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Cellular Responses to Exposure to Outdoor Air from the Chinese Spring Festival at the Air−Liquid Interface

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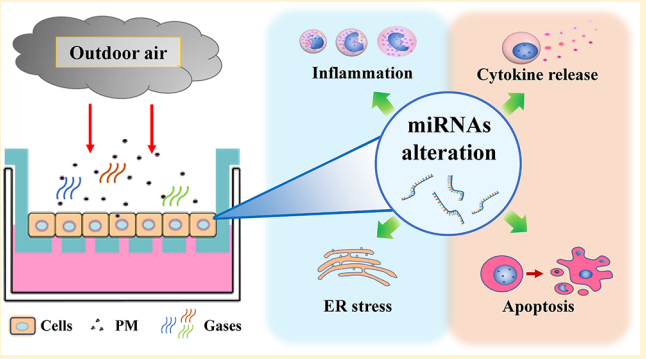
Supporting Information

ABSTRACT: The Spring Festival is the most important holiday in China. During this time, the levels of particulate matter (PM) as well as gaseous copollutants significantly increase because of the widespread enjoyment of fireworks. The expression patterns of microRNAs may serve as valuable signatures of exposure to environmental constituents. We exposed macrophages to the whole stream of outdoor air at the air−liquid interface aiming at closely approximating the physiological conditions and the inhalation situation in the lung. 58 miRNAs were up-regulated, and 68 miRNAs were down-regulated in the night of the New Year's Eve (exposure group E2N1) compared to filtered-air exposed control cells. The target genes of the up-regulated miRNAs were enriched in immunity- and inflammation-linked pathways, such as the TLR-NF-κB pathway. Compared to the E2N1 group, 29 miRNAs were up-regulated, and 23 miRNAs were down-regulated in the cells exposed to air from the daytime of the Chinese New Year with higher concentrations of particles, SO_2, and nitrogen oxide. The target genes of the up-regulated miRNAs were mostly enriched in apoptosis, adhesion, and junction-related pathways. These results preliminarily unravel part of the toxic mechanisms of air constituents and provide clues for discovering the main drivers of air pollution-induced disorders.



INTRODUCTION

Aerosol pollution has become a profound scientific and social problem with significant effects on the living environment.[1] Epidemiological evidence has revealed that exposure to atmospheric fine/ultrafine particles is closely associated with the morbidity and mortality rates of asthma, lung cancer, and other diseases.[2−4] Aerosol pollution is a mixture comprising mainly dust, sulfates, nitrates, ammonium salts, and organic matter, as well as water droplets, with aerodynamic diameters of less than 2.5 μm.[1] The Chinese Spring Festival is the most celebrated traditional holiday of China. During this special period, the composition of the atmosphere not only includes particulate matter (PM) but also gaseous copollutants, such as nitrogen oxide (NO, NO_2, NO_x) and sulfur dioxide (SO_2), because of the widespread enjoyment of fireworks.[5,6]

Due to the variety of emissions sources and formation processes, the particle size distribution of PM in the atmosphere spans several orders of magnitude. Most research has focused on PM with aerodynamic diameters below 2.5 or 10 μm ($PM_{2.5}$ or PM_{10}); health guidelines and national standards for $PM_{2.5}$ and PM_{10} have been established.[7,8] Studies in humans have also demonstrated moderately significant associations between the outdoor concentrations of PM_{10}, SO_2, and NO_2 and respiratory difficulties among asthmatic children and adolescents.[9] PM_1 (aerodynamic diameter <1 μm) may be

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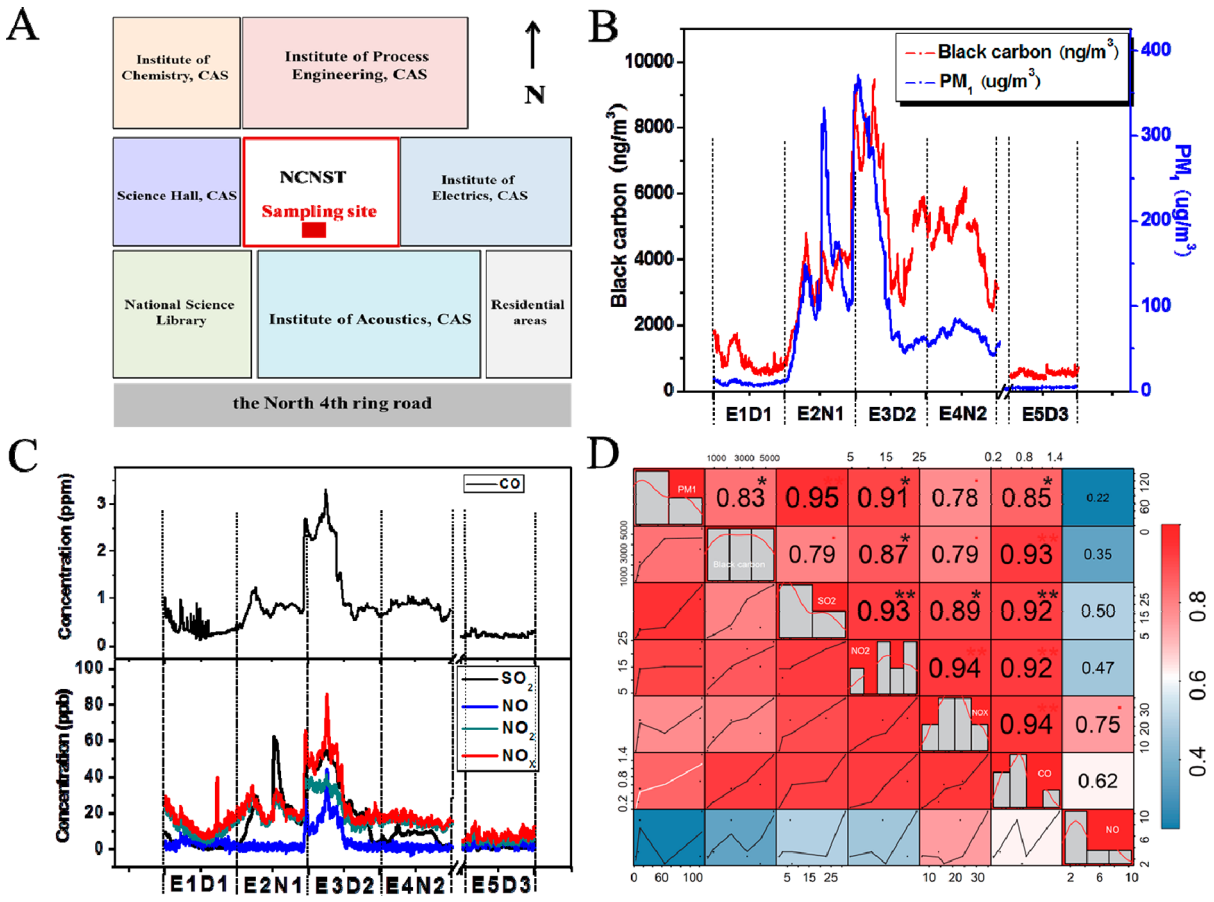

