The impact of inflammation and cytokine expression of PM2.5 in AML

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Abstract. Environmental and health issues have become a major focus of research worldwide in recent years. Particulate matter with diameter ≤2.5 μm (PM2.5) is a common air pollutant that has been demonstrated to be associated with various diseases, including acute myeloid leukemia (AML). In the present study, the effects of PM2.5 on the proliferation and inflammation were assessed using three human acute myeloid cell lines (U937, HL-60 and KG-1a) *in vitro*. Additionally, the levels of several cytokines [interleukin (IL)-2, *IL-10*, *IL-17A* and tumor necrosis factor (TNF)α] in AML cells and Sprague Dawley rats were evaluated to investigate the effects of PM2.5 on cytokine expression in AML. The results revealed that PM2.5 was capable of enhancing inflammatory responses in AML cells, and increasing *IL-2*, *IL-10*, *IL-17A* and *TNFα* mRNA expression in AML cells to different degrees. Furthermore, PM2.5 increased *IL-2* and *IL-10* contents in rats following 12 weeks of exposure. These results suggested that PM2.5 may serve a role in promoting the occurrence and progression of leukemia by affecting cytokine expression, and that there may be various mechanisms active in different AML subtypes.

Introduction

Air pollution, also known as smog, has become much more severe in China in recent years. Smog is the result of interactions between specific climatic conditions and human activities. The economic and social activities of high-density populations result in the emission of large amounts of fine particulate matter; one such common pollutant is known as particulate matter with diameter ≤2.5 μm (PM2.5) due to the aerodynamic diameter is ≤2.5 μm. When the discharge of PM2.5 exceeds the circulation and carrying capacity of the atmosphere, these fine particles accumulate in the air, leading to smog. Numerous studies have demonstrated that air pollution damages the respiratory system, cardiovascular system, and organs, including the heart and lungs (1-8) Additionally, PM2.5 increases the incidence of infectious diseases and decreases male fertility (9-13).

Leukemia, a malignant clonal disease originating from hematopoietic stem cells, is a major public health threat, and the sixth and eighth leading cause of cancer-associated mortality in men and women in China, respectively. Notably, leukemia is the leading cause of cancer-associated mortality in children and patients <35 years old. In recent years, several studies have demonstrated the association between PM2.5 and the risk of leukemia. Studies by Brosselin and Steffen have revealed that living near gas stations or garages may increase the risk of developing acute lymphoblastic leukemia and acute myeloid leukemia (AML) (14,15). Another study performed in Denmark indicated that there is an association between traffic-associated air pollution and the risk of developing AML (16).

Despite extensive studies, the mechanisms mediating the effects of PM2.5 on the occurrence and development of leukemia remain unclear. However, changes in the bone marrow microenvironment are considered to be involved in the progress of leukemia (17,18). A study performed in Taiyuan, China demonstrated that PM2.5 affects cell proliferation, but does not cause cell injury in exposed leukemia cells and that low doses of PM2.5 accelerates leukemia development through a reactive oxygen species-mediated pathway (19).

In the present study, the effects of various concentrations of PM2.5 on cell proliferation were investigated in three AML cell lines (U937, HL-60 and KG-1a). The expression levels of several cytokines, including interleukin-2 (*IL-2*), *IL-10*, *IL-17A* and tumor necrosis factor (TNF) α, after PM2.5 exposure in AML cell lines and rats were also evaluated, with the aim of elucidating the mechanisms through which PM2.5 affects the occurrence and development of leukemia.

Materials and methods

Reagents and cell lines. PM2.5 particles purchased from the National Institute of Standards and Technology (Gaithersburg,

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MD, USA) were diluted in sterilized PBS solution to 5 mg/ml and preserved at -20˚C in aliquots. The samples were then diluted to a working concentration in RPMI-1640 (cat. no. SH30809.01; Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) prior to use.

Three human AML cell lines (U937, HL-60 and KG-1a) were purchased from the National Infrastructure of Cell Line Resource (Beijing, China). U937 and HL-60 cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (cat. no. 10099141; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), whereas KG-1a cells were cultured in RPMI-1640 supplemented with 20% fetal bovine serum. All cell lines were cultured at 37˚C in a 5% CO_2 humidified atmosphere without antibiotics.

Treatment of U937, HL-60 and KG-1a cell lines. U937, HL-60 and KG-1a cells were treated with PM2.5 solution at concentrations of 0-20 mg/ml for 24, 48 and 72 h after reaching a steady-state of exponential growth in normal medium.

