(Auchincloss et al., 2008; Wu et al., 2013) A recent meta-analysis has shown an increase of 1.39 mmHg (95% confidence interval (CI):0.87–1.91) in SBP and 0.90 mmHg (95% CI 0.49–1.30) in diastolic blood pressure (DBP) per 10 μg/m^3 increase in $PM_{2.5}$ ambient levels.(Liang et al., 2014) In addition, an interquartile range increase in long-term (1-year average) BC exposure (0.32 μg/m^3) has been associated with an increase of 2.64 mmHg in SBP (95%CI 1.47 – 3.80) and 2.41 mmHg (95% CI 1.77 – 3.05) in DBP.(Mordukhovich et al., 2009)

Several physiological responses have been implicated in the association between PM and cardiovascular diseases (CVD), including the direct effect of PM on blood vessels, (Tamagawa et al., 2008) autonomic dysfunction,(Chuang et al., 2007) and inflammatory response after breaching endothelial integrity by PM.(Nemmar et al., 2001) Extracellular vesicles (EVs) are a recently characterized mechanism of cellular communication whereby active biological signals are being transferred between cells.(Sadik et al., 2018) Several studies have linked EVs and their cargo, including microRNAs (miRNAs), to several CVD-related processes such as cardiac fibrosis, hypertrophy, and coagulation.(Bei et al., 2017; Pergoli et al., 2017) We have recently reported changes in the levels of EV-enriched miRNAs (evmiRNAs) in response to long-term $PM_{2.5}$ ambient levels (e.g., miR-126–3p, miR-199a/b, miR-223–3p, and miR-150–5p) and their implications in CVD-related biological pathways in older individuals.(Rodosthenous et al., 2016) In addition, Bollati et al. showed that the levels of evmiRNAs (miR-128 and miR-302c) are increased in the blood of metal workers in response to short-term exposure to higher PM levels.(Bollati et al., 2015) Other changes, including those in DNA methylation marks, have also been described associated to long-term PM exposure.(Baccarelli et al., 2009) Recently, Motta et al., showed that miRNAs in the blood may mediate the impact of short-term exposure to PM_{10} on blood pressure in obese individuals.(Motta et al., 2016) However, whether evmiRNAs are epigenetically modified by long-term $PM_{2.5}$ exposures, and play a role in the association between long-term $PM_{2.5}$ exposures and blood pressure has not been previously evaluated.

2. Material and methods

2.1. Study population

The profile of the Veterans Affairs Normative Aging Study (NAS) is described elsewhere. (Rodosthenous et al., 2016) We used existing DNA methylation data from NAS participants (n=563) who also had $PM_{2.5}$ and BC data between years 2000–2008. Out of those, we randomly selected 22 participants and analyzed 42 serum samples for evmiRNAs. All 22 participants had a serum sample analyzed for evmiRNAs at first visit (n=22), and ten out of these participants had two additional serum samples analyzed for evmiRNAs that were collected during their second and third visit. Therefore, the total number of samples with evmiRNA data is n=42. Details of the study design, characteristics of the study participants, blood collection, EVs isolation and miRNA extraction, and miRNA profiling of this study have been published previously.(Rodosthenous et al., 2016) We screened for 800 known evmiRNAs in each serum sample and for statistical power reasons we only analyzed 31 evmiRNAs that were detected in ≥90% of all individuals. SBP and DBP of study

participants were measured in seated position and we used the means of the measurements in both left and right arm. The NAS study was approved by the Institutional Review Boards of participating institutions (#14027–102) and all participants provided informed consent according to the Declaration of Helsinki.

2.2. $PM_{2.5}$ and BC exposure assessment

We estimated daily $PM_{2.5}$ ambient levels at 1×1-km resolution using our recently developed spatio-temporal prediction models that incorporate satellite aerosol optical depth data, local predictors and spatial smoothing, across the Great Boston Area.(Kloog et al., 2011; 2014) Ten-fold cross-validation showed very good model predictions with out-of-sample R^2=0.88 for $PM_{2.5}$. BC was estimated using a time varying land use regression (R^2=0.83 for daily measures).(Gryparis et al., 2007) We defined our $PM_{2.5}$ and BC ambient levels as the average of daily estimates corresponding to 6-month and 1-year at the participant's residential address prior to the date of visit (moving average) that each serum sample was analyzed (time-varying $PM_{2.5}$ and BC measurement).

2.3. DNA methylation in peripheral blood

Molecular mechanisms regulating the expression of evmiRNAs in the context of environmental exposures have been largely unexplored. To examine whether air pollution-associated evmiRNAs expression was epigenetically affected by DNA methylation in peripheral blood,(Soto-Reyes et al., 2012) we utilized 450K methylation (Infinium HumanMethylation450K BeadChip, Illumina, San Diego, CA) data from the NAS study. More specifically, we determined the association between long-term $PM_{2.5}$ (moving average) ambient levels and DNA methylation levels in CpG sites located only within 2Mbp adjacent to promoter regions of the $PM_{2.5}$/BC-associated evmiRNAs. A total of 1,022 blood samples collected from 563 participants between 2000 and 2008 were analyzed for DNA methylation using the Infinium HumanMethylation450K BeadChip. Each participant has between one to four DNA methylation measurements from follow up visits (mean=2.2 measurements per participant). DNA methylation profiles were calculated using the β-mixture quantile normalization (BMIQ) method. Control samples were included in each plate and were analyzed for batch effects. Cross-reactive probes, probes with detection p-value>0.01, or those associated to single nucleotide polymorphisms were excluded from the analysis.