Figure 1. Online monitoring of the air pollution during the Spring Festival. (A) Location of the sampling site. (B and C) The air constituents of the exposure groups E1D1, E2N1, E3D2, and E4N2 were continuously measured from 8:00 a.m. on New Year's Eve to 8:00 a.m. on the second day of Chinese New Year, while the exposure period of group E5D3 was from 8:00 a.m. to 8:00 p.m. on the fourth day of Chinese New Year. (B) Black carbon and PM_1 concentrations were recorded every 5 min. C, CO, SO_2, NO, NO_2, and NO_x concentrations were recorded every 5 min. (D) Correlation analysis of the indicated factors in the atmosphere, including black carbon, PM_1, CO, SO_2, NO, NO_2, and NO_x.

Table 1. Mean Values of PM_1, Black Carbon, Gas Concentrations, and Conventional Meteorological Data during the Different Exposure Periods[a]

	Filtered-air	E1D1 (the daytime of the Chinese New Year's Eve	E2N1 (the Chinese New Year's Eve)	E3D2 (the Chinese New Year's Day)	E4N2 (the Chinese New Year's Night)	E5D3 (the fourth day of the Chinese New Year)
	2.16 6:00−18:00	**2.186:00−18:00**	**2.18 18:00−2.19 6:00**	**2.19 6:00−18:00**	**2.19 18:00−2.20 6:00**	**2.22 6:00−18:00**
Black carbon (ng m^{-3})	/	948	3544	5615	4599	477
PM_1 ($\mu g\ m^{-3}$)	/	10	142	146	6	4
SO_2 (pbb)	/	2.92	22.85	31.47	7.28	1.28
NO (pbb)	/	3.25	2.49	10.34	1.23	2.46
NO_2 (pbb)	/	10.93	21.86	24.59	15.16	4.61
NO_x (pbb)	/	14.18	24.35	34.94	16.39	7.08
CO (pbb)	/	0.376	0.893	1.535	0.813	0.247
O_3 ($\mu g\ m^{-3}$)	/	32	21.2	19.1	24.7	30.4
Vis (km)	/	4.3	3.8	1.29	1.06	15.8
TP (°C)	/	8.3	2.7	2.2	2.8	0.5
RH (%)	/	27	46	50	93	20
WS(m/s)	/	3.0	2.1	1.52	1.25	8.7
Estimated particle deposition ($\mu g\ cm^{-2}$)	0	0.08	1.16	1.19	0.54	0.03

[a]Vis: vibility; TP: temperature; RH: relative humidity; WS: wind speed.

more cytotoxic and genotoxic than $PM_{2.5}$ *in vitro*.[10] Smaller PMs, which often have larger surface to volume ratio, may carry more toxins than larger particles, eventually having a greater health impact.[11,12] PM_1 are almost completely derived

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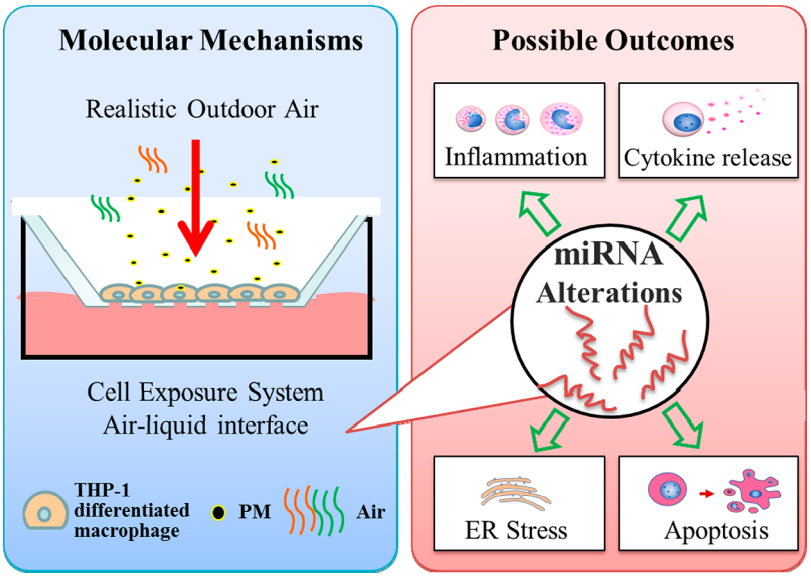

Figure 2. Schematic diagram of the cell exposure model and experimental design. Macrophages differentiated from the THP-1 cell line were cultured on Transwell inserts and directly exposed to the outdoor air. The cells and culture media from the basal chamber were harvested immediately after the 12-h exposure to the atmosphere. Alterations in miRNAs related to ER stress, apoptosis, and the immune response, as well as the release of cytokines, were examined.

from combustion processes and gaseous precursor-formed particles.[13] Increased clinical visits and cardiovascular mortality have been related to PM_1 pollution exposure.[14] In the present study, we focus primarily on the effects of PM_1 from the fireworks during the Chinese Spring Festival.

microRNAs (miRNAs) are noncoding RNAs comprising ~22 nucleotides, which bind to specific mRNAs (mRNAs) to promote their degradation and/or translational inhibition.[15] miRNAs play an essential role in the epigenetic regulation of biological and pathological processes and may thus be valuable signs of environmental exposure.[16] In the present study, we used an air−liquid interface (ALI) cell exposure model, exposing THP-1 differentiated macrophages to a whole air stream, with the aim of closely replicating the physiological conditions and inhalation paradigm in the lung. We performed several exposure experiments before, during, and after the Chinese New Year when the concentrations of PM and acidic gases are likely to be elevated due to fireworks-use, to assess alterations in miRNAs and target genes.