Measurement of cell proliferation. Cell proliferation rates were measured using a Cell Counting Kit-8 (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) according to the manufacturer's protocol. The relative cell proliferation ratio (%) following treatment was compared with controls (cultured cells without PM2.5 treatment or blank controls with RPMI-1640 with 10% FBS), and calculated as follows: $[(A_{control}-A_{blank\ of\ control})-(A_{sample}-A_{blank\ of\ sample})]/(A_{control}-A_{blank\ of\ control})$ x100%.

Measurement of cytokine mRNA expression levels in AML cell lines. The mRNA expression levels of *IL-2*, *IL-10*, *IL-17A*, and *TNFα* were detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in AML cells following treatment with 0.1 mg/ml PM2.5 solution for 0, 24, 48 and 72 h. The housekeeper gene actin β was used as a control. The RNA of cells was extracted using Trizol reagent (cat. no. 15596018; Invitrogen; Thermo Fisher Scientific, Inc.). cDNA templates from AML cell lines were prepared using a TIANScript RT kit (Tiangen Biotech, Co., Ltd., Beijing, China) according to the manufacture's protocol.

RT-qPCR cycling was performed in 96-well plates on a LightCycler 480 Real-Time PCR system (Roche Applied Science, Penzberg, Germany). The reaction was performed in a 20-μl total volume containing 10 μl SYBR Select Master mix (cat. no. 4472908; Thermo Fisher Scientific, Inc.), 1 μl of each primer (10 μM), and 2 μl template cDNA. The primer sequences used for PCR are presented in Table I. The amplification protocol consisted of an initial denaturation step at 95˚C for 5 min, followed by two-step PCR for 40 cycles at 95˚C for 30 sec and 60˚C for 30 sec. The mRNA expression levels of each target were determined based on the cycle threshold (Cq) value for the reference and each target and calculated as $2^{-\Delta Cq}$ (20). Three independent experiments were performed.

Measurement of protein expression levels in rats. All 26 Sprague Dawley (SD) rats were 6-week-old males (173-190 g), purchased from Charles River Laboratories (Beijing, China), and housed at a temperature of 25˚C, $1.013x10^5$ pa atmosphere, 12/12 h dark/light cycle and *ad libitum* access to food and water. The present study was approved by Ethics Committee of Beijing Luhe Hospital Affiliated to Capital Medical University (Beijing, China). Exposed chambers and clean chambers were constructed to raise rats. Air in the exposed chambers was the same as the true air outside, whereas air in the clean chambers was filtered to remove PM2.5 particles. Fourteen rats were kept in exposed chambers (n=8) or clean chambers (n=6) for 6 weeks (between August 9, 2016 and September 19, 2016), and 12 rats were kept in exposed chambers (n=8) or clean chambers (n=4) for 12 weeks (between August 9, 2016 and October 10, 2016). The PM2.5 concentration in the atmosphere during the study is presented in Table II.

Table I. Primer sequences and lengths of detected genes.

Primer name	Sequence (5'-3')	Product length (bp)
IL-2	F: TACAAGAACCCGAAACTGACTCG	29
	R: ACATGAAGGTAGTCTCACTGCC	28
IL-10	F: TCAAGGCGCATGTGAACTCC	26
	R: GATGTCAAACTCACTCATGGCT	28
IL-17A	F: AGATTACTACAACCGATCCACCT	29
	R: GGGGACAGAGTTCATGTGGTA	27
TNFα	F: CCTCTCTCTAATCAGCCCTCTG	28
	R: GAGGACCTGGGAGTAGATGAG	27
ACTB	F: CATGTACGTTGCTATCCAGGC	27
	R: CTCCTTAATGTCACGCACGAT	27

IL, interleukin; TNF, tumor necrosis factor; ACTB, actin β; F, forward; R, reverse.

The peripheral blood of rats was collected from hearts after feeding for 6 or 12 weeks. Blood samples were centrifuged at 1,000 x g for 20 min at room temperature to obtain serum. Milliplex Map Rat Cytokine/Chemokine Magnetic Bead Panel-Immunology Multiplex assays (cat. no. RECYTMAG-65K; EMD Millipore, Billerica, MA, USA) were used to detect the expression of cytokines in serum according to the manufacturer's protocol.