2.4. Statistical analysis

To improve normality in the residuals, we log-transformed (log_2) evmiRNAs data. Multivariate analysis was conducted between long-term (6-month and 1-year) $PM_{2.5}$ and BC moving averages and evmiRNAs. For the association between $PM_{2.5}$ and BC ambient levels and evmiRNAs, we included age (continuous), body mass index (continuous), tobacco smoking (current, never, former), number of platelets (continuous), C-reactive protein (≤10, >10), and white blood cell distribution (% of lymphocytes, monocytes, eosinophil, and basophil cells) as confounders. To determine associations between $PM_{2.5}$ and BC ambient levels and evmiRNAs, we used mixed effects models with random intercept and we adjusted for multiple comparisons using Bonferroni correction.(Noble, 2009) Statistical significance after Bonferroni correction was defined p-value<0.05. All analyses were performed in SAS

9.4 software (SAS Institute Inc., Cary, NC). For methylation analysis, we used mixed models, in which the independent variable was 1-year $PM_{2.5}$ ambient levels and as dependent variable the methylation values at 8,903 CpG sites for 6-month and 4,096 CpG sites from $PM_{2.5}$-associated evmiRNAs (the number and the miRNAs were different for each model) as independent variables, using the same set of covariates and batch for adjustment. All p-values were adjusted for multiple comparisons using the Bonferroni method. DNA methylation analysis was done in R software (R Project for Statistical Computing, Wien, packages CpGassoc, minfi, and methylumi). *In silico* analysis to identify experimentally validated mRNA targets and their biological relevance was performed using Ingenuity Pathway Analysis (Ingenuity Systems®, Redwood City, CA).

3. Results

3.1. Characteristics of study participants

Study participants with evmiRNAs data available (n=22) were all males between 60 and 82 (mean=72.63, SD= 6.31) years old, 18/22 had a body mass index (BMI) ≥25 (mean BMI=26.72, SD=2.89), and 72.73% were former smokers, at baseline (Table 1). Study participants with DNA methylation data available, (n=563) were all males between 50 and 90 (mean=75.46, SD= 6.86) years old, 436/563 had a body mass index (BMI) ≥25 (mean BMI=27.43, SD=4.09), and 68.38% were former smokers, at baseline (Table 1).

3.2. Associations of long-term $PM_{2.5}$ and BC ambient levels, evmiRNAs, and SBP

We found that evmiRNAs let-7g-5p, miR-1246, miR-126–3p, miR-142–3p, miR-150–5p, miR-15a-5p, miR-199a/b, and miR-223–3p were significantly associated with $PM_{2.5}$ 6-month ambient levels (Fig. 1, Panel a) and miR-130a-3p, miR-142–3p, miR-199a/b, miR-223–3p, and miR-23a-3p were significantly associated with $PM_{2.5}$ 1-year average (Fig. 1, Panel b). In these models, we used evmiRNA data from 36/42 samples because of missing $PM_{2.5}$ exposure values. The full list of the association estimates between long-term $PM_{2.5}$ and all evmiRNAs is shown in Supp. Table 1.

We also found that for each 2 standard deviations (2.76 $\mu g/m^3$) increase in 6-month $PM_{2.5}$, there was an increase of 0.19 mmHg (95% CI: 0.11, 0.28 mmHg; $p<0.01$) in SBP, whereas for each 2 standard deviations (2.39 $\mu g/m^3$) increase in 1-year $PM_{2.5}$ there was an increase of 0.12 mmHg (95%CI: 0.02, 0.19 mmHg; $p=0.01$) in SBP (Table 2). No associations were found between $PM_{2.5}$-associated evmiRNAs and SBP in study participants (Supp. Table 2). When we focused on the $PM_{2.5}$-associated evmiRNAs, we found significant interaction for miR-223–3p ($\beta=30.17$, SE=9.29, $p_{interaction}=0.01$ [no Bonferroni adjustment]) and borderline significant interaction (p-value lower than 0.1 and higher than 0.05) for miR-199a/b ($\beta=6.13$, SE=2.68, $p_{interaction}=0.07$ [no Bonferroni adjustment]) in the association of 1-year $PM_{2.5}$ ambient levels and SBP (Table 3). No associations were found between long-term BC and any of the evmiRNAs after multiple comparisons adjustment (Bonferroni) (Supp. Table 3). For the BC models, we used evmiRNA data from 33/42 samples because of missing BC exposure values.