METHODOLOGY

Dynamic Monitoring of the Air Pollution in the Spring Festival. Air pollution before, during, and after the Chinese New Year was measured from the roof of a five-story building (~15 m above the ground) situated about 100 m north of the North fourth Ring Road in Beijing (E 108.887°, N 34.229°; Figure 1A). The methods of real-time air pollution sampling were similar to those previously described.[17] Conventional meteorological data (Table 1) such as temperature (TP), relative humidity (RH), wind speed (WS), ambient O_3, and visibility (Vis) were recorded by the China Meteorological Administration about 5 km south of the sampling site.

Aerosol Deposition Chamber and Cell Exposure. We employed a portable instrument for the realistic toxicity testing of air pollutants, the Nano Aerosol Chamber for *In Vitro* toxicity (NACIVT), for short and long-term exposures with ALI cell culture (Figure 2).[18] As a model for differentiated macrophages, the cell-line THP-1, derived from human acute monocytic leukemia cells, was used. Well-differentiated macrophages, in the experimental groups (E1-E5), were sequentially exposed to the aerosols for 12 h, either during the daytime (E1D1, E3D2, E5D3) or during the night (E2N1, E4N2). Thus, the exposures of E1D1, E2N1, E3D2, and E4N2 covered the period from 8:00 a.m. on New Year's Eve to 8:00 a.m. on the second day of Chinese New Year, 2015. The exposure of E5D3 was performed from 8:00 a.m. to 8:00 p.m. on the fourth day of Chinese New Year. The control group was exposed to filtered air by mounting a filter and activated carbon to remove particles and gases.

miRNA Microarray Assay. miRNAs were extracted using the miRNA mirVana isolation kit (Ambion, Life Technologies, CA, USA), and the expression of miRNA was analyzed using Taqman microRNA assays (Applied Biosystems, Foster City, CA, USA) according to the product manufacture. Microarray results were extracted using the Agilent Feature Extraction software.

Cytokine Release. The release of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) into the medium on the basal side of the cells was analyzed using an enzyme-linked immunosorbent assay (ELISA) Ready-SET-Go! Kit (eBioscience, San Diego, CA, USA) following the manufacturer's instructions. The absorbance was measured in a Titertek Multiskan microplate reader (Germany) at 450 nm. The analysis of TNF-α and IL-6 was performed in seven replicates and three replicates, respectively.

Predicting Target Genes of miRNA. StarBase and miRBase were designed for decoding the interaction of miRNAs-mRNAs from large-scale CLIP-Seq (HITS-CLIP, PAR-CLIP, iCLIP, CLASH), microarray, or PCR data. These databases can be used to verify the interaction between miRNAs and targets.[19,20] In total, we obtained 57864 miRNA-mRNA interactions that included 14614 mRNAs and 456 miRNAs to predict the target genes of the miRNAs.

Statistical Analysis. The data are presented as the mean ± SD. Statistical significance was assessed using Student's *t* test

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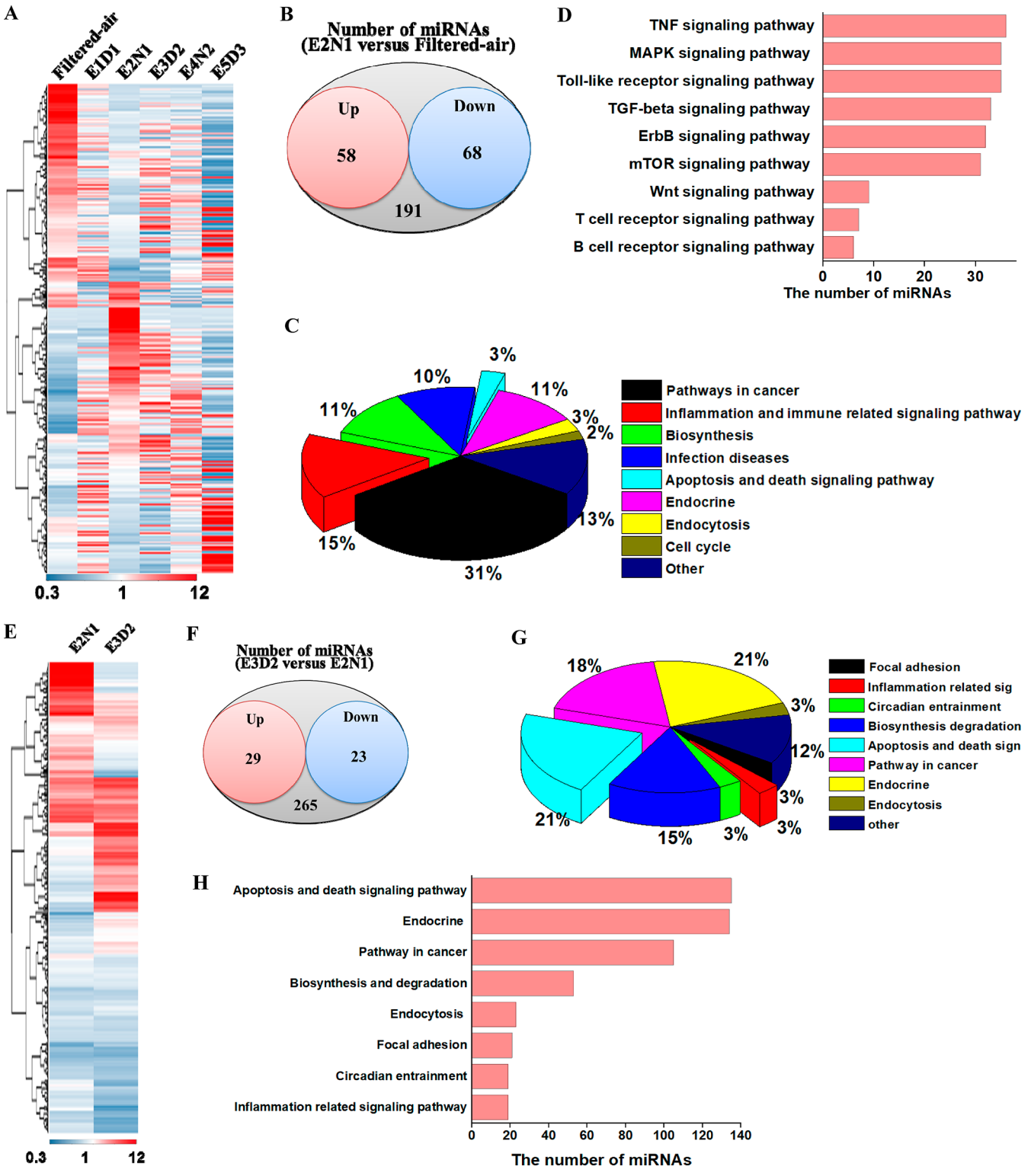

