Exposure to Metal-Rich Particulate Matter Modifies the Expression of Candidate MicroRNAs in Peripheral Blood Leukocytes

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Exposure to ambient particulate matter (PM) has been associated with increased morbidity and mortality from cardiovascular and respiratory diseases (Baccarelli et al. 2008; Brook et al. 2004; Ciocci and Thompson 1961). Epidemiologic and in vivo studies suggest that the transition metal components of PM may be responsible for such effects (Brook et al. 2004; Chang et al. 2005; Corey et al. 2006). Foundry work has also been associated with adverse health outcomes, including cardiovascular disease (Kuo et al. 1999), potentially linked with PM exposure. Although prior studies have associated inhalation of ambient or occupational PM with systemic activation of inflammatory pathways, production of reactive oxygen species (ROS), and enhanced coagulation (Baccarelli et al. 2007, 2008; Chahine et al. 2007; Guruge et al. 2002; Li et al. 2006), the underlying mechanisms linking PM exposure with adverse health outcomes still need to be clarified (Nel et al. 2006).

Inhaled PM pollutants have been shown to produce systemic changes in gene expression, which can be detected in peripheral blood of exposed individuals (Wang et al. 2005). Expression of human genes is controlled by several genetic and epigenetic mechanisms, including microRNA (miRNA) regulation (He and Hannon 2004). miRNAs are small, endogenous, single-stranded non-coding RNAs of 20–22 nucleotides (Baccarelli and Bollati 2009). They posttranscriptionally regulate gene expression by hybridization to messenger RNA (mRNA), leading to translational repression or degradation of the target mRNA (He and Hannon 2004). One single miRNA can regulate hundreds of mRNAs in interrelated gene pathways, and a single miRNA can be targeted by several different miRNAs (Lewis et al. 2005).

Changes in the expression of several miRNAs, including miR-222, miR-21, and miR-146a, have been implicated in disease mechanisms that may be related to PM exposure, such as oxidative stress (Babar et al. 2008) and regulation of inflammation (Xiao and Rajewsky 2009). In particular, miR-222 overexpression indirectly reduces the expression of the endothelial nitric oxide synthase Dicer small interfering RNA–transfected cells (Suarez et al. 2007), an inflammation-related hallmark of atherosclerosis and ischemic cardiomyopathy (Zehir 1996), and has been associated with altered redox signaling (Sen et al. 2009). miR-21 has been shown to respond to hydrogen peroxide stimulation and participates in coordinated protective responses to oxidative stress (Cheng et al. 2009), as well as in inflammatory responses as suggested by animal models of allergic airway (Lu et al. 2009) and lipopolysaccharide-induced inflammation (Moschos et al. 2007). Changes in miR-146a expression have been implicated in the negative regulation of inflammation induced via the innate immune response, which is also activated by PM (Shoenfeld et al. 2009). miR-146a expression during inflammation is under the control of the transcription factor NF-kB (nuclear factor-kappa B), a central mediator for PM-related inflammation and health effects (Williams et al. 2008). In the present study, we investigated the effects of PM exposure on miR-222, miR-21, and miR-146a measured in blood RNA from foundry workers with well-characterized exposure to a wide range of PM levels. To clarify the mechanisms activated by PM exposure, we evaluated whether the expression of miR-222, miR-21, and miR-146a was associated with oxidative stress levels, as reflected in 8-hydroxyguanine (8-OH-dG) measured in blood DNA.

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Materials and Methods

Study subjects. We recruited 63 healthy male workers (mean age, 44 years; range, 27–55 years), in a steel production plant in Brescia, Northern Italy. These workers were free of cancer, cardiovascular disease, and pulmonary disease, and all of them had been working in their current job position for at least 1 year. To discriminate short- and long-term effects of PM, we obtained blood samples for DNA methylation analysis at two different times: a) a baseline sample collected in the morning of the first day of a workweek (after 2 days off work) before the beginning of any work activity (time 1); and b) a postexposure sample collected at the same time on the fourth day of work, after 3 consecutive days of work (time 2).

Written informed consent and approval from the local institutional review board IRCCS Fondazione Ca’ Granda Ospedale Maggiore Policlinico were obtained before the study.