3.3. MiR-223–3p and miR-199a/b target several proteins related to cardiovascular diseases

In silico analysis using experimentally validated findings showed that miR-223–3p targets several proteins involved in cardiovascular functions, including proliferation of cardiomyocytes (SLC8A1 and TGFBR3), dysfunction of the heart (MEF2C, SLC8A1, and CD36), blood vessels remodeling (LIF, FBXW7, and MEF2C), heart hypertrophy (IL6ST, LIF, HDAC4), vasculogenesis, abnormal morphology of blood vessels, hypertrophy of cardiac muscle and contractibility of heart ventricles (Fig. 2). In addition, *in silico* analysis showed that miR-199a/b may target proteins involved in heart fibrosis (PTGS2), coronary artery disease (PDE3B, MTOR), ventricular hypertrophy (PTGS2 and MTOR), among others (Fig.3).

3.4. Long-term PM2.5 ambient levels are associated with DNA methylation in the enhancer region near miR-199a/b promoter

We found that DNA methylation levels of CpG cg23972892 were negatively associated with both 6-month ($\beta=-4.61$, SE=1.69, pBonferroni<0.05) and 1-year ($\beta=-13.11$, SE=2.34, pBonferroni <0.01) PM2.5 ambient levels (Fig. 4). This CpG site is located in a region enriched with histone-3 acetylation in lysine 27 (H3K27Ac) on chromosome 19 within the nearest miR-199a/b promoter region (chr19:10,928,102–10,928,172) (Fig. 5), which we found to be positively associated with both 6-month and 1-year PM2.5 levels.

4. Discussion

In this study, we found that EV-associated miR-223–3p and miR-199a/b modify positively the association between 1-year $PM_{2.5}$ moving average ambient levels and blood pressure in older male individuals, suggesting that the magnitude of the effect of $PM_{2.5}$ levels on blood pressure differs depending on the levels of these evmiRNAs. *In silico* analysis showed that EV-associated miRNA-223–3p and miR-199a/b can potentially target several proteins implicated in important cardiovascular functions. We also found that higher 1-year $PM_{2.5}$ levels were associated with lower DNA methylation in an enhancer region near miR-199a/b promoter and higher expression levels of EV- associated miR-199a/b circulating in the blood of the study participants. Together, these findings suggest that the effect of $PM_{2.5}$ on blood pressure may be modified by $PM_{2.5}$-asssociated epigenetic and expression changes of evmiRNAs in the blood circulation.

We have previously showed that long-term (6-month and 1-year) ambient $PM_{2.5}$ levels were associated with increased levels of multiple evmiRNAs in serum, including miR-223–3p, miR-199a/b, miR-23a, miR-150, miR-15a, miR-191, and let-7a.(Rodosthenous et al., 2016) In addition, Bollatti et al. found that short-term exposure to occupational PM was associated with increased levels of evmiRNAs miR-128 and miR-302, showing also an *in vitro* dose-response release of miR-128.(Bollati et al., 2015) Short-term exposure to PM ($PM_{2.5}$ and PM_{10}) has also been associated with the number of platelet-derived and annexin V-binding EV release in peripheral blood.(Emmerechts et al., 2012) Studies from Liu et al. found that PM increases release of macrophages EVs and induces selective neurotoxicity.(Liu et al., 2015) Although evmiRNAs have a high potential to mediate cell-to-cell communication and

potentially might link $PM_{2.5}$ induced-damage and cardiovascular diseases, no previous study had reported the interaction of evmiRNAs between long-term $PM_{2.5}$ exposure and blood pressure, especially in older individuals.

In agreement with our findings, several studies have previously reported changes in miR-223 expression levels in response to $PM_{2.5}$, as well as other environmental exposures such as including smoking and ozone.(Herberth et al., 2014) In the blood, miR-223 is one of the most abundant miRNAs released primarily from monocytes, macrophages and platelets after cell activation.(Gatsiou et al., 2012; Johnnidis et al., 2008) In atherosclerotic mice models, endothelial cells express high levels of miR-223, which attenuates the toll-like receptor 4 (TLR4)-dependent inflammatory response, lipid accumulation, and show anti-angiogenic activity through the RPS6KB1/hif-1a and β1 integrin pathway.(Shi et al., 2013) Dai et al., found that miR-223 inhibits angiogenesis in ischemic cardiac microvascular endothelial cells, suggesting involvement in the RPS6KB1/hif-1a signaling pathway, which affects endothelial migration and proliferation.(Dai et al., 2014) In addition, Wang et al. have also reported that miR-223 protects cardiomyocytes against hypertrophy by targeting cardiac troponin I-interacting kinase.(Y.-S. Wang et al., 2015) Last, other studies have shown that miR-223 regulates macrophage polarization,(Zhuang et al., 2012) suppress tissue factor expression, regulates thrombosis on disrupted atherosclerotic plaques, and inhibits its pro-coagulant activity.(Li et al., 2014) Altogether, these findings suggest that changes in miR-223 expression in the blood in response to toxic environmental exposures may modulate their effect on human health, including the effect of $PM_{2.5}$ on blood pressure.