Statistical analysis. Statistical analysis was performed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and Graphpad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). All data were derived from at least three independent experiments and are presented as means ± standard deviations. The results were evaluated using two-tailed unpaired Student's t-test and one-way analysis of variance (ANOVA) with Dunnett's multiple comparison post-hoc test. $P<0.05$ was considered to indicate a statistically significant difference. In RT-qPCR analysis, statistical significance was assumed if the $2^{-\Delta ct\ (target\ mean\ value-reference\ mean\ value)}$ value was >150% or <75%.

Results

Low concentrations of PM2.5 promote proliferation, whereas high concentrations of PM2.5 inhibit proliferation in AML cells. First, the effects of PM2.5 on the proliferation of AML

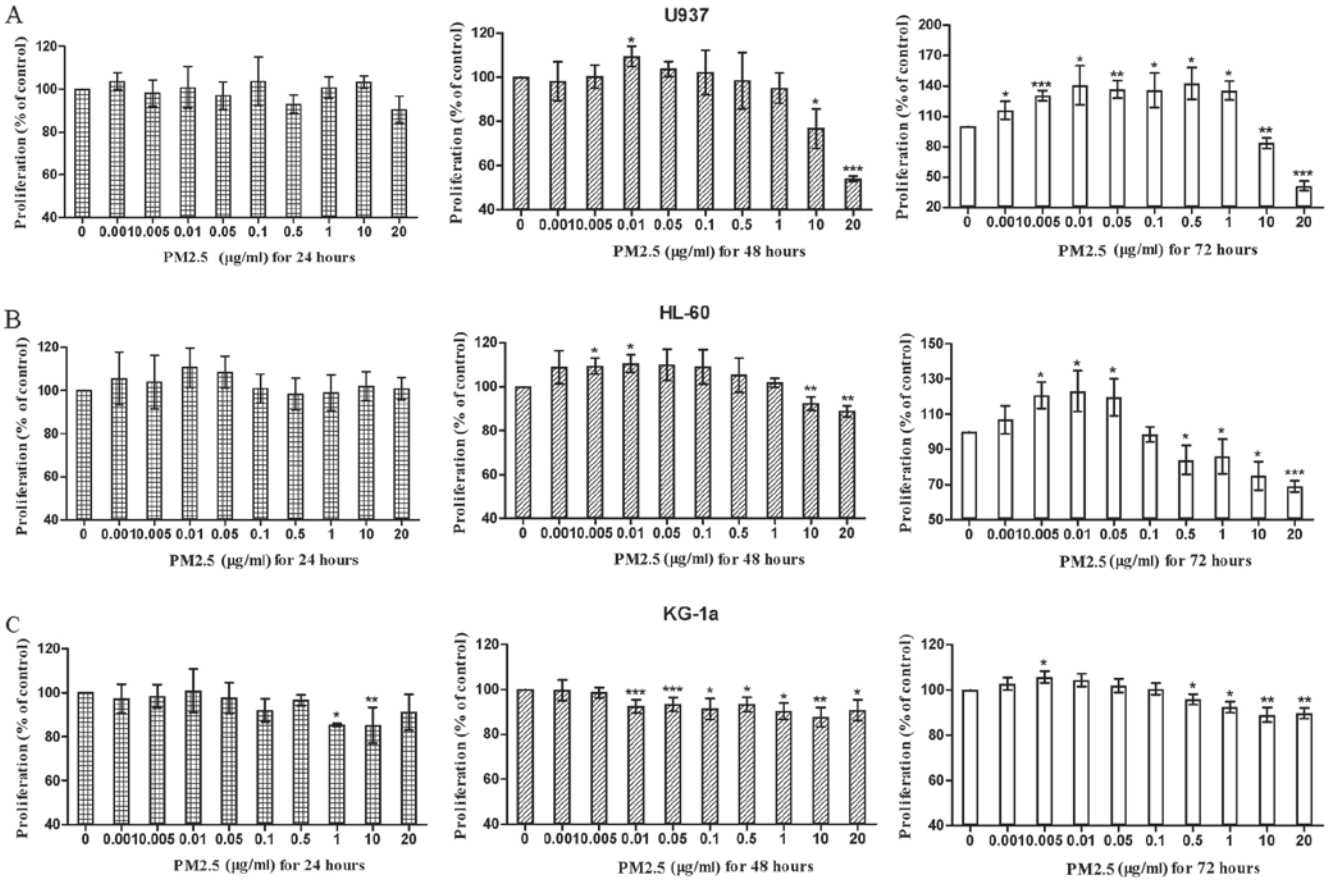

Figure 1. Stimulatory and inhibitory effects of PM2.5 on cell proliferation in (A) U937, (B) HL-60 and (C) KG-1a cells. Cells were treated with different concentrations of PM2.5 for 24, 48, or 72 h. Values represent the means ± standard deviations of three identical experiments with four replicates each. *P<0.05, **P<0.005, ***P<0.0005 vs. 0 μg/ml. PM2.5, particulate matter with diameter ≤2.5 μm.