Figure 3. Polluted air from the Spring Festival significantly altered miRNA expression. (A) Heatmap of the miRNA array data for each exposure group. The constituents of the air of E1D1, E2N1, E3D2, and E4N2 exposure group were continuously measured from 8:00 a.m. on New Year's Eve to 8:00 a.m. on the second day of Chinese New Year, while that of exposure group E5D3 was from 8:00 a.m. to 8:00 p.m. on the fourth day of Chinese New Year. The control group was exposed to filtered air by mounting a filter and activated carbon to respectively remove particles and gases. Up-regulated miRNAs are presented in red and down-regulated miRNAs in blue. (B) The number of the up-regulated or down-regulated miRNAs in the E2N1 exposure group (the night of New Year's Eve) versus the filtered-air control group. (C) The possible targeted pathways of the up-regulated miRNAs in the E2N1 exposure group. (D) The number of miRNAs in the inflammation- and immune-related signaling pathways. (E) Heatmap of the miRNA array ata for the E2N1 and E3D2 exposure groups (the night of New Year's Eve and the daytime on Chinese New Year). (F) The number of up-regulated or down-regulated miRNAs in the E3D2 group versus the E2N1 group. (G) The possible targeted pathways of the up-regulated miRNAs in the E3D2 exposure group. (H) The number of miRNAs in each possible pathway.

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Table 2. Up-regulated miRNAs and Targeted Genes in the E2N1 Group as Compared to the Filtered-Air Group

Up-regulated miRNAs examples	Number of miRNAs	Number of targeted genes	Targeted genes examples	Pathway
miR-125, miR-30a, miR-30c, miR-146b, miR-23c, miR-155-5p, miR-210, miR-320a, miR-320b, miR-483, miR-466, miR486-3p, miR-503, miR-1468, Let-7e-5p	36	79	NFKB1, ATF6B, PIK3R, NOD2, TRAF5, CXCL3, ICAM1, IKBKB, FAS, AKT3, TNFRSF1A	TNF signaling pathway
	35	41	MYD88, NFKB1, PIK3CB, MAPK, PIK3R3, IKBKE	Toll-like receptor signaling pathway
	35	29	EGFR, FGF, NFKB1, MAPK, TGFBR2	MAPK signaling pathway
	33	57	TGFBR1, MYC, MAPK3, TGFB2, BMPR1A, SMAD, E2F4, NOG, MAPK1	TGF-beta signaling pathway
	32	63	AKT2, PTK2, MAP2K7, STAT5A, PRKCA,PAK2, PIK3CB	ErpB signaling pathway
	31	51	BRAF, PRKCA, STK11, IKBKB, PIK3R3, EIF4E, AKT	mTOR signaling pathway
	9	21	SMAD, MAPK, WNT5A, WNT6, WNT8	Wnt signaling pathway
	7	15	NFKB1, PIK3CB, MAPK, PIK3R3	T cell receptor signaling pathway
	6	14	NFKB1, PIK3CB, MAPK, PIK3R3	B cell receptor signaling pathway

Table 3. Up-regulated miRNAs and Gene Target Examples in the E3D2 Group as Compared to the E2N1 Group

Up-regulated miRNAs Examples	Number of miRNAs	Number of targeted genes	Targeted gene examples	Pathway
miR-93, miR-186, miR-197, miR-342, miR-374b, miR-615, miR-1224-5p, miR-2861, miR-3654, miR-6722-3p	135	394	CHUK, FAS, IRAK1, NFKB, BCL2, BID, IKBKG	Apoptosis and death signaling pathway
	134	371	ESR1, ACTB, PRKCA, ATP1B2, MED13L, NRAS	Endocrine
	105	262	BAD, KRAS, RAF1, PRKCG, PIK3R2, RXRB	Pathway cancer
	69	123	FASN, ACACB, ACSL1, PRKCA, CAMK4, PIK3R2	Biosynthesis and degradation
	53	59	VPS4A, PSD4, PDGFRA, ADRBK1, SMAD2	Endocytosis
	23	86	PPP1CA, PARVG, BRAF, ACTB, PRKCA, ERBB2	Focal adhesion
	21	86	BRAF, PIM2, STAT5A, NRAS, PIK3R2,TGF	Inflammation related signaling pathway
	19	44	PRKCA, GNG13, CACNA1G, GNGT1, CAMK2G	Carcadian entrainment

for the PM_1 treatment and control groups. Differences in all tests were considered significant when $p < 0.05$ or $p < 0.01$.

RESULTS AND DISCUSSION

With the fireworks and firecrackers use during Chinese Lunar Eve and the Chinese New Year, the concentrations of PM_1, black carbon, and acidic gases SO_2, nitrogen oxide, and CO dramatically increased from 8:00 p.m. on New Year's Eve to 8:00 a.m. on the second day of Chinese New Year, especially at the Chinese Lunar Eve (E2N1, E3D2, and E4N2, with E3D2 being the most affected; Figure 1 and Table 1).

In the E2N1, E3D2, and E4N2 groups, the mean concentrations of PM_1 (142, 146, and 66 $\mu g/m^3$) and black carbon (3544, 5615, and 5615 ng/m^3) were 5−10 times higher than before and after the Chinese New Year, i.e., in the exposure groups E1D1 (10 $\mu g/m^3$ of PM_1 and 948 ng/m^3 of black carbon) and E5D3 (4 $\mu g/m^3$ of PM_1 and 477 ng/m^3 of black carbon). There were particularly high concentrations of particles and acidic gases (SO_2, NO_x, CO) in the E3D2 group (Table 1). The mean concentration of SO_2 was 31.47 ppb, which is 11 times the concentration in the E1D1 group and 25 times that in the E5D3 group. The estimated total particle dose deposited was 1.19 $\mu g/cm^2$ in the E3D2 group, with 0.08 and 0.03 $\mu g/cm^2$ before and after the Chinese New Year, i.e., in the E1D1 and E5D3 groups, all of which were considerably lower than the dose applied in the experiments under submerged exposure conditions.

The concentrations of acidic gases SO_2, nitrogen oxide, and CO, were highly elevated only in the E3D2 group (Figure 1B); the Chinese New Year day time had high concentrations of both particles and acidic gases (SO_2, NO_x, CO). PM_1 and SO_2 were the most important factors, highly correlating with most factors (Pearson coefficient >0.7).