We also found that EV-associated miR-199a/b expression levels are positively associated with long-term $PM_{2.5}$ levels. To the best of our knowledge no previous studies have associated miR-199a/b to $PM_{2.5}$ ambient levels, except from our previous study in the same population where increased levels of miR199a/b were associated with higher long-term $PM_{2.5}$ ambient levels.(Rodosthenous et al., 2016) In the present study, we used a higher resolution approach (1×1 km model) to assess $PM_{2.5}$ levels, confirming that miR-199a/b is associated with long-term $PM_{2.5}$ levels. In addition, we found that miR-199a/b is a potential effect modifier in the association between $PM_{2.5}$ and SBP. However, other exposures have been associated with this miRNA, including ozone(Fry et al., 2014) and in occupational workers exposed to perfluorooctanoic acid, suggesting a potential role as stress-induced biomarker.(J. Wang et al., 2012) This stress-induced expression may have a beneficial effect in the reduction of cardiovascular damage. In studies from Jansen et al, EV-associated miR-199a has been associated with a lower risk of major adverse cardiovascular events and revascularization in patients with stable coronary disease. They also demonstrated that the major source of EV-associated miR-199a was platelets.(Jansen et al., 2014) But stress-induced miR-199a overexpression may contribute also to angiogenesis and cardiac regeneration. Shatseva et al. have reported that miR-199a-3p promotes proliferation and survival of endothelial cells.(Shatseva et al., 2011) Remarkably, miR-199a promoted cell cycle re-entry of adult cardiomyocytes *ex vivo* in animal models, and promoted cardiomyocyte proliferation in both neonatal and adult animals. In fact, after myocardial infarction, miR-199a stimulated marked cardiac regeneration and almost completed recovery of cardiac functional parameters.(Eulalio et al., 2012) In cardiac hypoxia models, blocking miR-199a improves cardiac function and restores mitochondrial fatty acid oxidation.

(Azzouzi et al., 2013) These findings suggest a potential beneficial effect of miR-199 after $PM_{2.5}$ exposure and its effect on blood pressure.

To further explore whether the increase we observed in expression levels of $PM_{2.5}$-associated evmiRNAs was epigenetically regulated, we examined the association between $PM_{2.5}$ levels and DNA methylation levels in the promoter regions and up to 2Mbp adjacent to the promoter of each $PM_{2.5}$-associated evmiRNAs. We found a negative association between both 6-month and 1-year $PM_{2.5}$ ambient levels and DNA methylation in a CpG site (cg23972892) near miR-199a/b promoter. To our knowledge, this is the first study showing an epigenetic change linked to microRNAs in the context of PM levels. *In silico* analysis co-localized this CpG site with histone 3 acetylation in lysine 27 (H3K27Ac), a known mark for active enhancers.(Guo et al., 2014) These findings suggest a link between $PM_{2.5}$ ambient levels and DNA methylation changes with potential downstream effects for evmiRNAs expression levels. Previous studies have reported similar associations between PM exposure and DNA methylation changes in both repetitive sequences and other genes, which are in agreement with our findings that increased $PM_{2.5}$ levels are associated with decreased DNA methylation levels.(Hou et al., 2010)

Our study has certain limitations. To maximize our ability to detect as many evmiRNAs as possible we decided to use all the available sample volume, such that we could not for further validate our data using another method (e.g., real-time qPCR). Nevertheless, Knutsen et al., reported a relatively high correlation range (r = 0.7–0.8) between miRNA levels when measured with NanoString nCounter® and and other platforms, including real-time qPCR. (Knutsen et al., 2013) Also, although there is a difference in sample size when analyzing $PM_{2.5}$ effect on evmiRNAs than $PM_{2.5}$ effect on DNA methylation, demographic and clinical characteristics were similar among participants (Supp. Tables 1 and 2), except for CRP values which were higher in the methylation dataset (mean: 3.48 [SD: 8.52] vs. 2.77 [SD: 4.11] in the microRNAs dataset) but did not reach statistical significance (p=0.056). We also acknowledge the fact that evmiRNAs and DNA methylation cellular origin was not determined in our study. However, we evaluated whether the number of red blood cells, white blood cells, and platelets could confound our data, and whenever appropriate we adjusted for it in the analysis.

Strengths of this study include the use of higher resolution (1×1-km) $PM_{2.5}$ and BC models, which enabled us to assess ambient levels in our study population more precisely. In addition, the combination of DNA methylation data from 1,022 blood samples (n=563 participants) with evmiRNA expression data from 42 serum samples in a smaller subset (n=22 participants) enabled us to investigate the impact of $PM_{2.5}$ at both the epigenetic and expression levels of $PM_{2.5}$-associated evmiRNAs.

Conclusions

In conclusion, our findings suggest that evmiRNAs (i.e., miR-223–3p and miR-199a/b) are positively associated with long-term $PM_{2.5}$ ambient levels and can modify the association between long-term $PM_{2.5}$ levels and blood pressure in older individuals. Our findings also suggest that long-term $PM_{2.5}$ levels is associated with lower DNA methylation levels in

regulatory regions of PM-associated evmiRNAs that may explain their increase in expression levels in the peripheral blood. We encourage further research to determine the interplay between $PM_{2.5}$, epigenetic regulation of $PM_{2.5}$-associated evmiRNAs, and their impact on blood pressure in older individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- PM leads to increases in systemic blood pressure (SBP) by unknown mechanisms.
- Long-term $PM_{2.5}$ ambient levels were associated with evmiRNAs levels.
- evmiRNAs levels modified the magnitude of the association between $PM_{2.5}$ and SBP.
- evmiRNAs involved between PM5 and SBP could be regulated byepigenetic mechanisms.