Exposure assessment. Measures of airborne PM mass and PM metal components obtained in each of the 11 work areas of the steel production facility were used to estimate individual exposures. Measures of PM mass included levels of PM with aerodynamic diameters ≤ 10 μm (PM10) and ≤ 1 μm (PM1). We measured PM metal components on the PM10 fraction of PM mass through multielemental analysis by means of inductively coupled plasma mass spectrometer (Grimm Technologies, Inc., Douglassville, GA, USA). Concentrations of coarse particles were calculated from these measures as the difference between PM10 and PM1.

During the 3 working days between times 1 and 2, each of the study subjects recorded in a personal log the time that he spent in each work area. Individual exposures were calculated as the time-weighted averages of area concentrations (Tarantini et al. 2009).

miRNA analysis. Buffy coat samples were separated within 30 min of blood draw, immediately snap-frozen, and stored at –80°C. RNA was extracted from the buffy coats using the Ribopure Kit (Ambion, Inc., Austin, TX, USA), modified for miRNA extraction (see Supplemental Material [doi:10.1289/ehp.0901300]). We used specific TaqMan MicroRNA Assays (Applied Biosystems, Uppsala, Sweden) to detect and quantify mature miRNAs as recommended by the manufacturer, using ABI Prism 7900HT sequence detection systems (Applied Biosystems). In the reverse transcription (RT) step, 10 ng total RNA was employed in RT reactions (16°C for 30 min, 42°C for 30 min, 85°C for 5 min, and then to 4°C) using reagents from the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems) and specific miRNA primers provided with the TaqMan MicroRNA Assays. Real-time polymerase chain reaction (PCR) was performed using TaqMan MicroRNA Assays together with TaqMan Universal PCR Master Mix on an Applied Biosystems 7900 Sequence Detection System (95°C for 1 min and 40 cycles of 95°C for 15 sec and 60°C for 30 sec) (Chen 2005). Normalization was performed with RNU6B (RNA, U6 small nuclear 2) endogenous control (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed in triplicate, including no-template controls. The threshold cycle (Ct) was defined as the fractional cycle number at which the fluorescence passes the fixed threshold. The relative gene expression was calculated via a 2^−ΔΔCt method (Livak and Schmittgen 2001). Data are presented as relative quantity of target miRNA, normalized to RNU6B endogenous control and a calibrator built as a pool of 50 random samples. All laboratory analyses were performed in the same batch using samples that remained frozen until the analyses.

Determination of 8-OH-dG content in mitochondrial DNA (mtDNA). We measured the content of 8-OH-dG in mtDNA using quantitative real-time PCR to amplify different fragments of mtDNA on each sample with and without hOGG1 (human 8-oxoguanine DNA glycosylase 1) pretreatment, as previously described by Lin et al. (2008). All experimental DNA samples were assayed in triplicates, and the mean value of ΔCt was used in the statistical analyses.

Target prediction and pathway mining. We used the miRNA target prediction software miRanda (http://www.microrna.org/microrna/home.do) to predict the target genes of miR-222, miR-21, and miR-146a. A total of 1,056 target genes were annotated for miR-222, 1,065 for miR-21, and 1,038 for miR-146a. Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg) pathway searching was performed by mapping the predicted target genes from...
the KEGG human database. A total of 150 KEGG pathways were annotated for miR-222, 153 for miR-21, and 160 for miR-146a.

To find signal transduction pathways related to miR-222, miR-21, and miR-146a, we used LitInspector (http://www.litinspector.org), a literature search tool providing gene and signal transduction pathway mining within the National Center for Biotechnology Information’s PubMed database (Frisch et al. 2009). Two LitInspector pathways were annotated for miR-222, seven for miR-21, and six for miR-146a.

Statistical analysis. The Student’s paired t-test was used to assess differences in miRNA expression between baseline (time 1) and post-exposure (time 2). We evaluated the association of PM mass and PM metal component levels with miRNA expression measured in post-exposure samples using simple linear regression models and multivariable models. The adjusting variables were selected a priori based on the assumption that potential confounders had to be related to inflammation or oxidative stress generation. Age, body mass index (BMI), smoking, and number of cigarettes per day are all potential determinants of inflammation, oxidative stress, or both. In addition, we adjusted for percent granulocytes to control for possible shifts in leukocyte differential count.

As a sensitivity analysis, we also fitted duration of smoking in the model described above. The results of the models including duration of smoking were very similar to those including number of cigarettes per day.

