

Changes in gene expression in lungs of mice exposed to traffic-related air pollution

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ABSTRACT

Long-term exposure to traffic-related pollutants can lead to a variety of respiratory diseases, including inflammation, asthma, and lung cancer; however, the underlying biological mechanisms are not fully understood. We focused on the effects of exposure to different air pollutants on the expression of genes associated with inflammatory immune responses, allergic reactions and asthma, and lung cancer. In order to understand the cellular responses induced by exposure to different traffic-related pollutants, we performed PCR array to evaluate the mRNA expression of genes associated with inflammatory immune responses, allergic reactions and asthma, and lung cancer in the lungs of mice exposed to three different environments, including the laboratory (clean air), and polluted parking garages in Foshan and Guangzhou for four weeks. Cytokines (IFN- γ , IL-4, and IL-17A) were analyzed by Flow cytometry; the morphological structures were detected by Haematoxylin and eosin (H&E) staining. Our results revealed that the main pollutant in Guangzhou was PM_{2.5}, the main pollutants in Foshan were gaseous pollutants including CO, NO_x and SO₂. IFN- γ was significantly lower, and IL-4, and IL-17A were significantly higher in mice in the Guangzhou and Foshan groups compared with laboratory group. The morphological structures were damaged in Guangzhou and Foshan groups. In addition, we found that exposure to traffic-related pollutants triggered the expression of inflammatory genes (Cxcl11 and Tnfs4), allergy and asthma genes (Clca3 and Prg2), and lung cancer genes (Agr2, Col11a1, and Sostdc1). As such, our results demonstrate that persistent exposure to traffic-related pollutants may elevate the incidence of immune disorders and asthma, and may be as a risk factor for lung cancer.

1. Introduction

In recent years, studies have shown that exposure to air pollution is associated with numerous diseases, especially cardiopulmonary diseases and lung cancer. For example, Gehring et al. [1] and Schultz et al. [2] have suggested that exposure to air pollution increased the risk of respiratory and allergic disorders. Research conducted by Vermeulen [3] has also demonstrated that exposure to air pollution increased the morbidity and mortality of lung cancer. Currently, antioxidant and inflammatory responses are thought to be the underlying biological mechanisms for the increased risk of respiratory and allergic disorders caused by exposure to air pollution [1,2]. Previous studies have confirmed that long-term exposure to particulate matter (PM) with a diameter of less than 2.5 μ m (PM_{2.5}) can accelerate the progress of many diseases, including asthma and lung cancer [3–5]. Furthermore, inhaled particulate matter and gaseous pollutants, such as NO₂, may play

important roles in the developmental processes of diseases through stimulating the release of pro-inflammatory mediators [6,7].

Our previous study in cooperation with Sun Yat-sen University evaluated the effects of automobile fumes in underground parking lots on the immune systems of animals. The results indicated that long term exposure to a high concentration of pollutants promoted alveolar epithelium swelling and particle formation. Moreover, multiple inflammatory factors were increased in peripheral blood. We concluded that the air pollutants triggered immune disorders. However, the previous study did not further explore the expression changes of associated genes, and the parking space environment does not accurately represent the road traffic environment.

As such, this study expands on this previous research. In the current study, mice were divided into three groups, and each group was exposed to different kinds of air pollution in the laboratory, Guangzhou, and Foshan. Lung tissues and serum from mice in each group were

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collected. Cytokines (IFN- γ , IL-4, and IL-17A) were measured by Flow cytometry; the morphological structures were detected by Haematoxylin and eosin (H&E) staining. The expressions of genes associated with inflammatory immune responses, allergic reactions and asthma, and lung cancer were detected by PCR array and verified by RT-PCR. Our results demonstrated that the degree of inflammation was lower, and the morphological structure was more complete in laboratory group; persistent exposure to traffic-related pollutants may elevate the incidence of immune disorders, asthma, and lung cancer. Furthermore, the *Clca3* gene and the *Col11a1* gene were demonstrated to be related to the pathogenesis of diseases induced by exposure to atmospheric pollutants.

2. Materials and methods

2.1. Animals

Adult male BALB/c mice (8 weeks of age) were obtained from the animal experimental center in the North Campus of Sun Yat-sen University. Experiments were approved by the animal ethics committee. The mice were divided into three treatment groups (laboratory, Foshan, Guangzhou). Mice in the three groups were housed in the laboratory in the North Campus of Sun Yat-sen University (a clean air environment), site A (an underground parking lot in Guangzhou, Haizhu district), and site B (an underground parking lot in Foshan, Sanshui district) respectively. There were 15 mice in each group. All mice were maintained in a normal light-dark cycle, with available food and water for four weeks. Animals were euthanized by cervical dislocation, and the lung tissue was collected and stored at liquid nitrogen for gene expression analyses.

2.2. RNA purification

The total RNA of lung tissues was isolated using TRIzol[®] reagent (Gibco BRL) according to the manufacturer's specifications. First, the lung tissues were pulverized and homogenized in 1 ml of TRIzol. Then, the RNA was precipitated using isopropanol after chloroform addition and centrifugation. The RNA concentration was determined with a NanoDrop[®] ND-1000, and the integrity and purity of RNA were assessed using an Agilent 2100 Bioanalyzer.