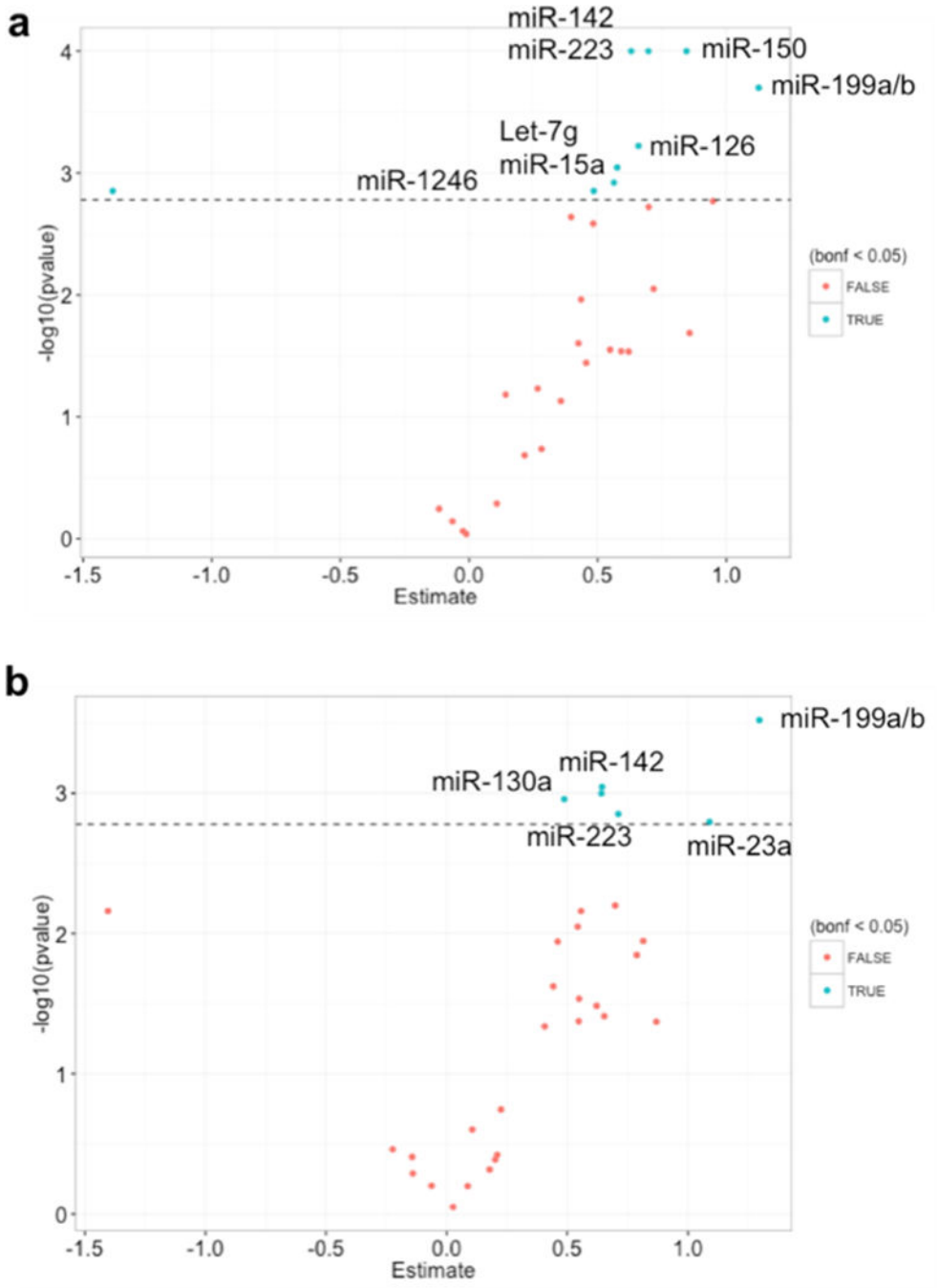

Fig 1.

Associations between 6-month and 1-year exposure to $PM_{2.5}$ ambient levels and extracellular vesicle-associated miRNAs (n=36). Dotted line represents the Bonferroni-adjusted threshold for statistical significance (a=0.05).