Air Pollution in the Spring Festival Induces Post-Transcriptional Regulation. Macrophages are important cells in the respiratory tract, which come directly into contact with inhaled particles and gases. We mimicked this scenario by culturing macrophages on Transwell inserts and exposing them at the ALI directly to the ambient air from the Spring Festival. A miRNA array was then employed to evaluate miRNA variation after each exposure period in comparison to filtered-

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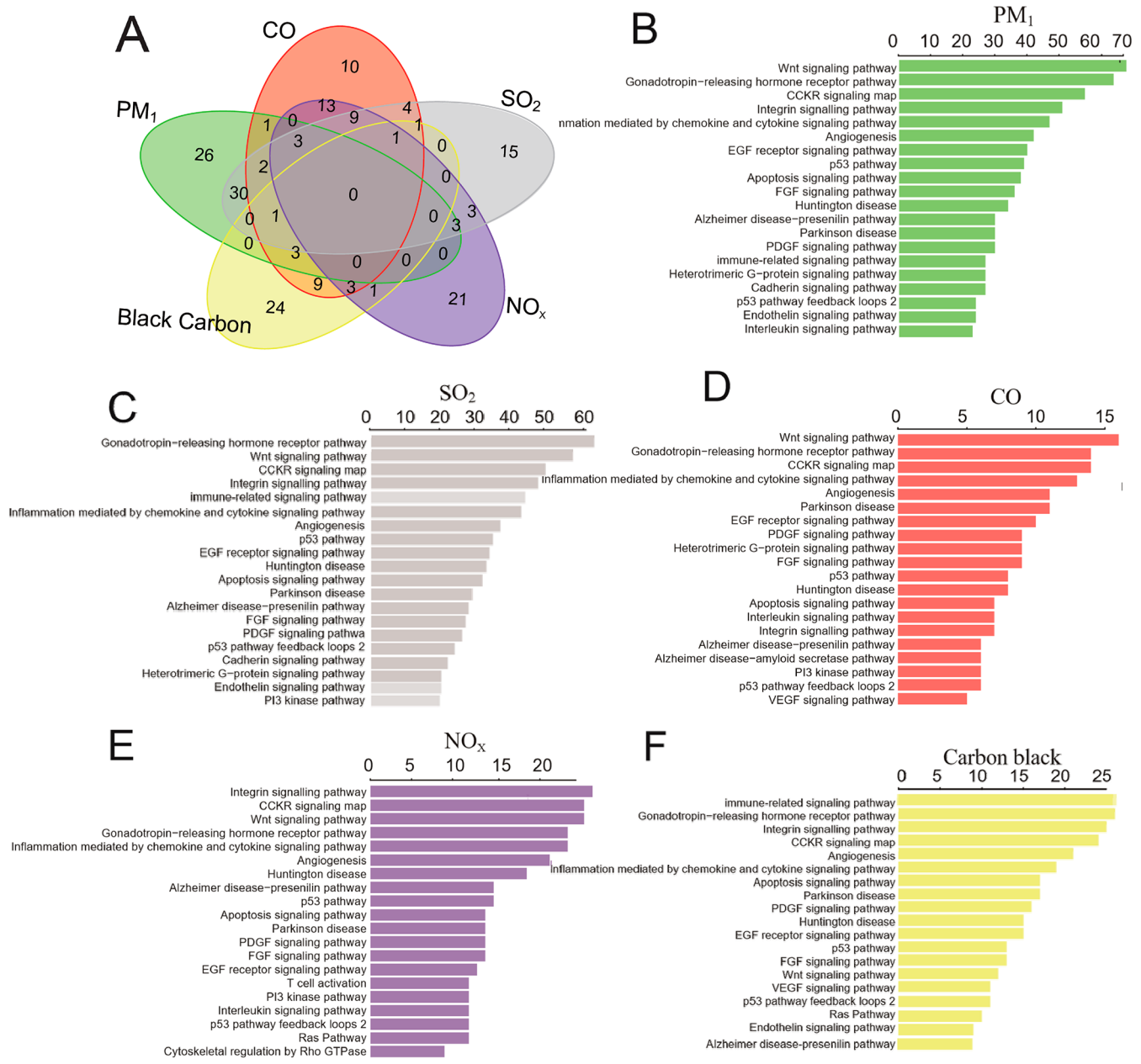

Figure 4. miRNAs and targeted signaling pathways associated with air constituents. (A) Venn diagram illustrating the number of miRNAs associated with the factors in the air (PM1, black carbon, SO_2, nitrogen oxide or CO). (B−F) KEGG pathways enriched for gene targets of miRNAs associated with PM1, black carbon, SO_2, nitrogen oxide, or CO. The abscissas represent the number of gene targets of miRNAs enriched in the pathways.

air exposed control cells. We obtained 317 microRNAs exhibiting elevated expression (Signal >100, Figure 3A).

We focused on the miRNAs that were altered in the E2N1 group (fold change >2). The health of cells in the filtered-air control group had been demonstrated to be similar to cells in the cell incubator control group before the exposure period (Figures S1 and S2). Compared to the filtered-air control cells, 58 miRNAs were up-regulated, and 68 miRNAs (fold change >2) were down-regulated in the E2N1 group (Figure 3B). The target genes of the up-regulated miRNAs were enriched in immunity- and inflammation-linked pathways, such as the TGF-beta, toll-like receptor, insulin, wnt, and chemokine signaling pathways as well as others (Figure 3C, 3D, and Table 2). Thus, the air pollution during the Spring Festival induced differential expression and post-transcriptional regulation of miRNAs.

Compared to the exposure group E2N1, there were differentially up- and down-regulated miRNAs in the cells exposed to air from the daytime of the Chinese New Year (E3D2; Figure 3E). In this group, 29 miRNAs were up-regulated, and 23 miRNAs were down-regulated compared to the E2N1 group (Figure 3F). The target genes of the up-regulated miRNAs in this case were mostly enriched in apoptosis, adhesion, and junction-related pathways, such as adherens junction, focal adhesion, actin cytoskeleton, and apoptosis (Figure 3G, 3H, and Table 3). The different air constituents played important roles in the effects on miRNA levels. In all exposure groups, there were higher concentrations of particles and acidic gases than in the E2N1 group.