To compare the magnitude of the associations of miRNA expression with different exposures, we calculated standardized regression coefficients. Outliers were excluded from regression analysis by dropping observations with studentized residuals that exceeded +3 or –3. We checked regression assumptions by performing diagnostic tests for each model, including the Shapiro–Wilk test to verify normality of residuals and the White test to verify the homogeneity of variance of the residuals. For miRNAs that did not show differences in their expression between time 1 and time 2, we also used mixed models that regressed PM and metal exposures against all measures of miRNA expression, regardless of whether they were measured at baseline or postexposure samples (Tarantini et al. 2009). A two-sided p-value < 0.05 was considered statistically significant. All statistical analyses were performed using SAS (version 9.1.3; SAS Institute Inc., Cary, NC, USA).

Results

Subject characteristics and exposure. The characteristics of the 63 study subjects are summarized in Table 1. miRNA expression did not show significant correlations with any of these characteristics (see Supplemental Material, Table S1). Supplemental Material, Table S2, shows the average levels and distributions of the individual exposures to PM and PM metal components during the 3 workdays between the baseline and postexposure miRNA measurements.

Differences in miR-222, miR-21, and miR-146a expression between baseline (time 1) and postexposure (time 2). miR-222 expression was significantly increased in blood samples taken after 3 days of work (time 2) compared with the measurement at time 1 (mean ± SD; mean_{time1} = 0.68 ± 3.41; mean_{time2} = 2.16 ± 2.25; p = 0.002; Figure 1). miR-21 expression was also significantly increased in postexposure samples (mean_{time1} = 4.1 ± 3.04; mean_{time2} = 4.7 ± 2.63; p = 0.05). miR-146a was not significantly different between baseline and postexposure blood samples (mean_{time1} = 0.61 ± 2.42; mean_{time2} = 1.9 ± 3.94; p = 0.19).

Associations of individual exposure levels to PM and PM metal components with miR-222, miR-21, and miR-146a expression. miR-222 expression exhibited a positive association with the levels of lead exposure (unadjusted analysis: β_{std} = 0.33; 95% confidence interval (CI), 0.07 to 0.76; p = 0.02; multivariable regression: β_{std} = 0.41; 95% CI, 0.01 to 0.87; p = 0.06, adjusting for age, BMI, current smoking, number of cigarettes per day, and percent granulocytes). miR-21 expression was not significantly associated with any of the measures of exposure to PM and PM metal components (Table 2). miR-146a expression showed a negative significant association with lead exposure (unadjusted models: β = –0.51; 95% CI, –0.88 to –0.14; p = 0.008; multivariable regression: β = –0.51; 95% CI, –0.88 to –0.13; p = 0.011; Table 2).

Table 2. Unadjusted and multivariable regression models (adjusted for age, BMI, smoking, number of cigarettes/day, percentage of granulocytes) estimating effects of PM mass and PM metal component exposure on miR-222, miR-21, and miR-146a expression (n = 63).