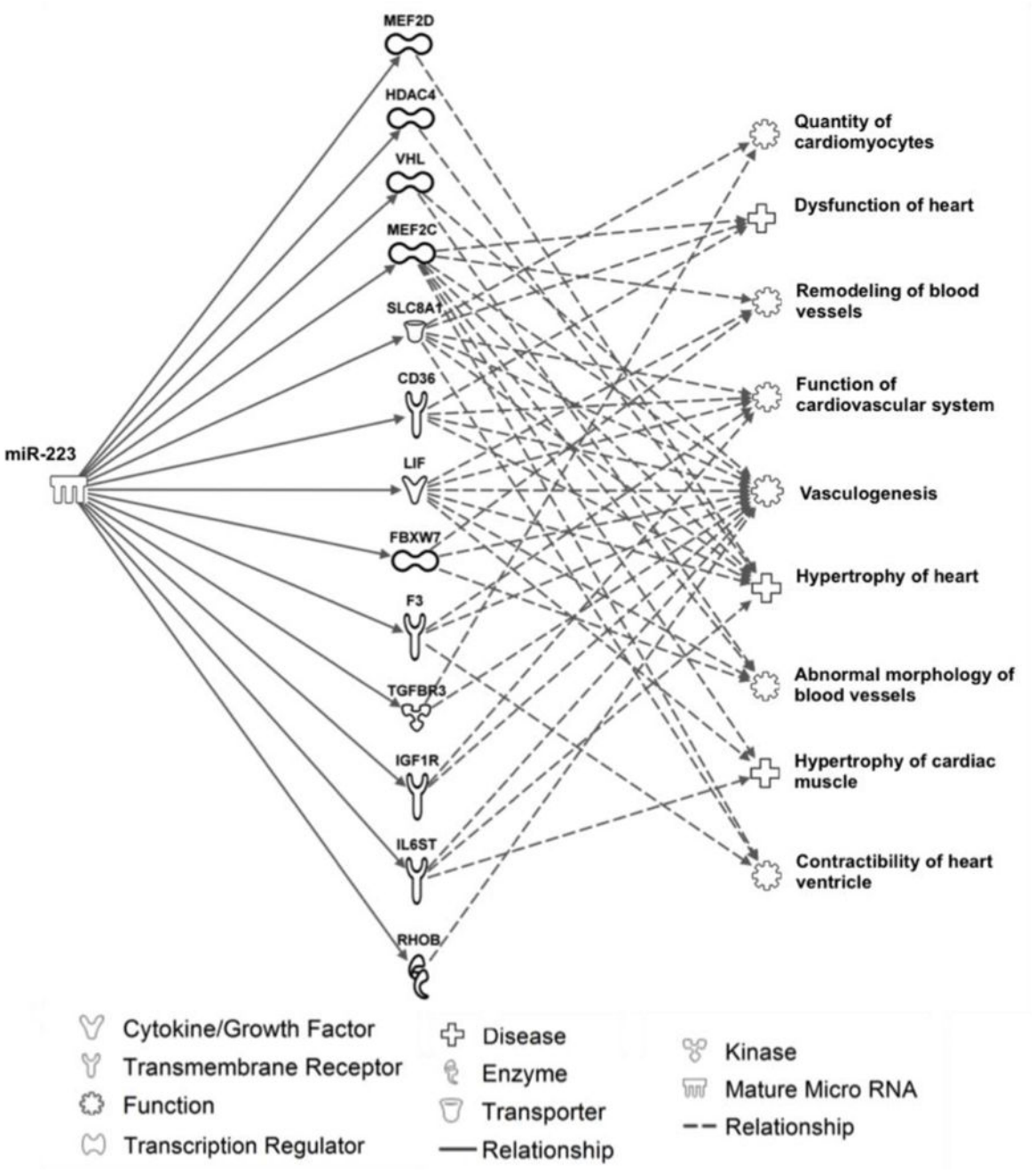

Fig 2.

In silico analysis of experimentally validated targets and cardiovascular pathways linked to miR-223 using Ingenuity Pathway Analysis®.