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Compared to the exposure group, there were higher concentrations of particles, SO_2, and nitrogen oxide in the E3D2 group (Table 1).

Compared to exposure group E2N1, the alterations in miRNA levels were also different in the E4N2 group, though there were elevated concentrations of black carbon in both exposure groups (Figure 3A). Interestingly, there were higher concentrations of PM_1, SO_2, and nitrogen oxide in the E2N1 group than in the E4N2 group (Table 1).

We also observed a significant correlation of miRNA expression with the air constituents (PM_1, black carbon, SO_2, nitrogen oxide, and CO). Compared to the filtered-air control group, 200 miRNAs were associated with the air pollution from the Spring Festival: there were 77, 75, 62, 59, and 43 miRNAs associated with SO_2, PM_1, CO, nitrogen oxide, and black carbon, respectively ($p < 0.05$; Figure 4A, Figures S3 and S4). From another point of view, PM_1 and SO_2, which correlated with a greater number of miRNAs, were indeed the most critical factors. On New Year's Eve, there was a northerly wind in the daytime, with a relative humidity of about 35% (E1D1). However, the wind direction shifted at night, and the diffusion conditions worsened. Firecrackers on New Year's Eve further exacerbated the air pollution. Short-term use of firecrackers superimposed on the unfavorable meteorological situation, resulting in continuously growing pollutant concentrations from the first day to the third day of the Chinese New Year (E2N1 to E4N2). It is important to note that sharp changes in the meteorological conditions did occur on E5D3 when a large area of dust resulted in high pollution levels of PM_{10} (but low $PM_{2.5}$ and PM_1) (Table S1). However, because we cut the particle size under PM_1, E5D3 turned out to be a low-dose exposure group.

We performed enrichment analysis of the target genes and corresponding miRNAs to examine the effects on different pathways, such as Wnt signaling, the gonadotropin-releasing hormone receptor pathway, and immunity- and inflammation-linked pathways. Notably, the number of genes targeted by miRNAs associated with PM_1 and SO_2 in the immunity or inflammation-linked pathways amounted to 95 and 87, respectively (Figure 4B, C), which was much greater than that of other influencing constituents (40 for black carbon, 36 for nitrogen oxide, and 18 for CO; Figure 4D, E, F).

We verified selected miRNAs from the array results by real-time PCR. Thus, we confirmed that the expressions of miR-125a, miR-125b, miR-21, miR-30a, and miR-105 were significantly up-regulated after exposure to air from the nights of New Year's Eve and the Chinese New Year (E2N1 and E4N2, Figure 5A). The expressions of let-7e, let-7i, miR-30c, miR-107, and miR-146b were significantly up-regulated in samples treated with air from the night of Chinese New Year (E4N2) but not the night of New Year's Eve (E2N1; Figure 5B). Thereby, miR-125a, miR-125b, miR-21, miR-30a, and miR-105 may have a role in promoting apoptosis, while let-7e, let-7i, miR-30c, miR-107, and miR-146b have been implicated in protecting cells from apoptosis. The concentrations of PM_1 and SO_2 in exposure group E2N1 were higher than that of E4N2. Therefore, exposure to the mixture of a high concentration of particles and acidic gases may lead to the up-regulation of miRNAs associated with both inflammation and apoptosis. In addition, only miRNAs related to inflammatory responses were altered in exposure group E4N2.

High Particulate Concentration and Low Acidic Gas Levels Activate the Toll-like Receptor Pathway. As shown in Figure 5, miRNAs exhibiting significant changes, such as miR-125a, miR-125b, miR-21, miR-30a, and miR-105, have all been reported to be closely related to toll-like receptor (TLR) pathway activation. TLRs are membrane receptors playing crucial roles in innate immunity.[22] The adaptor protein MyD88-dependent response occurs upon TLR receptor dimerization to activate nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK).[21] The miRNAs related to the TLR pathway in macrophages may serve a fine-tuning regulatory role in inflammation.[22] miRNAs have also been shown to perform a negative feedback regulatory role in the TLR-NF-κB pathway, and downstream immune responses, in macrophages.[23,24]

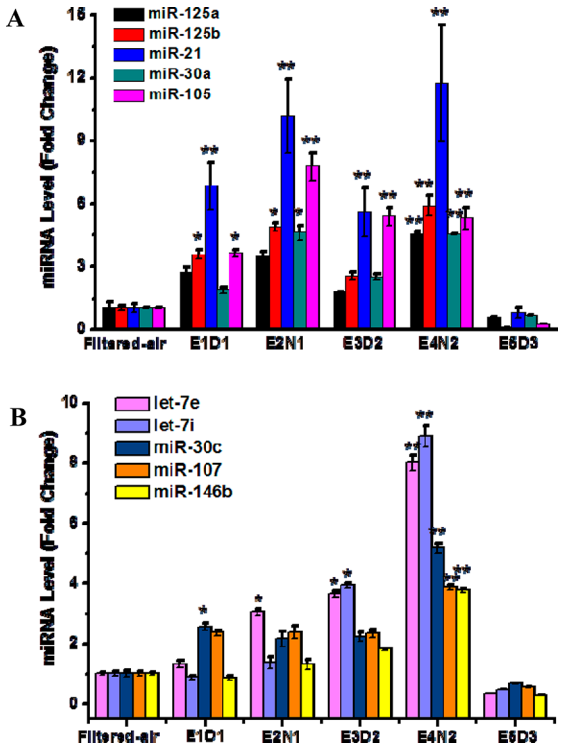

Figure 5. Alterations in miRNAs induced by the Spring Festival air pollution. miR-125a, miR-125b, miR-21, miR-30a, miR-105 (A) and let-7e, let-7i, miR-30c, miR-107, miR-146b (B) as quantified by real-time PCR. The miRNAs are normalized to the levels of U6. Data are expressed as mean ± SD, $n = 6$, $^*p < 0.05$, $^{**}p < 0.01$ compared to the filtered-air control group.

miR-21 has been demonstrated to decrease the TLR2 agonist-induced lung inflammatory response.[25] miR-125b and miR-105 inhibit immune responses by targeting the TLR2/MyD88 signaling pathway.[26] In addition, miR-125a and miR-30a also play essential roles in regulating inflammatory responses.[27] Let-7i and miR-30c negatively regulate inflammation, while let-7e and miR-107 are also related to immune regulation.[28] miR-146b targets the TLR signaling pathway molecule TRAF6 and may decrease inflammation.[29] The miR-320d and miR-124-3p may target IRAK and TRAF6, which are crucial molecules downstream in the TLR signaling pathway.[30,31]