2.3. Reverse-transcription real-time PCR and array analysis

The reactions of RT-PCR were carried out using the 2X SuperArray PCR master mix, and the PCR arrays were amplified with the ABI QuantStudio 12K Flex system. PCR arrays (96 wells) were designed for the evaluation of mice genes associated with inflammatory immune responses, allergic reactions and asthma, and lung cancer. The PCR arrays were conducted using the following kits from SABiosciences: mouse inflammatory cytokines and receptors, PAMM-011Z, mouse allergy and asthma, PAMM-067Z, and mouse lung cancer, PAMM-134Z. In each PCR array, relative expression of 84 target genes and 12 actin housekeeping genes was determined according to the comparative Ct method. qRT-PCR assays were performed to validate the amplification efficiencies of the target genes, and the $2^{-\Delta\Delta C_t}$ method was used to calculate the relative gene expression (presented as fold change) [8]. A change greater than 1.5-fold indicates upregulation, and a change of less than 0.5-fold indicates downregulation. GAPDH was used as a control. Divergent primers were designed and synthesized by Invitrogen Custom Oligo Service (Invitrogen, USA). The primer sequences of objective genes and GAPDH are shown in Table 1.

2.4. Flow cytometry analysis

5 ml of peripheral blood was collected, and the serum was separated by centrifugation after 2 h. According to the manufacturer's

Table 1

Primer sequences for qRT-PCR analysis.

Gene	Primer sequences	Product (bp)
GAPDH	Forward: 5'-GGGTCCCAGCTTAGGTTTCAT-3' Reverse: 5'-CATTTCTCGGCCTTGACTGTG-3'	248
Clca3	Forward: 5'-CGTTGATCTCAGGGACCACT-3' Reverse: 5'-GGGAGTTGAGTCTTCGGGAA-3'	250
Prg2	Forward: 5'-ATGGGTGACTCTGGATGCAA-3' Reverse: 5'-GATCCTGCCTCCCAATCCAGA-3'	195
Areg	Forward: 5'-CTTTGGTGAACGGTGTGGAG-3' Reverse: 5'-TCGTTTCCAAAGGTGCACTG-3'	160
Il13	Forward: 5'-ACTGCGTCTCCTGAGACAA-3' Reverse: 5'-GCTTCCGTCCAGTTCAAAGG-3'	196
Agr2	Forward: 5'-CCTGCTTCTTGTGGCCATT-3' Reverse: 5'-GGGTCTGTTGCTTGTCTTGG-3'	187
Col11a1	Forward: 5'-TCCTGGAACCATGCTGATGT-3' Reverse: 5'-ATCCCCACTCTCACCTTGG-3'	208
Sostdc1	Forward: 5'-TGGAGGCAGGCATTTCAGTA-3' Reverse: 5'-TCCGGCTCCAGTACTTTGTT-3'	212
Ccl1	Forward: 5'-AGCATGCTTACGGTCTCCAA-3' Reverse: 5'-CGTTTGTGTAGTTGAGGCGC-3'	168
Cxcl11	Forward: 5'-AACAGGAAGGTACAGCCAT-3' Reverse: 5'-ACTTCAACTTTGTGCGAGCC-3'	182
Tnfsf4	Forward: 5'-CTGGAACAGGATCAAGGCC-3' Reverse: 5'-GCCATCTCACATCTGGTA-3'	184

instructions, the serum levels of cytokines (IFN- γ , IL-4, and IL-17A) were detected by using a cytometric bead array (CBA) mouse Th1/Th2/Th17 cytokine kit (BD Biosciences). The standards of each cytokine were prepared, the cytokine capture beads were freshly mixed by equal quantity. The mixed beads (50 μ L) were incubated with cytokine standard dilutions (50 μ L) and Phycoerythrin (PE) Detection Reagent (50 μ L) at room temperature for 2 h. The beads were centrifuged (200g for 5 min) and resuspended in 120 μ L of wash buffer. The results were obtained by using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA).

2.5. Haematoxylin and eosin (H&E) staining

All samples were immersed in the haematoxylin (Surgipath, Richmond, USA) for 10 min, and decolorized with 70% ethanol containing 1% hydrochloric acid. After washing, these sections were then placed in 0.1% eosin (Surgipath) for 5 min, and dehydrated in 80%, 90%, and 100% ethanol. After washing, these sections were treated with Oil Red O solution (Sigma-Aldrich, USA) for 10 min. The sections were stained with 1% Picrosirius Red (PSR, Sigma-Aldrich, India) for 10 min, and then dehydrated by using ethanol.

2.6. Statistical analysis

The manufacturer's online tool was used to analyze the array data. Other data were analyzed using Student's t-test and analysis of variance (ANOVA) using GraphPad Prism 6 software. Each experiment was repeated at least three times. The data are expressed as the mean $\pm$ standard deviation (mean $\pm$ SD). $P < 0.05$ was considered statistically significant.

3. Results

3.1. The distribution of different air pollutants in the laboratory, Guangzhou, and Foshan

We found that the main pollutant in Guangzhou was PM_{2.5}. However, the main pollutants in Foshan were gaseous pollutants including CO, NO_x and SO₂ (Fig. 1).