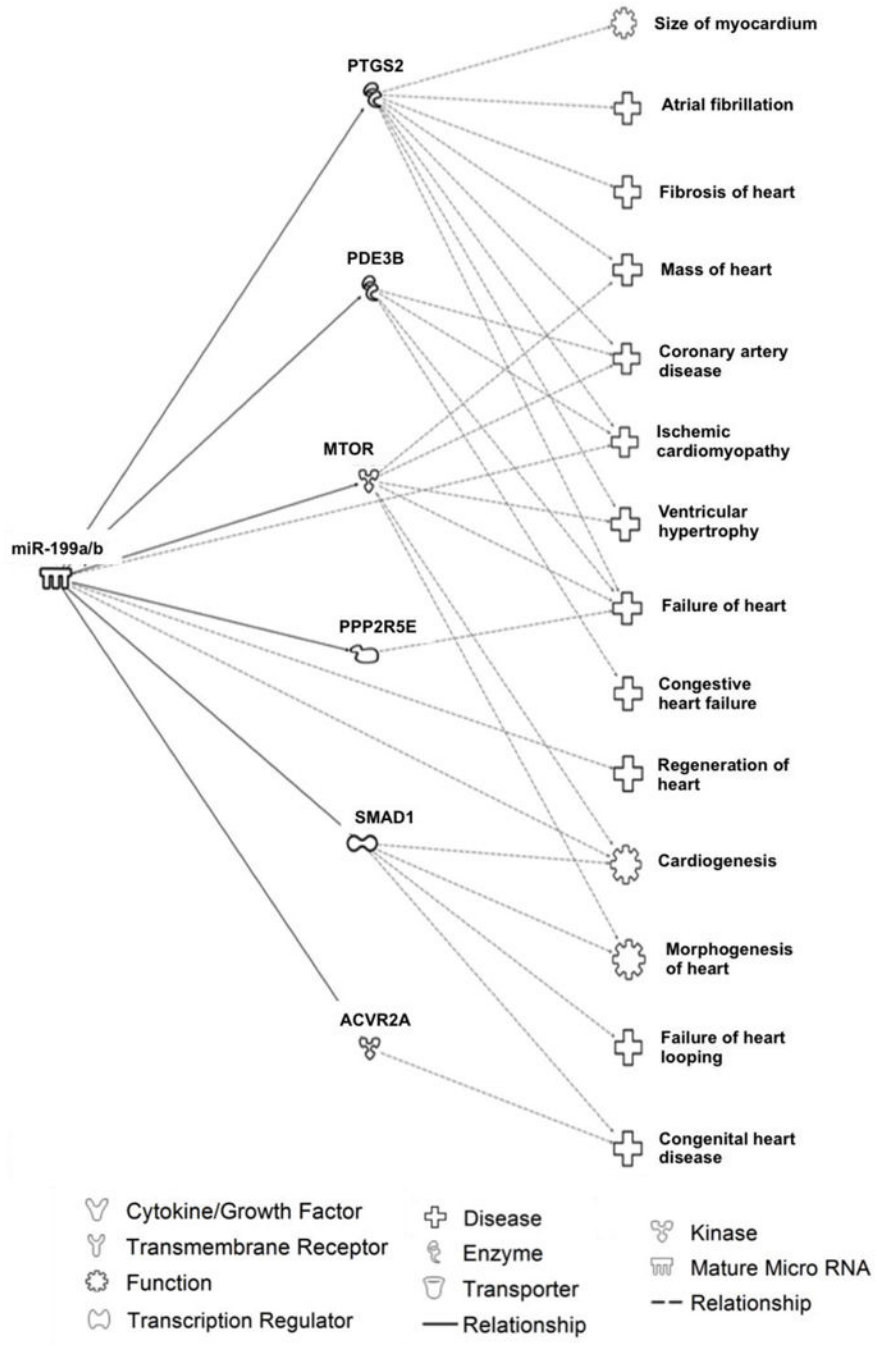

Fig 3.

In silico analysis of experimentally validated targets and cardiovascularpathways linked to miR-4199a/b using Ingenuity Pathway Analysis®.

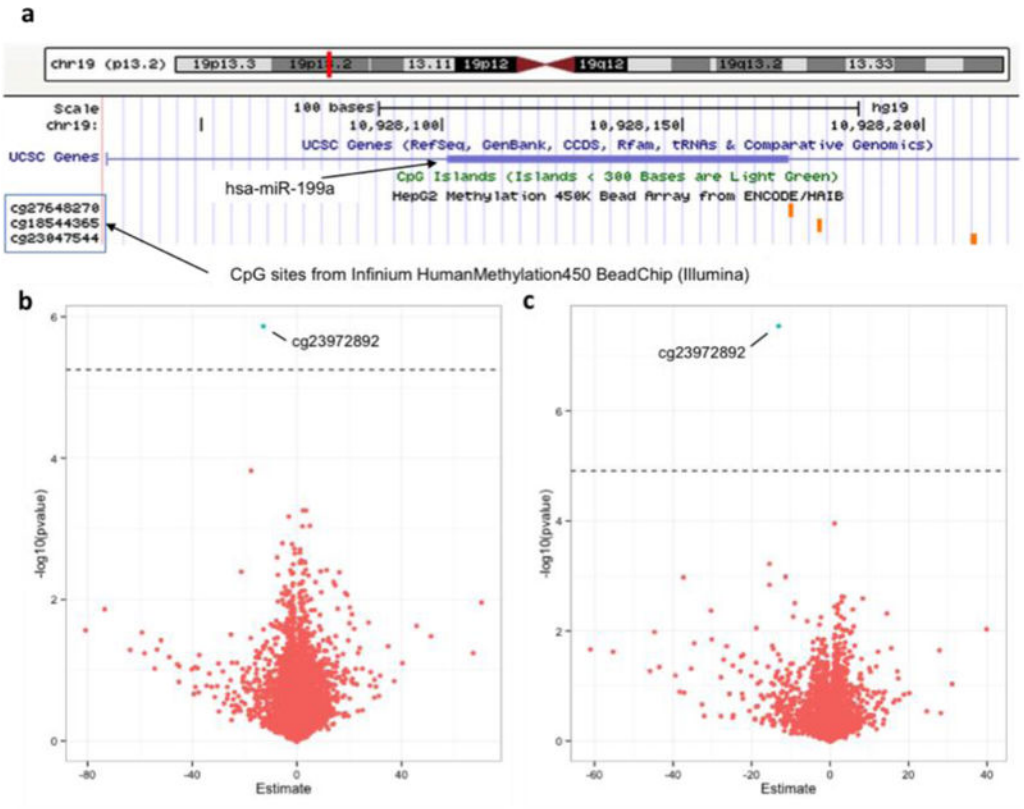

Fig 4.