Consistent with the observed effects implicating TLR2 and MyD88, we found that the expressions of NF-κB signaling molecules p65, IκBα, and IKKβ were significantly up-regulated in the E2N1 and E4N2 groups (Figure 6), along with activation of the TLR-NF-κB pathway. No significant up-

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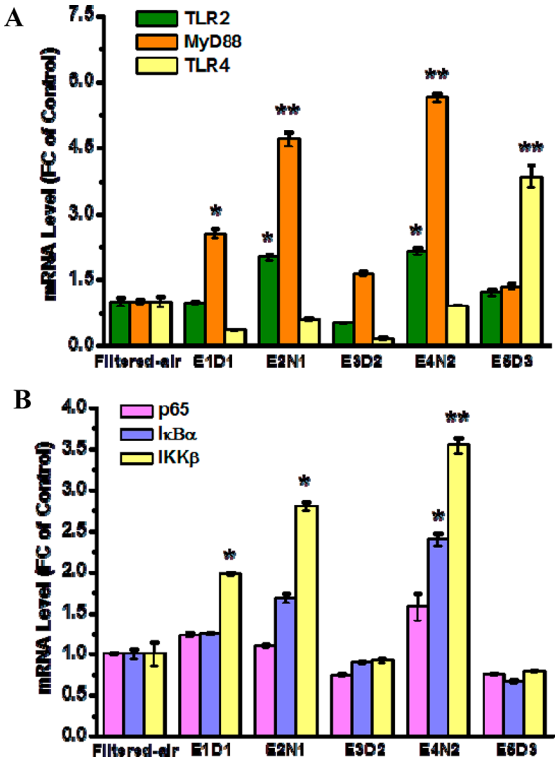

Figure 6. Toll-like receptor 2 pathway activated by the Spring Festival air pollution. The expression of TLR2, TLR4, MyD88 (A), p65, IκBα, and IKKβ genes (B), as analyzed by real-time PCR. The data are expressed as the mean ± SD, $n = 6$, $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the filtered-air control group.

regulation of TLR2/MyD88 or NF-κB pathway genes was detected in the E3D2 group. It is notable that another important toll-like receptor, TLR4, was only up-regulated in the E5D3 group. High particle concentration and low acidic gas levels mainly induced TLR2 but did not activate TLR4. Compared with the other groups, there were low concentrations of particles and acidic gases, but the ions from the acidic gases (SO_4^{2-}, NO^{3-} and Cl^{-}) were 6-fold greater in the PM in the E5D3 group, which may have caused the up-regulation of TLR4. Changes in the levels of many more miRNAs were observed when the air possessed a high particle concentration and low acidic gas level (E2N1 and E4N2). Many of these miRNAs have feedback regulatory roles in the prevention of excessive inflammation.

TLR2 and MyD88 were activated in the high PM_1 exposure groups, followed by NF-κB signaling pathway activation, both of which indicate an inflammatory response. The TLR2/MyD88 signaling is crucial for the development of lung eosinophilia.[32] In addition, ultrafine PM stimulates macrophages' inflammatory response and activates the TLR pathway, both of which have been implicated in exacerbating asthma and chronic inflammatory diseases.[33] Therefore, inhalation of the air pollution in the Spring Festival may induce inflammation in the respiratory tract, possibly exacerbating asthma and chronic respiratory eosinophilia as a result.

High Particulate Concentration and Acidic Gas Levels Induce Cytokine Release. NF-κB integrates inflammatory pathway networks, prolonging activation, which may regulate the release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and interleukin 1 (IL-1), leading to endoplasmic reticulum (ER) stress response and cell death.[26] After the air pollution exposure, TNF-α and IL-6 secretion increased in the E2N1, E3D2, and E4N2 groups, indicating an activated immune response cascade, most markedly in the macrophages of the E3D2 group, which exhibited ultrahigh concentrations of black carbon, PM_1, and acidic gases (Figure 7). It was previously

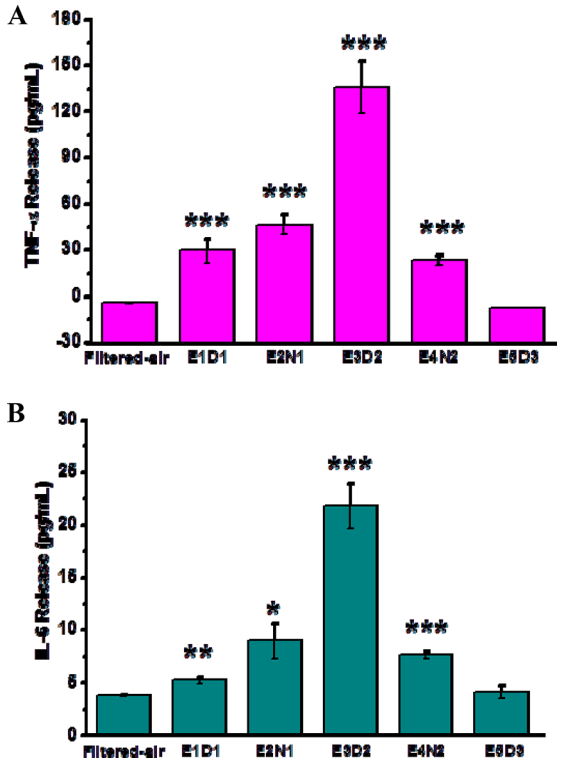

Figure 7. Increased TNF-α and IL-6 secretion was induced by the Spring Festival air pollution. The amounts of TNF-α (A) and IL-6 (B) in the basal chamber as determined by ELISA, after exposure to air from the Spring Festival. TNF-α and IL-6 secretion were most significantly increased during the daytime of the Chinese New Year (E3D2). The data are expressed as the mean ± SD, $n = 6$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

demonstrated that apoptosis may be a TNF-α-dependent pathway.[26] PM_{10} and SO_2 exposures have been shown to lead to apoptosis, while PM_{10} or SO_2 alone does not damage cells.[27] Thus, the condition most closely associated with the expression of TNF-α and IL-6 appears to be attributed to high concentrations of both PM and acidic gases.