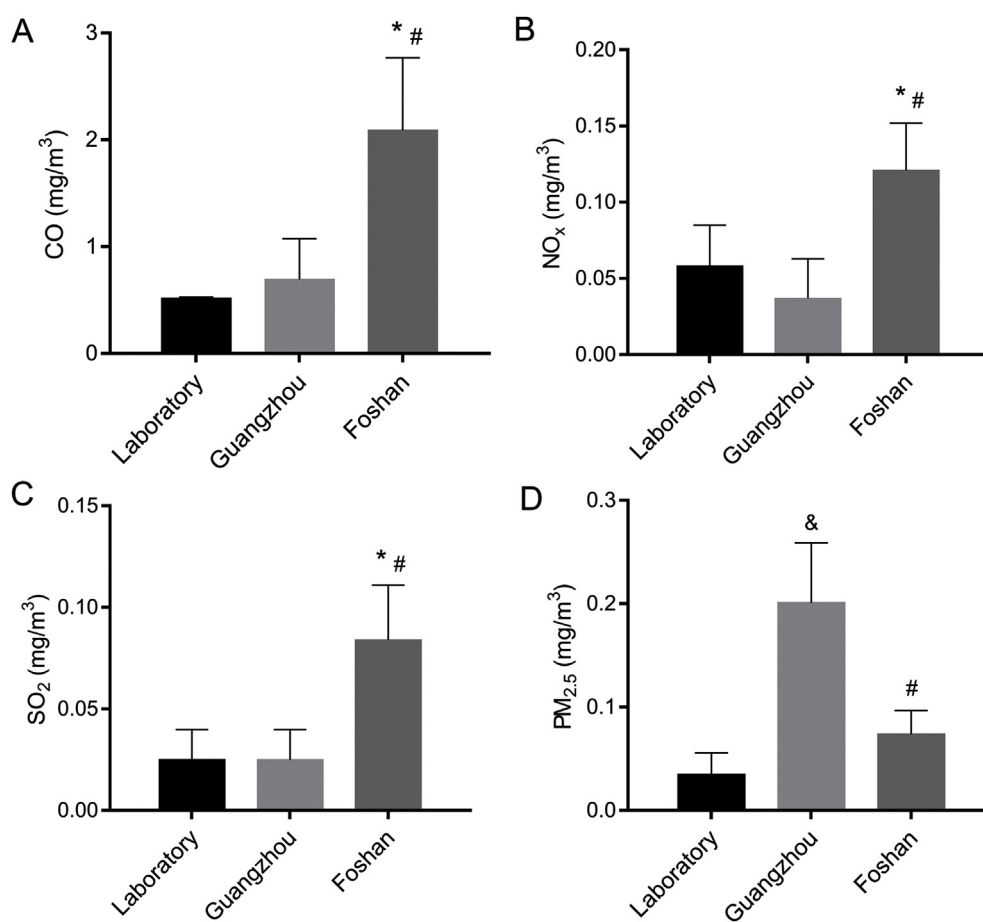


Fig. 1. The distribution of different air pollutants in Laboratory, Guangzhou and Foshan. (A–C) The concentration of CO, NO_x and SO₂ in Foshan is significantly higher compared to Laboratory or Guangzhou. (D) The concentration of PM_{2.5} in Guangzhou is significantly higher compared to Laboratory or Foshan.

3.2. Comparative levels of IFN- γ , IL-4, and IL-17A in laboratory, Foshan, or Guangzhou groups

In addition, Flow cytometry was used to detect the levels of cytokines (IFN- γ , IL-4, and IL-17A). The results found that the percentage of IFN- γ was 6.22%, 3.31% and 3.14% in laboratory, Foshan, or Guangzhou groups, respectively (Fig. 2A); the percentage of IL-4 was 2.37%, 6.27% and 7.41% in laboratory, Foshan, or Guangzhou groups, respectively (Fig. 2B); the percentage of IL-17A was 1.66%, 3.92% and 3.75% in laboratory, Foshan, or Guangzhou groups, respectively (Fig. 2C). Therefore, our results indicated that IFN- γ was significantly lower in mice in the Guangzhou and Foshan groups compared with laboratory group, and IL-4, and IL-17A were higher in mice in the Guangzhou and Foshan groups compared with laboratory group, suggesting that the degree of inflammation was higher in Guangzhou and Foshan groups.

3.3. Comparative morphological structures in laboratory, Foshan, or Guangzhou groups

To further detect the morphological structures, H&E staining was performed in laboratory, Foshan, or Guangzhou groups. As shown in Fig. 2D, the tissue in laboratory group was damaged. The tissues were comparatively complete, and the nucleus was clearly visible in Foshan, or Guangzhou groups. The quantities of inflammation surrounding bronchial epithelial cells (BEC), neutrophils, lymphocytes, eosinophils were increased in the pollution environment.

3.4. Differentially expressed inflammatory genes for a comparison between mice in the laboratory, Foshan, or Guangzhou groups

To compare effects of different types of air pollution, we used a PCR array to evaluate the mRNA expression of 84 inflammatory genes in the lungs of mice in the laboratory, Foshan, or Guangzhou groups. Heat map profiling revealed that the expression of inflammatory genes was different in mice in the laboratory, Foshan, and Guangzhou groups (Fig. 3A). Next, we evaluated the differences between mice in the Guangzhou group vs. laboratory group, Foshan group vs. laboratory group, and Guangzhou group vs. Foshan group with regards to lung inflammatory gene expression in mice. A scatter plot indicates the comparison of gene expression values for two groups and displays genes differentially regulated in the experiment (Fig. 3B). As shown in Table 2, we found that 28 genes were expressed differently in mice in the Guangzhou vs. laboratory group. The expression of four genes was significantly upregulated, and the expression of 24 genes was down-regulated. We found that nine genes were expressed differently in mice in the Foshan vs. laboratory group, and six of these genes were significantly upregulated, and three genes were down-regulated. There were 36 genes that were expressed differently between Guangzhou group and Foshan group lung tissues. Eleven genes were significantly upregulated, and 25 genes were down-regulated. qRT-PCR analysis was used to validate the gene microarray results for Ccl1, Cxcl11, and Tnfs4. As shown in Fig. 3C, Ccl1 gene expression was lower in mice in the Guangzhou and Foshan groups. However, Cxcl11 and Tnfs4 expression was significantly higher in mice in the Guangzhou and Foshan groups. The differential expression of three selected genes was in accordance with gene microarray data.