Association between 6-month and 1-year $PM_{2.5}$ ambient levels and CpG methylation within 2Mbp adjacent to extracellular vesicle-associated miRNAs (evmiRNAs) promoter in peripheral blood of study participants (n=563). (a) Diagram (adapted from USCS-Genome Browser) showing miR-199a/b near-promoter CpG sites included in Infinium HumanMethylation450 BeadChip (Illumina). (b) Volcano plot showing the association between 6-month $PM_{2.5}$ ambient levels and CpG sites within 2Mbp adjacent to evmiRNAs promoter (8,903 CpG sites); (c) Volcano plot showing the association between 1-year $PM_{2.5}$ ambient levels and CpG sites within 2Mbp adjacent to evmiRNAs promoter (4,096 CpG sites). Dotted line represents the Bonferroni-adjusted threshold for statistical significance (a=0.05).

Fig 5.

Representation of CpG (cg23972892) near miR-199a/b promoter. Upper panel: Location at chromosome level (chr19:10,928,102–10,928,172). Lower panel: Zoom-in to show Histone 3 Lysine 24 acetylated (H3K27Ac) mark and other transcription factors (e.g. CTCF, TBP, ZNF143, RUNX3) in cells H1-hESC cells, suggesting a regulatory region. Adapted from UCSC-Genome Browser.

Table 1.

Demographic, physical, clinical characteristics, $PM_{2.5}$ and black carbon levels of participants at the baseline visit evaluated for extracellular vesicles-enriched microRNAs (n=22) and DNA methylation analysis (n=563).

Characteristic	evmiRNAs analysis Mean (SD)					DNA methylation analysis Mean (SD)		
	N (%) or Mean (SD)	$PM_{2.5}$, µg/m^3		BC[†], µg/m^3		N (%) or Mean (SD)	$PM_{2.5}$ (µg/m^3)	
		6-month	1-year	6-month	1-year		6-month	1-year
Age, years	72.63 (6.31)	11.67 (1.01)	11.56 (0.83)	0.58 (0.07)	0.59 (0.07)	75.46 (6.86)	10.47 (1.60)	10.45 (1.48)
BMI[*], kg/m^2	26.72 (2.89)					27.43 (4.09)		
Platelets (thousands/mm^3)	231.90 (0.15)					219.60 (52.03)		
C-reactive protein (mg/L)	0.02 (0.15)					3.48 (8.52)		
Tobacco smoking								
Never	6 (27.27%)	11.63 (0.71)	11.60 (0.89)	0.61 (0.06)	0.63 (0.07)	154 (27.35%)	11.42 (1.08)	11.62 (1.22)
Current	0 (0%)	-	-	-	-	24 (4.26%)	11.33 (0.96)	11.67 (1.15)
Former	16 (72.73%)	10.81 (1.47)	10.83 (1.23)	0.60 (0.08)	0.61 (0.07)	385 (68.38%)	11.24 (1.07)	11.33 (1.18)

[*] BMI: Body mass index.

[†] BC: Black carbon

Table 2.

Linear mixed-effects models estimating the change in blood pressure of study participants associated with a 2-SD increase* in long-term exposure to $PM_{2.5}$ ambient levels (n=36).

	$PM_{2.5}$ 6-month			$PM_{2.5}$ 1-year		
	mmHg	**95% CI**	**p-value**	**mmHg**	**95% CI**	**p-value**
Systolic blood pressure	0.19	(0.11, 0.28)	<0.01	0.12	(0.02, 0.19)	0.01
Diastolic blood pressure	0.11	(−0.03, 0.28)	0.13	0.05	(−0.07, 0.17)	0.43

*Corresponding to a 2.76 $\mu g/m^3$ increase in 6-month and to a 2.39 $\mu g/m^3$ increase in $PM_{2.5}$ levels. Models were adjusted for age, body mass index, smoking, C-reactive protein, white blood cells distribution (percentage of B-cells, monocytes, NK-cells, CD4, and CD8), and number of platelets. 95% CI: 95% Confidence interval.

Table 3.

Interaction of extracellular vesicle-associated miRNAs in the association between long-term $PM_{2.5}$ ambient levels and systolic blood pressure in study participants (n=36).

PM2.5	microRNA	β*	95% CI	$p_{interaction}$
6-month	let-7g-5p	−1.40	(−25.36, 22.55)	0.93
	miR-1246	−3.11	(−9.17, 2.94)	0.36
	miR-126–3p	−8.92	(−25.45, 7.62)	0.46
	miR-142–3p	−7.91	(−32.28, 16.46)	0.69
	miR-150–5p	6.89	(−7.41, 21.20)	0.42
	miR-15a-5p	11.03	(−7.75, 29.82)	0.21
	miR-199a/b	2.29	(−1.01,5.59)	0.14
	miR-223–3p	2.01	(−14.52, 18.55)	0.72
	miR-23a-3p	−3.07	(−14.23, 8.09)	0.67
1-year	miR-130a-3p	−2.01	(−44.62, 40.60)	0.92
	miR-142–3p	3.77	(−25.41, 32.94)	0.76
	miR-199a/b	6.13	(0.87, 11.39)	0.07
	miR-223–3p	30.17	(11.96, 48.39)	0.01
	miR-23a-3p	5.46	(−23.23, 34.14)	0.74

*β=Estimate for the interaction between long-term $PM_{2.5}$ ambient levels and systolic blood pressure. Models were adjusted for age, body mass index, smoking, C-reactive protein, white blood cell distribution, and number of platelets.