| Exposure | miR-222 | miR-21 | miR-146 |
|----------|---------|--------|---------|
|          | β_{std} | 95% CI | β_{std} | 95% CI | β_{std} | 95% CI |
| Unadjusted regression | | | | | | |
| PM_{10}  | –0.08   | –0.36 to 0.21 | 0.17 | –0.12 to 0.45 | –0.58 | –1.35 to 0.19 |
| PM_{1}   | –0.10   | –0.41 to 0.21 | 0.18 | –0.18 to 0.45 | 0.06 | –0.40 to 0.52 |
| Coarse PM | –0.07   | –0.36 to 0.21 | 0.17 | –0.12 to 0.45 | –0.62 | –1.40 to 0.16 |
| Chromium | 0.02    | –0.30 to 0.33 | 0.29 | –0.06 to 0.58 | –0.17 | –0.48 to 0.15 |
| Lead     | –0.08   | –0.46 to 0.29 | 0.39b | 0.07 to 0.76 | –0.51b | –0.88 to –0.14 |
| Cadmium  | 0.31    | –0.10 to 0.71 | 0.10 | –0.23 to 0.46 | –0.41c | –0.74 to –0.07 |
| Arsenic  | –0.41   | –0.74 to 0.24 | –0.03 | –0.16 to 0.71 | –0.12 | –0.49 to 0.25 |
| Nickel   | –0.04   | –0.44 to 0.36 | 0.13 | –0.18 to 0.61 | –0.15 | –0.55 to 0.26 |
| Manganese | 0.24   | –0.06 to 0.53 | –0.11 | –0.41 to 0.25 | –0.03 | –0.33 to 0.27 |
| B-DHG    | 0.11f   | 0.01 to 0.21 | –0.01 | –0.10 to 0.11 | 0.02 | –0.05 to 0.09 |
| Multivariable regression | | | | | | |
| PM_{10}  | –0.03   | –0.33 to 0.27 | 0.16 | –0.11 to 0.44 | –0.60 | –1.48 to 0.27 |
| PM_{1}   | –0.06   | –0.39 to 0.27 | 0.13 | –0.11 to 0.46 | 0.16 | –0.40 to 0.72 |
| Coarse PM | –0.03   | –0.33 to 0.27 | 0.16 | –0.11 to 0.44 | –0.65 | –1.53 to 0.24 |
| Chromium | –0.03   | –0.40 to 0.33 | 0.26b | 0.00 to 0.58 | –0.16 | –0.54 to 0.21 |
| Lead     | –0.07   | –0.49 to 0.35 | 0.41 | –0.01 to 0.67 | –0.51f | –0.88 to –0.13 |
| Cadmium  | 0.19    | –0.32 to 0.69 | 0.12 | –0.22 to 0.42 | –0.42g | –0.83 to –0.02 |
| Arsenic  | –0.11   | –0.57 to 0.36 | 0.27 | –0.38 to 0.32 | –0.26 | –0.69 to 0.18 |
| Nickel   | 0.00    | –0.45 to 0.45 | 0.21 | –0.25 to 0.50 | –0.15 | –0.58 to 0.29 |
| Manganese | 0.25   | –0.09 to 0.63 | –0.08 | –0.40 to 0.19 | –0.04 | –0.38 to 0.30 |
| B-DHG    | 0.11f   | 0.02 to 0.20 | 0.01 | –0.11 to 0.08 | 0.03 | –0.03 to 0.10 |

*p = 0.02; *p = 0.008; *p = 0.018; *p = 0.03; *p = 0.05; *p = 0.001; *p = 0.043; *p = 0.01.

Effects of particulate matter on miRNA expression
**Associations of miR-222, miR-21, and miR-146a expression with 8-OH-DG.** In univariate analysis, we found a positive correlation between miR-21 expression and 8-OH-DG (β = 0.11; 95% CI, 0.01–0.21; p = 0.03), which was confirmed in multivariable regression (β = 0.11; 95% CI, 0.02–0.20; p = 0.01). miR-222 and miR-146a were not associated with 8-OH-DG (Table 2).

**Associations between exposures and miRNA expression stratified by smoking and age.** In noncurrent smokers, miR-222 expression was up-regulated in response to chromium (β = 37.09; 95% CI, 13.72 to 60.46; p = 0.003), lead (β = 0.19; 95% CI, 0.03 to 0.34; p = 0.020), and cadmium (β = 141.34; 95% CI, 7.32 to 275.36; p = 0.039), whereas in current smokers we observed no significant association. In noncurrent smokers, miR-146a expression was down-regulated in response to lead (β = −0.58; 95% CI, −0.87 to −0.29; p < 0.001) and cadmium (β = −414.26; 95% CI, −726.87 to −101.64; p = 0.01), whereas in current smokers we observed no significant association.

The results stratified by age showed that in subjects 25–45 years of age miR-222 was up-regulated in association with lead (β = 0.21; 95% CI, 0.08 to 0.34; p = 0.003), whereas miR-146a expression was down-regulated in association with lead (β = −0.38; 95% CI, −0.59 to −0.17; p = 0.001). We found no significant associations among individuals between 46 and 65 years of age. The complete analyses stratified by smoking and age are reported in Supplemental Material, Tables S3 and S4 (doi:10.1289/ehp.0901300).

**Table 3.** Selected KEGG biological pathways potentially affected by miR-222, miR-21, and miR-146a that have established biological roles in oxidative stress and inflammatory processes.