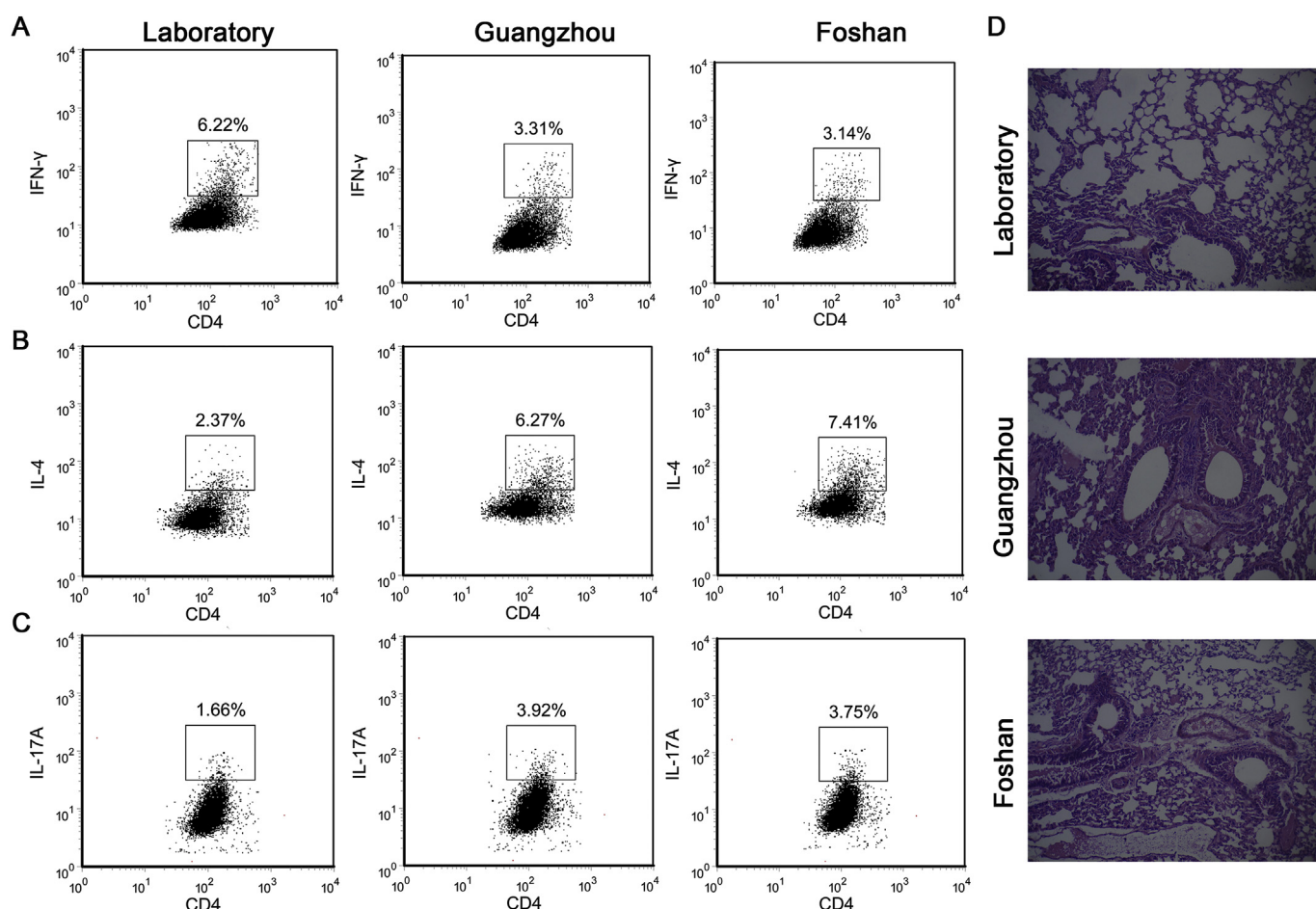


Fig. 2. Comparative levels of IFN- γ , IL-4, and IL-17A and morphological structures in laboratory, Foshan, or Guangzhou groups. (A–C) The levels of cytokines (IFN- γ , IL-4, and IL-17A) were detected by flow cytometry. D the morphological structure was measured by H&E staining.

3.5. Differentially expressed allergy and asthma genes for a comparison between mice in the laboratory, Foshan, or Guangzhou groups

A mouse allergy and asthma response PCR array was used to evaluate the mRNA expression of 84 allergy and asthma genes in the lungs of mice in the laboratory, Foshan, or Guangzhou groups. Heat map profiling revealed that the gene expression of allergy and asthma genes was different in mice in the laboratory, Foshan, and Guangzhou groups (Fig. 4A). Next, we evaluated the differences between mice in the Guangzhou vs. laboratory groups, Foshan vs. laboratory groups, and Guangzhou vs. Foshan groups in terms of lung allergy and asthma gene expression in mice. A scatter plot indicates the comparison of gene expression values for two groups, and displays genes differentially regulated in the experiment (Fig. 4B). Differentially expressed genes between lung tissues from mice in the laboratory, Guangzhou, and Foshan groups are shown in Table 3. We found that 22 genes were differentially expressed in mice in the Guangzhou vs. control group. Seven genes were significantly upregulated, and 15 genes were down-regulated. We found that 11 genes were differentially expressed in mice in the Foshan vs. laboratory group, and eight genes were significantly upregulated, and three genes were down-regulated. There were 26 differentially expressed genes in lung tissues from mice in the Guangzhou and Foshan groups. Nine genes were significantly upregulated, and 17 genes were down-regulated. As shown in Fig. 4C, differential gene expression analysis by real-time RT-PCR of *Areg*, *Clca3*, *Il13*, and *Prg2* in lungs of mice in the three different groups matched the data obtained by PCR array. The results demonstrate that expression of *Areg* and *Il13* genes was lower in mice in the Guangzhou and Foshan groups.