High Particulate Concentration and Acidic Gas Levels Induce the Trend of Cell Apoptosis. PM is able to induce ER stress and the expression of the transcription factor CHOP in murine macrophages.[34,35] CHOP is generally a primary factor and regulates ER stress-induced apoptosis.[36] Up-regulation of the chaperone protein BiP is another recognized marker of ER stress.[37] BiP induction closely follows the time course of CHOP induction. However, overexpression of BiP prevents CHOP induction and may attenuate cell death. Additionally, PM may also cause apoptosis by activating the extrinsic apoptosis pathway (TNF-α secretion and caspase-8 and -3 activation).[38] Individually, NO_2 or SO_2 exposure at relatively high concentrations can lead to apoptosis.[39] In our study, besides affecting an immune response and release of cytokines, we also observed that ALI exposure with ambient air

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from the Spring Festival induced ER stress and the trend of apoptosis.

Exposure to air pollution from the Spring Festival induced ER stress and the trend of apoptosis to different extents in macrophages at the various exposure periods (Figure 8). The

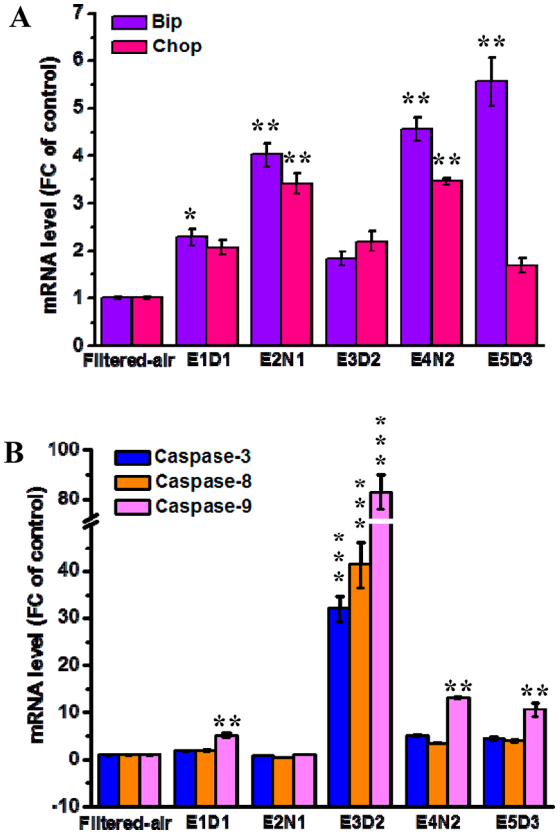

Figure 8. ER stress and apoptosis were induced by the Spring Festival air pollution. mRNA levels of ER stress markers (BiP and CHOP) and apoptosis related genes (caspase-3, caspase-8, and caspase-9), as analyzed by real-time PCR. (A) Both BiP and CHOP genes were significantly increased in the Chinese New Year's Eve and the Chinese New Year's Night exposure samples (E2N1 and E4N2). (B) Caspase-3, caspase-8, and caspase-9 were significantly up-regulated in the samples exposed to air from the daytime on Chinese New Year (E3D2). The data are expressed as the mean ± SD, $n = 6$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the filtered-air control group.

gene expression profile of macrophages in the E3D2 group was different from that in the E2N1 and E4N2 groups. Both the BiP and CHOP genes were significantly up-regulated in the New Year's Eve night and Chinese New Year night exposure groups (E2N1 and E4N2), both of which exhibited high PM_1 and black carbon levels (Figure 8A), suggesting that high PM may induce the ER stress response with BiP and Chop balancing each other's activity. Air in the E5D3 group (few fireworks), induced the expression of BiP, while there was no change in the expression of CHOP. It may be deduced that a low concentration of air pollution is tolerable for the macrophages. Only apoptosis-related genes, such as caspase-3, -8, and -9, were remarkably decreased in the E3D2 group (Figure 8B), which is consistent with the TNF-α and IL-6 secretion data. Based on the real-time monitoring of the air components, high concentrations of particles and SO_2, NO_x, and CO gases were present in the E3D2 group, while only the concentrations of particles, and not acidic gases, were elevated in the E2N1 and E4N2 groups. The high concentration PM_1 and acidic gases may surpass the cell self-defense threshold to induce the trend of apoptosis.

In our present study, we combined real-time monitoring of the atmosphere and ALI exposure of macrophages to investigate the biological effects of air pollution during the Spring Festival in Beijing. Our data demonstrate that (1) the air pollution during the Spring Festival may lead to the alteration of distinct miRNAs that target genes of the TLR-NF-κB pathway, fine-tuning inflammatory responses at a post-transcriptional level; (2) air pollutant conditions of high PM but low acidic gas concentrations may induce inflammation in macrophages, while high concentrations of PM and acidic gases may lead to the trend of apoptosis (Figure 9). Our results may help to elucidate the toxic mechanisms of air pollution and provide clues for discovering the main drivers of air pollution-induced disorders.

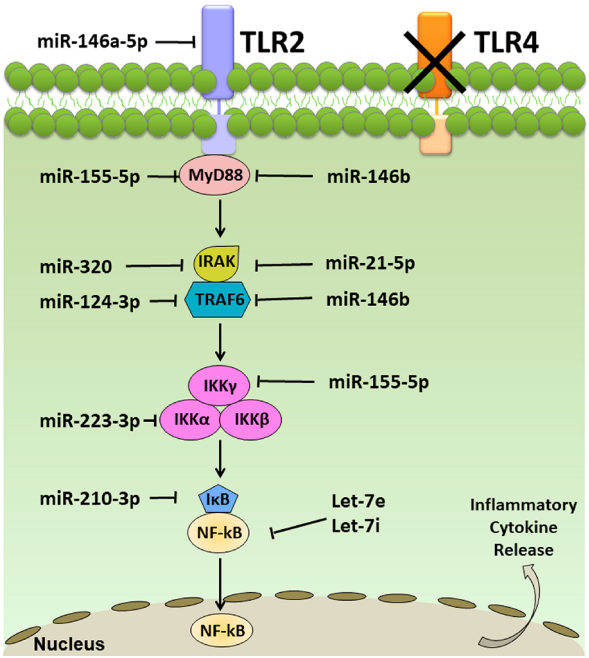

Figure 9. Affected miRNAs and their gene targets. The air pollution during the Spring Festival may lead to the alteration of distinct miRNAs targeting genes of the TLR2-NF-κB pathway, which may fine-tune inflammatory responses at a post-transcriptional level.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b00399.

Part of the methodology, Figure S1−S4, and Table S1−S3 (PDF)

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Notes

The authors declare no competing financial interest.

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