cell lines (U937, HL-60, and KG-1a) were evaluated. The results demonstrated that 24 h of treatment with PM2.5 did not induce or inhibit the proliferation of U937 cells at any concentration. However, after 48 or 72 h of treatment, PM2.5 first promoted and then significantly inhibited the proliferation of U937 cells as the concentration of PM2.5 increased. These effects were more evident at 72 h compared with at 48 h. A similar situation was observed in HL-60 and KG-1a cells. However, the stimulatory and inhibitory effects of PM2.5 on KG-1a cells were less evident compared with those on U937 and HL-60 cells (Fig. 1).

PM2.5 significantly alters the expression of cytokines in AML cells. Next, the expression levels of *IL-2*, *IL-10*, *IL-17A* and *TNFα* were analyzed in U937, HL-60 and KG-1a cells following treatment with PM2.5. The results revealed that the mRNA expression levels of *IL-2* in U937 cells, *IL-17A* in HL-60 cells, and *IL-2* and *IL-17A* in KG-1a cells were below the limit of detection (data not shown). In contrast, the expression of *IL-10*, *IL-17A* and *TNFα* in U937 cells; *IL-10* and *TNFα* in HL-60 cells; and *IL-10* in KG-1a cells significantly increased in a time- and concentration-dependent manner (Figs. 2 and 3). Notably, *IL-2* expression in HL-60 cells and *TNFα* expression in KG-1a also increased after treatment with 0.1 μg/ml PM2.5 for 24 h or ≥0.01 μg/ml for 72 h; however, these effects did not persist over time (Fig. 2) and did not increase as the concentration was increased (Fig. 3).

PM2.5 significantly increases serum IL-2 and IL-6 in SD rats following treatment for 12 weeks. Four cytokines, *IL-2*, *IL-10*, *IL-17A* and *TNFα*, were detected in SD rat serum using enzyme-linked immunosorbent assays following treatment with PM2.5 for 6 or 12 weeks. The results demonstrated that exposure to PM2.5 for 6 weeks did not significantly alter serum levels of *IL-2*, *IL-10*, *IL-17A* and *TNFα* in SD rats (Table III). However, when the exposure time was increased to 12 weeks, serum *IL-2* and *IL-10* levels in rats were significantly higher compared with those of untreated rats (Table IV).

Discussion

Several epidemiological studies have demonstrated positive associations between PM2.5 exposure and increased leukemia risk (14-16,21-23). However, the mechanism explaining this association has not yet been elucidated. The present study aimed to explain the effects of PM2.5 on the occurrence and development of leukemia through its influence on cytokines *in vitro* and *in vivo*.

In the current study, low doses of PM2.5 promoted the proliferation of AML cells (U937, HL-60, and KG-1a), whereas high doses of PM2.5 resulted in cytotoxicity, thereby inhibiting cell proliferation. Furthermore, PM2.5 exposure resulted in significantly increased levels of *IL-2*, *IL-10*, *IL-17A* and *TNFα* in AML cells. Thus, these results suggested that PM2.5 was capable of inducing an inflammatory response in human AML cells.

Table II. PM2.5 concentration of the atmosphere during the study.

A, PM2.5 concentration between August 09 2016 and September 19 2016

Date	PM2.5 ($\mu g/m^3$) within chambers	PM2.5 ($\mu g/m^3$) in Beijing outside	Date	PM2.5 ($\mu g/m^3$)	Beijing
Aug-09-2016	30	44	Aug-30-2016	25	28
Aug-10-2016	41	82	Aug-31-2016	15	16
Aug-11-2016	36	85	Sep-01-2016	6	7
Aug-12-2016	23	59	Sep-02-2016	8	11
Aug-13-2016	10	21	Sep-03-2016	13	18
Aug-14-2016	9	18	Sep-04-2016	56	80
Aug-15-2016	15	20	Sep-05-2016	25	27
Aug-16-2016	18	22	Sep-06-2016	22	17
Aug-17-2016	35	59	Sep-07-2016	28	41
Aug-18-2016	38	74	Sep-08-2016	17	20
Aug-19-2016	11	17	Sep-09-2016	19	17
Aug-20-2016	31	52	Sep-10-2016	18	15
Aug-21-2016	40	51	Sep-11-2016	28	32
Aug-22-2016	37	51	Sep-12-2016	33	31
Aug-23-2016	35	69	Sep-13-2016	62	86
Aug-24-2016	41	79	Sep-14-2016	66	101
Aug-25-2016	10	19	Sep-15-2016	72	69
Aug-26-2016	9	9	Sep-16-2016	82	133
Aug-27-2016	12	14	Sep-17-2016	28	41
Aug-28-2016	9	10	Sep-18-2016	17	22
Aug-29-2016	14	17	Sep-19-2016	12	10
Average value	44	61	Average value	44	61