However, expression of *Clca3* and *Prg2* was significantly higher in mice in the Guangzhou and Foshan groups.

3.6. Differentially expressed lung cancer genes for a comparison between mice in the laboratory, Foshan, or Guangzhou groups

Mouse lung cancer PCR array was used to evaluate the mRNA expression of 84 lung cancer genes in the lungs of mice in laboratory, Foshan, or Guangzhou groups. Heat map profiling revealed that the lung cancer genes expression was different in mice in the laboratory, Foshan, and Guangzhou groups (Fig. 5A). Next, we evaluated the differences between mice in the Guangzhou vs. laboratory group, Foshan vs. laboratory group, and Guangzhou vs. Foshan group in terms of lung cancer gene expression in mice. A scatter plot shows the comparison of gene expression values for two groups, and displays genes differentially regulated in the experiment (Fig. 5B). Differentially expressed genes between lung tissues from mice in laboratory, Guangzhou, and Foshan groups are shown in Table 4. We found that 22 genes were differentially expressed in mice in the Guangzhou vs. laboratory group. Seventeen genes were significantly upregulated, and five genes were down-regulated. We found that nine genes were differentially expressed in mice in the Foshan vs. laboratory group, and all of them were significantly upregulated. There were 24 genes that were differentially expressed between lung tissues from mice in the Guangzhou and Foshan groups. Twenty-one genes were significantly upregulated, and three genes were down-regulated. As shown in Fig. 5C, differential gene expression analysis by real-time RT-PCR of *Agr2*, *Col11a1*, and *Sostdc1* in lungs of mice in the three different groups matched the data obtained by PCR

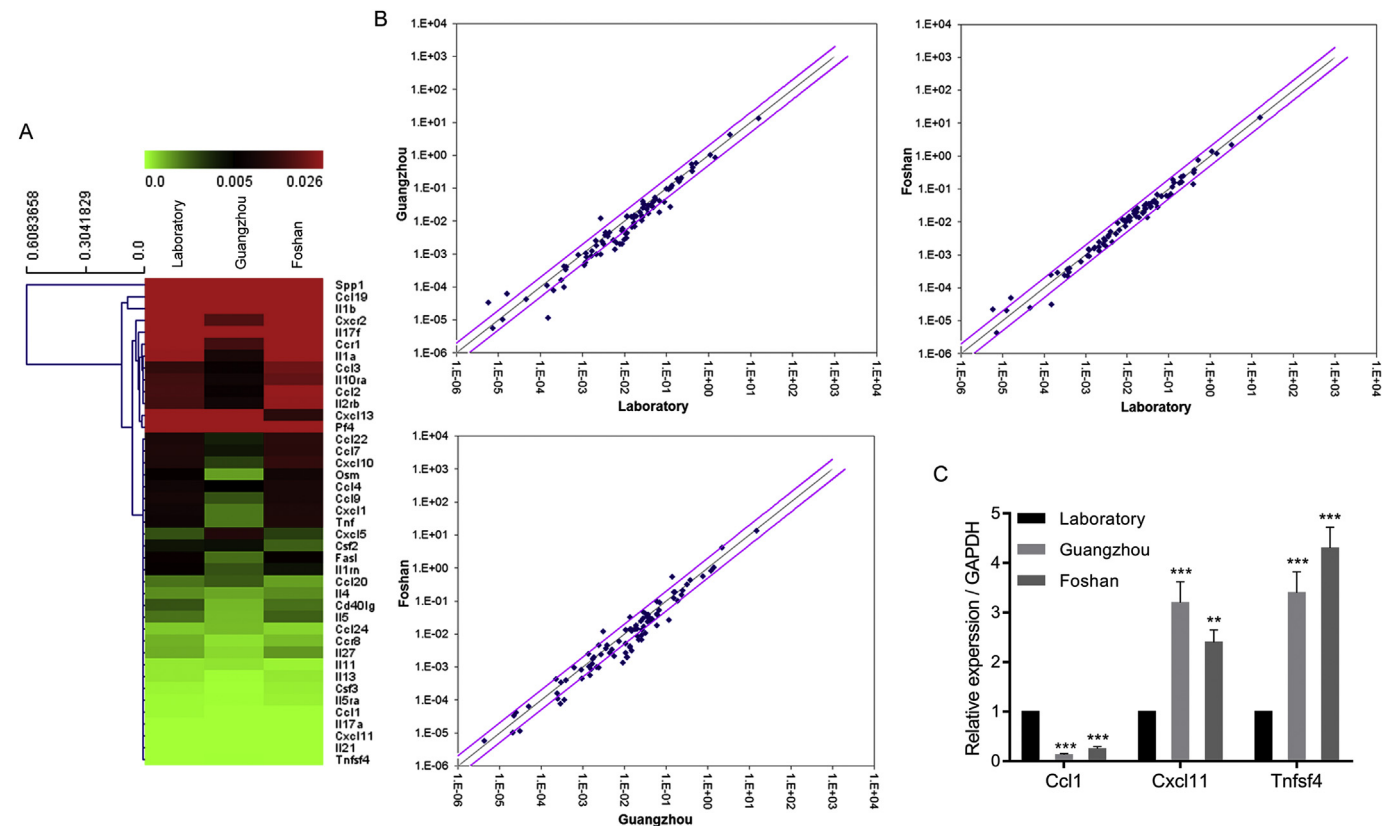


Fig. 3. Differentially expressed inflammatory genes for a comparison among Laboratory, Foshan or Guangzhou groups. (A) Cluster analysis of 72 inflammatory genes in lungs of mice exposed to Laboratory, Foshan or Guangzhou. ($P \leq 0.05$; ≥ 1.5 -fold or ≤ 0.5 -fold change). Red and green cells signify genes that were either up- or down-regulated, respectively. (B) Comparison of gene expression data from microarrays between Guangzhou and Laboratory, Foshan and Laboratory, and Foshan and Guangzhou. (C) Q-RT-PCR validation of selected genes expression levels in the mice lung in different regions exposure to different air particles. Note that Ccl1 expression is significantly decreased in Guangzhou and Foshan groups, however, Cxcl11 and Tnfsf4 expression is significantly elevated in Guangzhou and Foshan groups. *** $P < 0.001$, ** $P < 0.01$ (all vs. Laboratory). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Analysis of differentially expressed genes between Laboratory, Guangzhou and Foshan lung tissues. Inflammatory Cytokines & Receptors.