| Pathway ID       | KEGG pathway name                        | No. of target genes involved in the pathway | miR-222 | miR-21 | miR-146a |
|------------------|------------------------------------------|--------------------------------------------|---------|--------|----------|
| hsa04010         | MAPK signaling pathway                    | 14                                         | 11      | 9      |          |
| hsa04062         | Chemokine signaling pathway               | 10                                         | 9       | 10     |          |
| hsa04060         | Cytokine-cytokine receptor interaction    | 7                                          | 17      | 14     |          |
| hsa04510         | Focal adhesion                            | 7                                          | 8       | 10     |          |
| hsa04210         | Apoptosis                                 | 6                                          | 3       | 6      |          |
| hsa04270         | Vascular smooth muscle contraction        | 6                                          | 7       | 6      |          |
| hsa04660         | T cell receptor signaling pathway         | 6                                          | 5       | 3      |          |
| hsa04670         | Leukocyte transendothelial migration      | 6                                          | 1       | 5      |          |
| hsa04620         | Toll-like receptor signaling pathway      | 5                                          | 3       | 7      |          |
| hsa04682         | B cell receptor signaling pathway         | 5                                          | 5       | 3      |          |
| hsa04514         | Cell adhesion molecules                   | 4                                          | 3       | 1      |          |
| hsa04350         | TGF-beta signaling pathway                | 3                                          | 4       | 5      |          |
| hsa04610         | Complement and coagulation cascades       | 3                                          | 5       | 3      |          |
| hsa09010         | Nitrogen metabolism                       | 1                                          | 1       | 1      |          |
| hsa04370         | VEGF signaling pathway                    | 1                                          | 2       | 3      |          |
| hsa04650         | Natural killer cell mediated cytotoxicity | 4                                          | 8       | 7      |          |

See also Supplemental Material, Table S5 (doi:10.1289/ehp.0901300).

**Table 4.** Signaling pathways potentially related to miR-222, miR-21, and miR-146a, identified by the literature search tool List INSPECTor.

| Pathway component | Signaling pathway                                                                 | References |
|-------------------|-----------------------------------------------------------------------------------|------------|
| **miR-222**       | MAPK Mitogen activated protein kinase signaling                                   | Terasawa et al. 2009 |
|                   | NRGN Nerve growth factor signaling                                                | Terasawa et al. 2009 |
| **miR-21**        | PTEN Phosphatase and tension homolog signaling                                    | Meng et al. 2006, 2007; Roy et al. 2009 |
|                   | TGFB TGFβ signaling                                                               | Davis et al. 2008, Papagiannakopoulos et al. 2008 |
| **miR-146a**      | BMP TGFβ signaling                                                                | Davis et al. 2008 |
|                   | FAK Focal adhesion kinase 1 signaling                                             | Davis et al. 2008 |
|                   | MAPK Mitogen activated protein kinase signaling                                   | Thum et al. 2008 |
|                   | P13K Phosphatidylinositol signaling                                               | Meng et al. 2006 |
|                   | TP53 p53 signaling                                                                | Papagiannakopoulos et al. 2008 |
| **miR-146a**      | BCL XL bc12 like 1 signaling                                                      | Liu et al. 2009 |
|                   | CXCR4 Chemokine c c motif receptor 4 signaling                                    | Llabaye et al. 2008 |
|                   | EGFFR Epidermal growth factor receptor signaling                                  | Hurst et al. 2009 |
|                   | NFKB Nuclear factor kappaB signaling                                              | Schmelzer et al. 2009 |
|                   | STAT Signal transducer and activator of transcription signaling                   | Liu et al. 2009 |
|                   | TLR Toll-like receptor signaling                                                  | Dai et al. 2008 |

**Discussion**

In this study of foundry workers in an electric-furnace steel plant, we evaluated the effect of exposure to PM and PM metal components on the expression of three candidate miRNAs that regulate genes in pathways related to oxidative stress and inflammation. We found that miR-222 and miR-21 expression was increased in postexposure samples collected after 3 workdays, compared with baseline samples. miR-222 expression in postexposure samples was positively associated with the mean lead exposure measured in the PM10 fraction of the PM mass during the 3 workdays, whereas we found no significant association of miR-21 expression with the exposures levels we evaluated. In addition, although miR-146a expression did not differ in postexposure and baseline samples, we observed that individual levels of exposure to lead and cadmium in the PM10 fraction were associated with miR-146a expression in post-exposure samples, as well as in a pooled data analysis that included both baseline and post-exposure samples.

Although many studies have focused on comparing miRNA expression between pathologic samples and normal tissues, very few studies have evaluated changes in miRNA expression in response to environmental stimuli (Baccarelli and Bollati 2009). A recent in vitro study by Jardim et al. (2009) showed that miRNA expression profiles in human airway cells change in response to diesel exhaust particles. In that study, 197 of the 313 detectable miRNAs (62.9%) were either up- or down-regulated ≥ 1.5 times, including many miRNAs associated with responses in inflammatory pathways. To the best of our knowledge, ours is the first human study to show that PM modifies the in vivo expression of candidate miRNAs.

miR-222 expression has been related with nitric oxide (Suarez et al. 2007) and redox...
signaling (Sen et al. 2009). An up-regulation of miR-222, as measured in our study, may suggest an increased proliferation rate of blood leukocytes in response to environmental stimuli that are able to induce inflammation responses.