B, PM2.5 concentration between September 19 2016 and October 31 2016

Date	PM2.5 ($\mu g/m^3$) within chambers	PM2.5 ($\mu g/m^3$) in Beijing outside	Date	PM2.5 ($\mu g/m^3$)	Beijing
Sep-20-2016	32	18	Oct-11-2016	75	116
Sep-21-2016	56	63	Oct-12-2016	77	77
Sep-22-2016	73	99	Oct-13-2016	148	150
Sep-23-2016	77	121	Oct-14-2016	133	241
Sep-24-2016	85	111	Oct-15-2016	96	190
Sep-25-2016	80	162	Oct-16-2016	83	119
Sep-26-2016	44	58	Oct-17-2016	52	59
Sep-27-2016	10	24	Oct-18-2016	80	117
Sep-28-2016	20	13	Oct-19-2016	130	225
Sep-29-2016	56	51	Oct-20-2016	42	94
Sep-30-2016	76	97	Oct-21-2016	49	42
Oct-01-2016	106	165	Oct-22-2016	23	14
Oct-02-2016	96	183	Oct-23-2016	43	26
Oct-03-2016	62	120	Oct-24-2016	79	60
Oct-04-2016	37	33	Oct-25-2016	87	96
Oct-05-2016	66	55	Oct-26-2016	24	43
Oct-06-2016	48	72	Oct-27-2016	36	30
Oct-07-2016	35	39	Oct-28-2016	17	10
Oct-08-2016	26	11	Oct-29-2016	38	23
Oct-09-2016	52	31	Oct-30-2016	34	43
Oct-10-2016	88	87	Oct-31-2016	10	8
Average value	44	61	Average value	44	61

PM2.5, particulate matter with diameter ≤2.5 μm.