	Guangzhou vs. Laboratory	Foshan vs. Laboratory	Guangzhou vs. Foshan
Differentially expressed	28 (84)	9 (84)	36 (84)
Up-regulated	4 (14.3%)	6 (66.7%)	11 (30.6%)
Down-regulated	24 (85.7%)	3 (33.3%)	25 (69.4%)

array. The results demonstrate that gene expression of Agr2, Col11a1, and Sostdc1 genes was up-regulated in mice in the Guangzhou and Foshan groups.

4. Discussion

In the current study, mice were placed in a laboratory environment, and in underground parking lots in the Guangzhou Haizhu district and the Foshan Sanshui district. We found that the mice in the Guangzhou and Foshan groups were exposed to high levels of air pollution, compared to the laboratory environment, which had clean air. Our results indicate that the distribution of air pollutants in Guangzhou and Foshan are different. The main pollutant in Guangzhou was PM2.5, whereas the main pollutants in Foshan were gaseous pollutants including CO, NO_x, and SO₂.

IL-17A, belongs to IL-17 protein family, is a potent inflammatory cytokine. It can be generated by activated NK T (NKT) cells, $\gamma\delta$ T cells,

natural killer (NK) cells, T cells and innate lymphoid cells [9]. The role of IL-17A is to accumulate neutrophils and to promote the production of antimicrobial peptides and proinflammatory cytokines [10]. Interferon γ (IFN- γ), acts as a type of Th1 cytokines, has a protective effect in clearing E. IL-4, serves as kind of Th2 cytokines, is responsible for chronic infections [11]. In our study, we found that IFN- γ was significantly higher, and IL-4, and IL-17A were significantly lower in laboratory group, suggesting that the degree of inflammation was lower in laboratory group. In addition, we found that the morphological structures were more complete in laboratory group, BEC, neutrophils, lymphocytes, eosinophils were increased in the pollution environment.

Reports have demonstrated that nitrogen dioxide (NO₂), particulate matter less than 10 μ m in diameter (PM10), and particulate matter less than 2.5 μ m in diameter (PM2.5) are the main pollutants, and that these account for 80%, 9%–53%, and 9%–66% of traffic-related air pollution (TRAP), respectively. Long-term exposure to NO₂ or PM2.5 elevated the IL-6 expression level in mice, which was associated with inflammation and pulmonary diseases [12–17]. Asthma is a chronic inflammatory disease, and the occurrence and development progress of asthma is associated with a series of environmental factors. Growing evidence indicates that systemic inflammation plays an important role in the pathogenesis of asthma [17]. Moreover, in asthma pathogenesis, exposure to air pollutants, and enhancement of respiratory sensitization to allergens were suggested to play a key role [18]. A recent study indicated that TRAP elevated the incidence of childhood asthma [19]. Over the last decade, with the rapid development of technology, an increasing number of studies have focused on the changes in gene expression in relation to the mechanisms of action of air pollutants. For