Expression of miR-21 is part of a response aimed at limiting injuries from ROS (Cheng et al. 2009). In the present study, we observed an up-regulation of miR-21 after 3 days of work compared with the baseline measurement. This may reflect a nonspecific response to ROS production in blood due to increased PM-induced oxidative stress (Brauner et al. 2007), as also suggested by the positive association that we observed between miR-21 expression and 8-OH-dG. However, we found no significant associations between miR-21 expression and PM or PM metal components. Other unknown biological changes intervening during the 3 workdays—or other unmeasured exposures that are present in foundry facilities, such as heat, carbon monoxide, and nonionizing radiation (Tarantini et al. 2009)—might have modified miR-21 expression.

Studies in myeloid cells activated by bacterial and fungal components or other exposure to the proinflammatory cytokines tumor necrosis factor α or interleukin-1β have shown that miR-146a is involved in limiting inflammatory responses triggered through the innate immune system (Perry et al. 2008; Williams et al. 2008). The negative association of miR-146a expression with lead and cadmium levels that we observed in the present study may indicate that PM metal components enhance inflammatory processes initiated by organic PM antigens through the innate system (Descotes 1992; Li et al. 2008).

Bioinformatic strategies, such as those implemented in miRanda software (Bentwich 2005), are now available to identify potential miRNA target sites in the 3’ untranslated region (UTR) of a protein-coding gene. The potential targets of miRNAs often include hundreds of genes because the reverse complement of some “seeds” (bases 2–8 of the mature miRNA) appears in multiple locations in many pre-miRNA 3’ UTRs. With the understanding that recognition of miRNA targets is speculative, we explored miR-222, miR-21, and miR-146a targets and examined whether these targeted genes are overrepresented in pathways annotated in the KEGG database.

miRanda identified > 3,000 genes that are potential targets of the three miRNAs evaluated in our study. Using the KEGG pathway database, we identified several pathways that are involved in immune/inflammatory and oxidative stress. Transition metals are common components of ambient PM and have been shown to interact with the immune system in antigen nonspecific fashion (Mishra 2009). MacNee and Donaldson (2000) proposed that the generation of oxidative stress, either directly by transition metal components of PM or indirectly from the recruitment into the airspaces and activation of blood leukocytes, is a primary mechanism determining the inflammation-related health outcomes of PM. Our results indicate novel pathways through which metals may elicit specific PM-related responses and help explain previous studies that have specifically indicated a role of metals in promoting PM effects (Arisawa et al. 2001; Magari et al. 2002; Messner et al. 2009; Park et al. 2008).

Results of the present study show that associations of metals with miR-222 and miR-146a expression were limited to nonsmokers. In addition, when we divided the study subjects in two subgroups according to age (25–45 years vs. 45–60 years), we observed stronger associations among younger subjects. Identification of sets of individuals who have enhanced responses to PM may suggest possible mechanisms of physiologic assault, and provide data that can be used for more detailed risk assessment (Bateson and Schwartz 2004).

In the present study we investigated a population with well-characterized exposure to PM and PM metal components that allowed for contrasting subjects over a wide range of different exposure levels. Because of the limited number of study subjects, it is possible that the associations observed were due to chance. However, the occupational exposure and relatively controlled environment of a foundry provide a good setting for evaluating these mechanistic questions and reduce bias and chance findings. Our study was based on subjects working in several areas of the same factory but did not include a different population of subjects without exposure to PM. Limiting our investigation to individuals who have all been working in the same facility avoided potential concerns related to the selection of external referents who might have differed from the exposed population in terms of socioeconomic factors and other characteristics determining hiring into the plant (Pearce et al. 2007). However, the differences in the individual levels of exposure within our study group were large, providing sufficient contrast for identifying exposure-related changes in miRNA expression (Anselmi and Patelli 2006).

In summary, our findings suggest that air particles, particularly those rich in lead and cadmium, are able to modify miRNAs expression. Further studies are required to determine the role of such alterations along the pathways determining the effects of PM on human health.

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