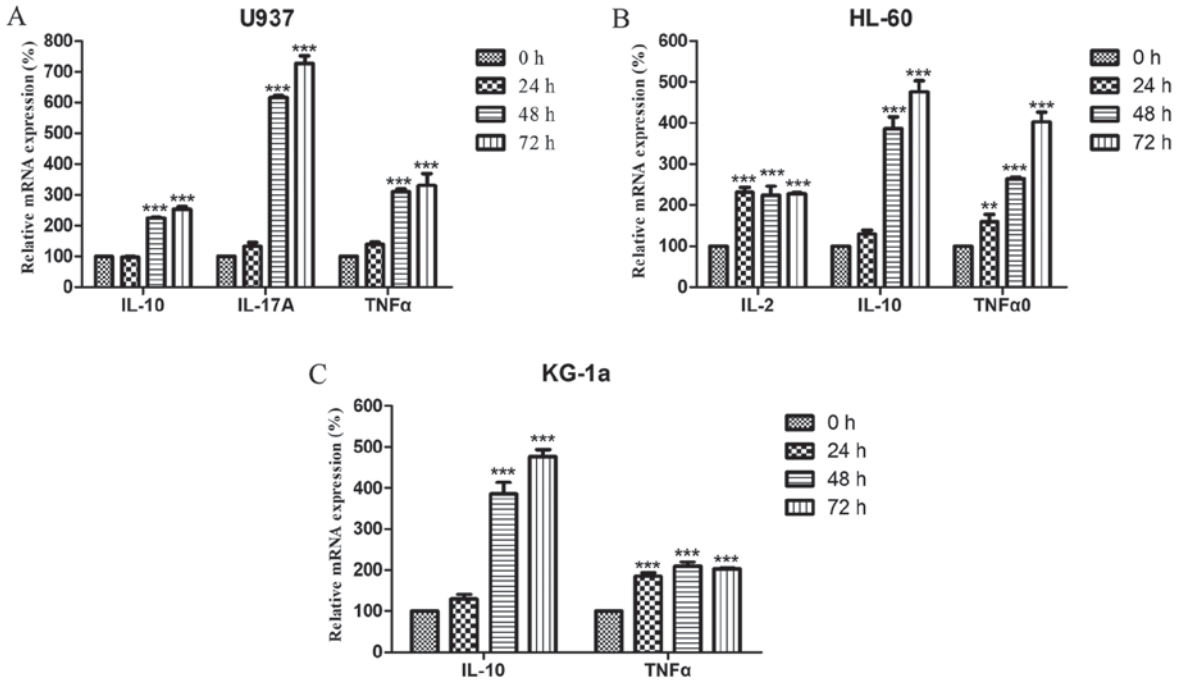

Figure 2. Relative mRNA expression levels of *IL-10, IL-17A,* and *TNFα* in (A) U937 cells; (B) *IL-2, IL-10,* and *TNFα* in HL-60 cells; and (C) *IL-10* and *TNFα* in KG-1a cells treated with 0.1 μg/ml PM2.5 for 24, 48, or 72 h. mRNA expression levels are relative to actin β. Data are presented as the means ± standard deviations of three identical experiments with four replicates each. **P<0.005, ***P<0.0005 vs. 0h. IL, interleukin; TNF, tumor necrosis factor; PM2.5, particulate matter with diameter ≤2.5 μm.

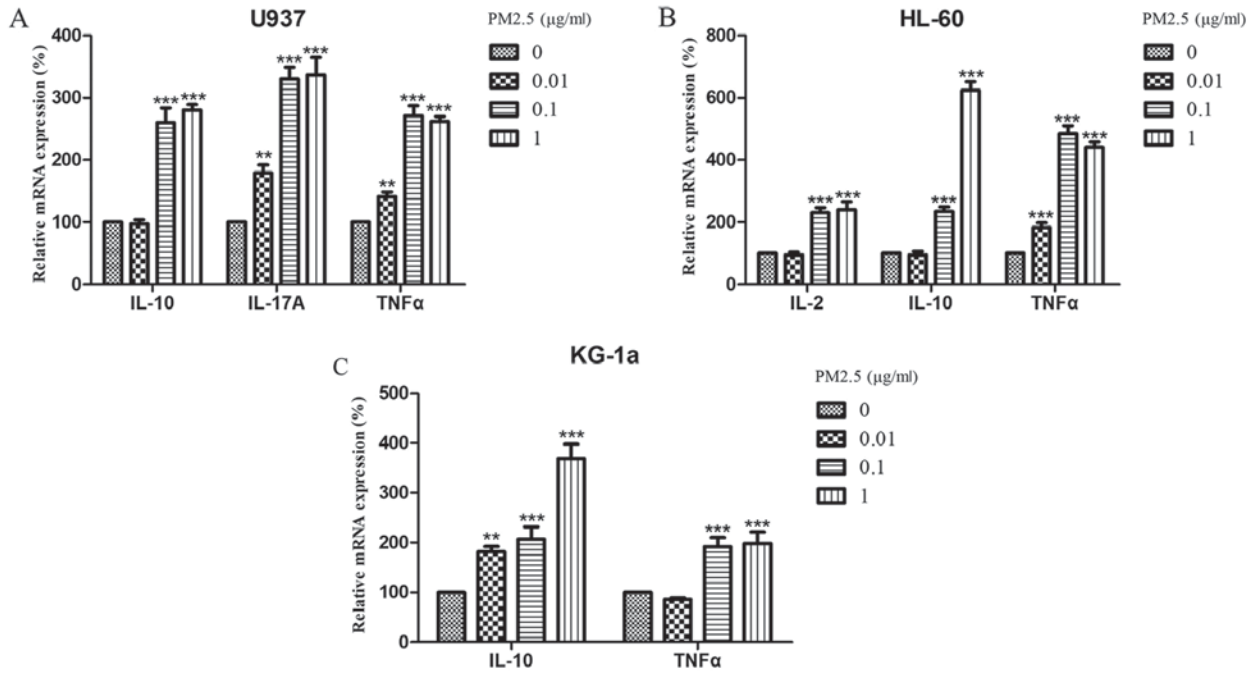

Figure 3. Relative mRNA expression levels of *IL-10, IL-17A,* and *TNFα* in (A) U937 cells; (B) *IL-2, IL-10,* and *TNFα* in HL-60 cells; and (C) *IL-10* and *TNFα* in KG-1a cells treated with 0, 0.01, 0.1, or 1 μg/ml PM2.5 for 72 h. mRNA expression levels are relative to actin β. Data are presented as the means ± standard deviations of three identical experiments with four replicates each. **P<0.005, ***P<0.0005 vs. 0 μg/ml. IL, interleukin; TNF, tumor necrosis factor; PM2.5, particulate matter with diameter ≤2.5 μm.