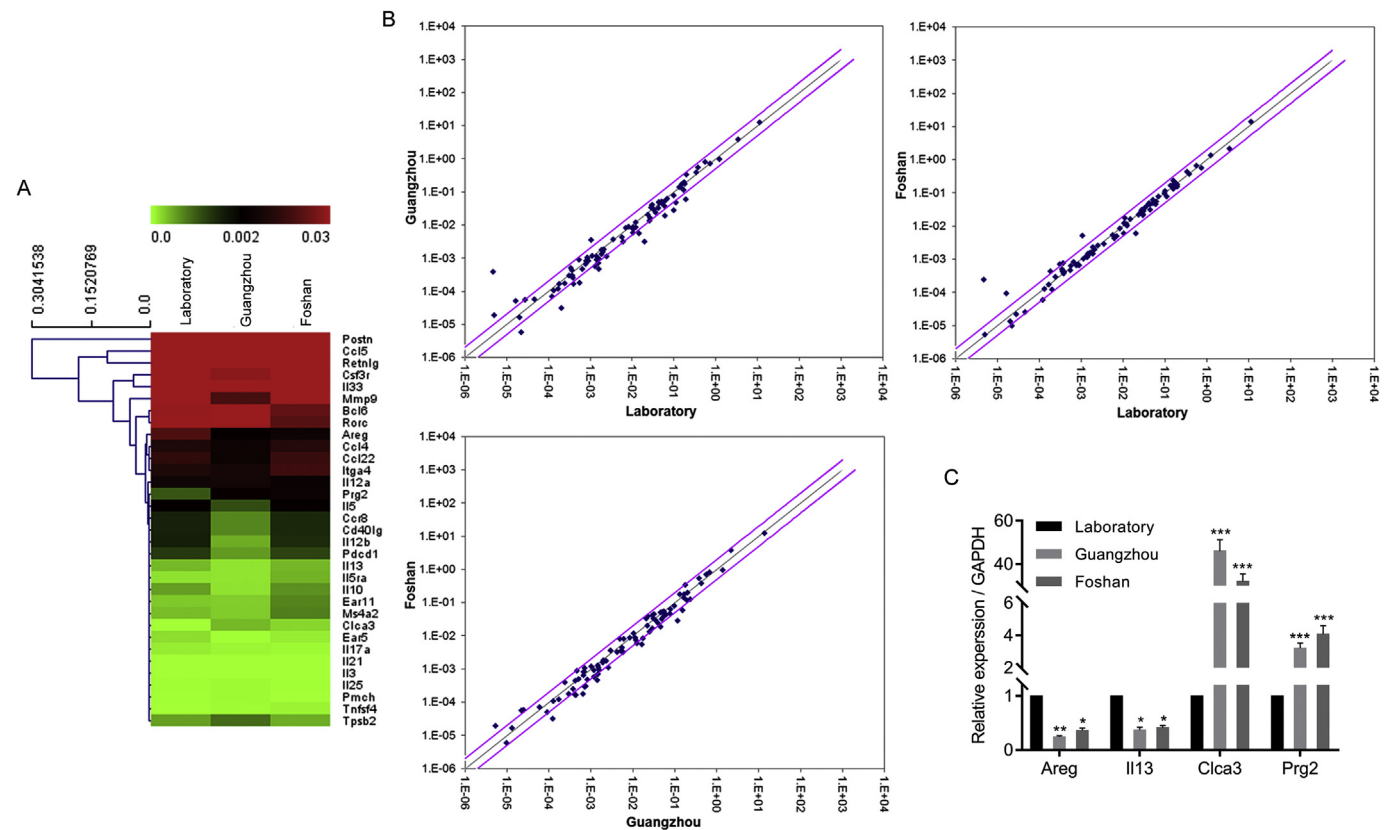


Fig. 4. Differentially expressed Allergy & Asthma genes for a comparison among Laboratory, Foshan or Guangzhou groups. (A) Cluster analysis of 84 Allergy & Asthma genes in lungs of mice exposed to Laboratory, Foshan or Guangzhou. ($P \leq 0.05$; ≥ 1.5 -fold or ≤ 0.5 -fold change). Red and green cells signify genes that were either up- or down-regulated, respectively. (B) Comparison of gene expression data from microarrays between Guangzhou and Laboratory, Foshan and Laboratory, and Foshan and Guangzhou. (C) Q-RT-PCR validation of selected genes expression levels in the mice lung in different regions exposure to different air particles. Note that Areg and Il13 expression is significantly decreased in Guangzhou and Foshan groups, however, Clca3 and Prg2 expression is significantly elevated in Guangzhou and Foshan groups. *** $P < 0.001$, ** $P < 0.01$; * $P < 0.05$ (all vs. Laboratory). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Analysis of differentially expressed genes between Laboratory, Guangzhou and Foshan lung tissues. Allergy & Asthma.

	Guangzhou vs. Laboratory	Foshan vs. Laboratory	Guangzhou vs. Foshan
Differentially expressed	22 (84)	11 (84)	26 (84)
Up-regulated	7 (31.8%)	8 (72.7%)	9 (34.6%)
Down-regulated	15 (68.2%)	3 (27.3%)	17 (65.4%)

example, studies conducted on cell and animal models demonstrated the effects of PM on the expression of genes associated with inflammatory responses, oxidative stress, vascular dysfunction, or progression of atherosclerosis [20–23].

In this paper, we aimed to describe the different expression of genes associated with inflammatory immune responses, allergic reactions and asthma, and lung cancer in lungs of mice exposed to TRAP in different regions with different pollutants. First, we investigated 84 inflammatory genes to evaluate the effects of long-term TRAP exposure on inflammation. Our results indicate that were significant gene expression changes in lung tissues in response to exposure to different pollutants (i.e., comparing the test group with control group). We found that in the lungs of mice in the Guangzhou group, exposure to a high concentration of PM_{2.5} induced a significantly higher expression of inflammatory genes Cxcl11 and Tnfsf4, although the expression of inflammatory gene Ccl1 was much lower in this group. Mice in the Foshan group were exposed to high concentrations of gaseous

pollutants, including CO, NO_x, and SO₂, and results from mice in this group were similar to those from mice in the Guangzhou group. However, the change in gene expression was a little smaller in the Foshan group compared to the Guangzhou group. We believe this may be due to differences in pollutants.

The mice in the Guangzhou and Foshan groups also had higher expression of allergy and asthma genes Clca3 and Prg2, but lower expression of allergy and asthma genes Areg and Il13 in their lung tissue, compared to mice in the laboratory group. As a member of calcium-activated chloride channels, Clca3 was found to be associated with the development of mucous cell metaplasia in asthmatic models [24]. The overexpression of Clca3 can induce goblet cell hyperplasia and mucous cell hypersecretion in asthmatic mice [25]. Consequently, we believe that the effects of air pollutants on asthma may be related to the increased expression of Clca3.

Moreover, we found that lung cancer genes Agr2, Coll11a1, and Sostdc1 expression was up-regulated in mice in the Guangzhou and Foshan groups, compared to mice in the laboratory group. Coll11a1 is a minor fibrillar collagen involved in tumorigenesis, such as ovarian cancer, head and neck squamous cell cancer, and non-small cell lung carcinoma (NSCLC) [26–28]. Lihuashen et al. [29] have confirmed that COL11A1 was up-regulated in non-small cell lung cancer, and promotes the proliferation, migration, and invasion of cancer cells. Based on our results, we believe that continuous exposure to atmospheric pollutants may increase the expression of a series of lung cancer genes, and the up-regulated expression of the Coll11a1 gene may be an important mechanism.