CD4$^+$ T cells, also known as helper T cells, are divided into three classes: Th1, Th2, and Th17, according to their different cytokine secretion patterns. The differentiation of Th1 and Th2 cells is influenced by local environmental cytokines, and these two groups complement or antagonize each other to regulate immune functions.

IL-2 is a Th1 cytokine that is indispensable in immune system regulation. *IL-2* is secreted by activated T or natural

Table III. Content of rat serum cell factors after raising in exposed and clean chambers for 6 weeks.

		Content (pg/ml) in each rat								
Cell factors	Chamber	1	2	3	4	5	6	7	8	P-value
IL-2	Exposed chamber	35.38	16.18	33.35	9.6	25.42	13.61	9.6	8.71	0.33
	Clean chamber	17.95	23.5	41.6	16.18	17.95	31.33			
IL-10	Exposed chamber	25.53	89.42	222.9	56.52	23.3	23.3	155.46	41.05	0.79
	Clean chamber	28.97	72.71	115.15	9.45	129.69	67.25			
IL-17A	Exposed chamber	3.49	5.23	5.23	8.31	19.99	10.63	3.49	6.2	0.42
	Clean chamber	5.23	13.13	5.23	8.31	11.86	17.15			
TNFα	Exposed chamber	1.35	2.45	2.17	3.02	3.02	2.17	1.35	1.9	0.25
	Clean chamber	2.73	2.45	1.9	1.9	3.02	3.62			

IL, interleukin; TNF, tumor necrosis factor.

Table IV. Content of rat serum cell factors following raising in exposed and clean chambers for 12 weeks.

		Content (pg/ml) in each rat								
Cell factors	Chamber	1	2	3	4	5	6	7	8	P-value
IL-2	Exposed chamber	9.6	8.71	6.78	14.45	12.78	9.6	6.78	5.92	0.01
	Clean chamber	21.61	16.18	14.45	11.16					
IL-10	Exposed chamber	14.95	82.4	59.17	22.2	10.31	17.96	28.97	39.81	0.04
	Clean chamber	351.6	192.02	66.11	14.95					
IL-17A	Exposed chamber	4.33	5.23	2.7	5.23	8.31	5.23	2.7	4.33	0.08
	Clean chamber	5.23	11.86	6.2	6.2					
TNFα	Exposed chamber	1.9	1.68	1.68	1.51	2.17	1.9	1.68	1.9	0.68
	Clean chamber	1.9	1.68	1.68	2.17					

IL, interleukin; TNF, tumor necrosis factor.

killer (NK) cells by autocrine or paracrine secretion, and serves an important role in the activation and maintenance of the immune response and in the promotion of lymphocyte development. Numerous studies have confirmed that *IL-2* induces NK cells and enhances their antitumor activity (24,25). The application of *IL-2* as an antitumor drug in the clinical treatment of patients with AML began in the 1980s (26). In the present study, it was revealed that *IL-2* levels increased in a time- and concentration-dependent manner in HL-60 cells treated with PM2.5, but was not detected in U937 or KG-1a cells. In rats, *IL-2* expression was significantly enhanced after 12 weeks of exposure to PM2.5. These results suggested that PM2.5 may affect different subtypes of AML cells in different ways; thus, there may be multiple complex cytokine networks *in vivo*.

IL-10 is a Th2 cytokine that has multiple pleiotropic effects on immunoregulation and inflammation, and is capable of inhibiting the synthesis of pro-inflammatory cytokines, including interferon-γ, *IL-2*, IL-3, *TNFα* and granulocyte-macrophage colony-stimulating factor, produced by macrophages and Th1 T cells (27-29). Despite the inhibitory effects of *IL-10* on Th1 cytokines, certain Th1 cells are also able to produce *IL-10*, and a negative feedback pathway is formed when *IL-10* regulates Th1 cell activation (27). Previous studies have demonstrated that *IL-10* directly inhibits the proliferation and migration of effector T cells, and blocks the production of associated cytokines, serving an important role in inducing tumor immune escape. When the *IL-10* content is increased, the killing effect of T cells on tumor cells is markedly inhibited (30-32). Blocking *IL-10* expression in animal models improves the killing ability of the immune system on tumor cells (33,34). In addition, *IL-10* has been shown to inhibit T cells, forming an immunosuppressive environment and inducing tumor immune escape by inhibiting antigen-presenting cells activation (35). In the present study, the mRNA expression levels of *IL-10* were significantly increased in a time- and concentration-dependent manner following treatment of AML cell lines with PM2.5 solution. Similar results were also obtained in an *in vivo* experiment; that is, serum *IL-10* contents were significantly increased in rats exposed to PM2.5 compared with those of unexposed rats after 12 weeks. These

results demonstrate the potentially carcinogenetic effects of PM2.5 in AML.

IL-17A is a pro-inflammatory cytokine produced by activated Th17 cells, which function to amplify inflammation, and activate neutrophils to engulf and digest extracellular bacteria and molds by releasing pro-inflammatory cytokines (36). IL-17 and Th17 cells mediate carcinogenesis in rat tumor models and patients with cancer. The mechanisms through which IL-17 and Th17 cells mediate carcinogenesis involve angiogenesis and the induction of cytokines in the tumor microenvironment, which promotes tumor growth (37-40). Several studies have demonstrated that IL-17 induces IL-6 production, and IL-6 activates the signal transducer and activator of transcription 3 pathway, which then upregulates the expression of prosurvival and angiogenic genes to modulate tumor angiogenesis (41-45). The tumor promoting effects of IL-17 and Th1 exist in various types of common tumors (40,46-48). Wu *et al* (49) demonstrated that Th17 cells were significantly increased in the peripheral blood of patients with AML compared with that in normal healthy individuals. In addition, IL-17 content increased as the number of Th17 cells increased, resulting in promotion of bone marrow cell proliferation and inhibition of immune function. Additionally, the slow growth of *TNFα* in the tumor microenvironment enhanced the recruitment of IL-17-dependent bone marrow cells and promoted tumor development. In the present study, the mRNA expression of *IL-17A* was significantly increased in U937 AML cells a time- and concentration-dependent manner following treatment with PM2.5 solution; however, the *IL-17A* content in HL-60 and KG-1a cells was below the detection range. Thus, these findings indicated that different mechanisms mediated the effects of PM2.5 on AML cells. The *in vivo* experiment revealed no significant increases in exposed rats compared with unexposed rats after 6 or 12 weeks, indicating that the mechanisms through which PM2.5 affected AML differed *in vitro* and *in vivo*. However, further studies with increased numbers of animals are required to confirm these findings.

TNFα, which is also a Th1 cytokine, is produced ectopically by malignant and immune cells in the tumor-associated microenvironment, creating a tumor-supportive inflammatory niche that modulates the development and progression of malignant disease (50). *TNFα* is produced by various types of leukemia cells (51-55). In several clinical studies, a positive association between the expression levels of *TNFα* and adverse clinical parameters in leukemia was observed (53-57) In AML, high levels of *TNFα* expression are associated with greater fatigue and poorer quality of life (58). Previous studies have revealed that *TNFα* activates nuclear factor κB and c-Jun N-terminal kinase/activator protein-1 to exert anti-apoptotic and proproliferative effects in leukemia cells, thereby facilitating leukemia cell survival and progression (32-34, 59-61). In the current study, *TNFα* levels were significantly increased in U937 and HL-60 cells in a time- and concentration-dependent manner following PM2.5 treatment. In KG-1a cells, *TNFα* levels were also increased, although the time- and concentration-dependent effects were not as evident. This *in vitro* experiment reflects the potential ability of PM2.5 to promote the occurrence and development of leukemia by regulating intracellular *TNFα*. *In vivo*, significant changes in *TNFα* expression were not observed after 6 or 12 weeks of treatment with PM2.5, further supporting that there may be different mechanisms mediating the effects of PM2.5 on AML *in vitro* and *in vivo*. However, again, further studies with greater numbers of animals are required.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

TC performed the cell and animal experiments of the present study and analyzed the obtained data. JZ and HuZ interpreted the data. YuZ, YoZ and XZ helped TC to perform the animal experiments. DZ and YF helped with the animal experiments and participated in writing the manuscript. HeZ was a major contributor to the idea of the study and participated in writing the manuscript.

Ethics approval and consent to participate

The present study was approved by Ethics Committee of Beijing Luhe Hospital Affiliated to Capital Medical University (Beijing, China).

Consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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