Based on all the results described above, we demonstrated that

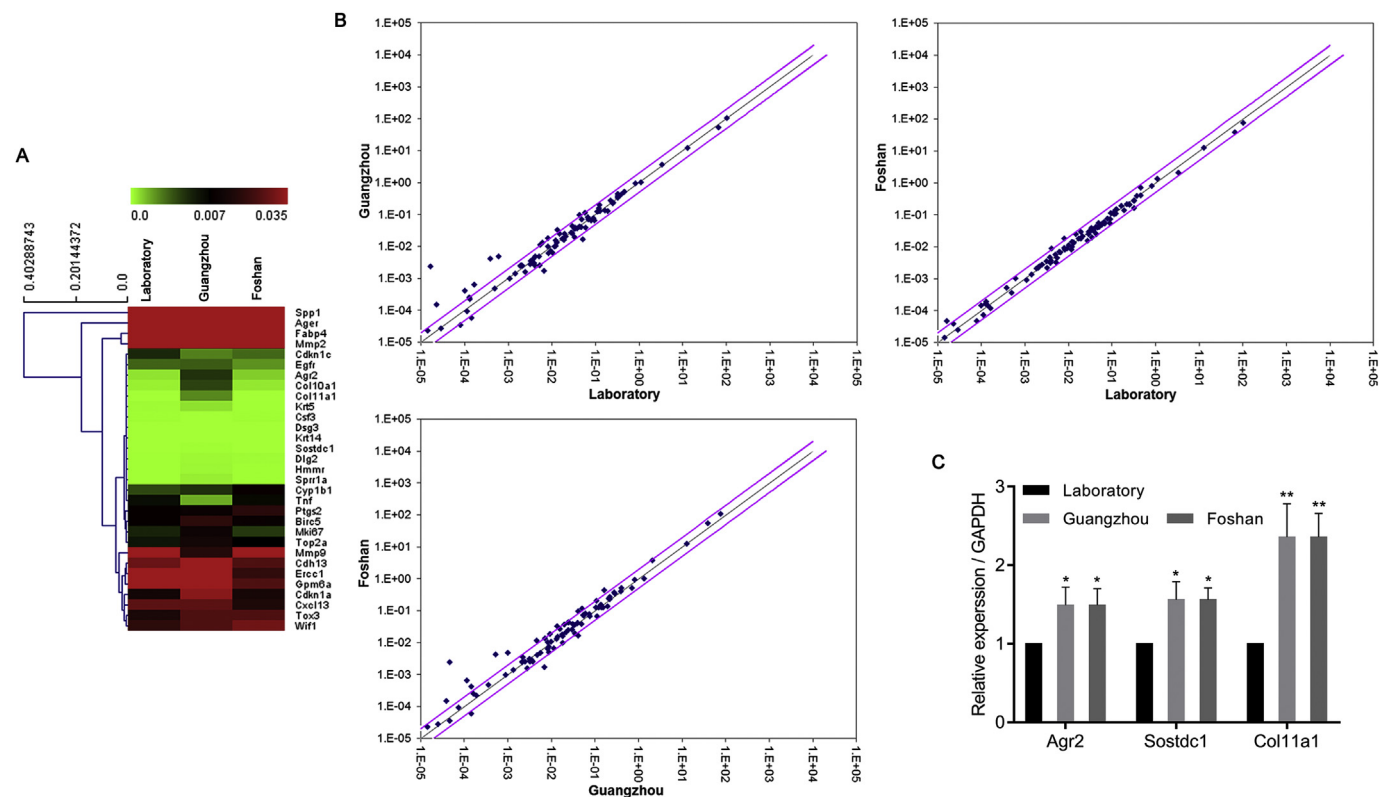


Fig. 5. Differentially expressed lung cancer genes for a comparison among Laboratory, Foshan or Guangzhou air. (A) Cluster analysis of 84 lung cancer genes in lungs of mice exposed to Laboratory, Foshan or Guangzhou. ($P \leq 0.05$; ≥ 1.5 -fold or ≤ 0.5 -fold change). Red and green cells signify genes that were either up- or down-regulated, respectively. (B) Comparison of gene expression data from microarrays between Guangzhou and Laboratory, Foshan and Laboratory, and Foshan and Guangzhou. (C) Q-RT-PCR validation of selected genes expression levels in the mice lung in different regions exposure to different air particles. Note that Agr2, Col11a1 and Sostdc1 expressions were significantly elevated in Guangzhou and Foshan groups. $**P < 0.01$ (all vs. Laboratory). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Analysis of differentially expressed genes between Laboratory, Guangzhou and Foshan lung tissues. Lung cancer.

	Guangzhou vs. Laboratory	Foshan vs. Laboratory	Guangzhou vs. Foshan
Differentially expressed	22 (84)	9 (84)	24 (84)
Up-regulated	17 (77.3%)	9 (100%)	21 (87.5%)
Down-regulated	5 (22.7%)	0 (0%)	3 (12.5%)

persistent exposure to traffic-related pollutants may elevate the incidence of immune disorders, and asthma, and may be as a risk factor for lung cancer. Furthermore, the Clca3 gene and the Col11a1 gene were demonstrated to be related to the pathogenesis of diseases induced by exposure to atmospheric pollutants. We believe that a large epidemiological investigation is needed to confirm the correlation between exposure to traffic-related pollutants and human disease.

Declaration of interest

None.

Author contributions

Jie Yang, Yi Chen and Zhongfu Ma contributed with the conception, design.
Yi Chen and Zhi Yu contributed with the analysis and interpretation.
Jie Yang and Hui Ding contributed with drafting the manuscript and critically reading it.

Jie Yang, Yi Chen and Zhongfu Ma contributed with reading and final approval of the version to be published.

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