

The Impact of Organic Extracts of Seasonal PM2.5 on Primary Human Lung Epithelial Cells and Their Chemical Characterization

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Research

Keywords: PM2.5, organic compounds, lung epithelial cells, cytokine, senescence, macroautophagy

Posted Date: January 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-137404/v1>

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Abstract

Background: Lung epithelial cells serve as first line of defense against various inhaled pollutant particles. This study focused on assessing the impact of organic extracts of PM_{2.5} collected in Seoul, South Korea on primary human lung epithelial cells and identifying the relevant components and sources which induced lung epithelial cell injury.

Results: We used primary lung epithelial cells isolated directly from healthy donors and evaluated the effects of organic compounds of twelve selected, seasonal PM_{2.5} on inflammation, cellular aging, and macroautophagy in primary lung epithelial cells. Organic extracts of PM_{2.5} specifically induced neutrophilic chemokine, interleukin-8, via extracellular signal regulated kinase activation. Moreover, PM_{2.5} significantly increased the expression of aging markers (p16, p21, and p27) and activated macroautophagy. Average mass concentrations, OC and EC, had no significant correlations with PM_{2.5} effects. However, regression analysis showed that polycyclic aromatic hydrocarbons and n-alkanes were the most relevant components of PM_{2.5} correlated with neutrophilic inflammation. Vegetative detritus and residential bituminous coal combustion sources were strongly correlated with neutrophilic inflammation, aging and macroautophagy activation.

Conclusions: These data suggest that the chemical composition of PM_{2.5} is crucially important to determine the adverse health effects of PM_{2.5}. Our study provides encouraging evidence to regulate the harmful components of PM_{2.5} in Seoul.

Background

The persistent occurrence of ambient air pollution has attracted a large amount of attention as a global environmental issue. The International Agency for Research on Cancer (IARC) classified particulate matter from outdoor air pollution as a Group 1 carcinogen in 2013 [1]. In particular, ambient fine particulate matter (PM_{2.5}) which has an aerodynamic diameter of 2.5 μm or less is well known to be correlated with an increase in mortality and morbidity caused by cardiovascular and pulmonary impairments [2–6]. As the airway is the first line of defense against inhaled PM_{2.5}, studies have discovered that particulate matter elicits oxidative stress and inflammation, which induce inflammatory lung diseases such as chronic obstructive pulmonary disease (COPD) and lung cancer [7–10]. Potential mechanisms of PM_{2.5}-induced adverse health effects on the human respiratory system have been consistently found in toxicological, experimental-based studies and in epidemiological studies [3, 11–13]. To investigate the effects of PM_{2.5}, number of toxicological studies used commercial lung epithelial cells [14–17] and Standard Reference Materials (SRM) urban particulate matter. However, the effects of collected ambient particulate matter, especially in Seoul, South Korea, on primary human airway epithelial cells (HAECs) isolated directly from healthy donors are not studied.

Due to the complexity of PM_{2.5} itself, the adverse health effects of PM_{2.5} may be different depending on chemical characteristics, sources, and regions. While PM_{2.5} is composed of various chemical constituents, organic material is chemical constituents that comprise about 20–40% of PM_{2.5} mass in urban area [18–20] and organic carbon (OC) and elemental carbon (EC) are highly related to adverse health effects such as emergency room visits and cardiopulmonary diseases [21–23]. In addition, organic compounds such as polycyclic aromatic hydrocarbons (PAHs) are prominent carcinogens [24–26]. Thus, finding the sources of PM_{2.5} based on local chemical characteristics and linking to toxicological effects is needed. With the supposition that the PM_{2.5} in Seoul will have distinct organic compounds and contributing sources, we analyzed organic compounds in PM_{2.5} and identified potential contributing sources by using a receptor model. Recently, the frequency of high concentration events (HCEs) has been increasing in Seoul. According to *The 2016 Environmental Performance Index Report*, more than 50% of Korean people are exposed to dangerous levels of PM_{2.5} [27]. In the present study, we investigated the impact of organic extracts of PM_{2.5} collected in Seoul, South Korea on primary human lung epithelial cells and identified the relevant components and sources in PM_{2.5}.

Results

The Effect of PM_{2.5} Organic Compounds on Cell Viability in Lung Epithelial Cells (BEAS-2B)

As PM_{2.5} organic compounds have been shown to be cytotoxic, we first evaluated the dose dependent effect of PM_{2.5} organic compounds on the viability of lung epithelial cells. BEAS-2B cells were treated with vehicle control (V.C., dichloromethane) and PM_{2.5} organic extracts (0.1, 0.5, 1, 2%) of single sample (sample collected on November 8th, 2016) for 24 h, and cell viability assays (MTT and LDH release assays) were performed. PM_{2.5} organic extracts (1%) or less did not affect cell viability (Fig. 1). Based on this result, we used PM_{2.5} (1%) in all experiments.

The Effect of PM_{2.5} Organic Compounds on Cytokine Production and the Expression of Aging and Macroautophagy Markers in BEAS-2B Cells

To test whether PM_{2.5} organic compounds (collected on November 8th 2016) induce the production of pro-inflammatory or anti-inflammatory cytokines in lung epithelial cells, BEAS-2B cells were incubated with V.C. and PM_{2.5} (1%) for 24 h. The levels of interleukin-1 β (IL-1 β), IL-6, IL-8, tumor necrosis factor- α (TNF- α), IL-17, basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) in cell culture media were determined by a multiplex bead assay. PM_{2.5} organic compounds exclusively induced the production of IL-8 but not IL-1 β , IL-6, TNF- α , IL-17, bFGF, or VEGF (Fig. 2A and B). IL-8 is the primary cytokine involved in the recruitment of neutrophils to the site of infection or damage [34]. IL-8 released from lung epithelial cells is well known to recruit neutrophils to the lung, thus further amplifying inflammation. It has been suggested that mitogen-activated protein (MAP) kinases, especially

extracellular signal-regulated kinase (ERK), play a role in PM_{2.5}-induced pro-inflammatory signaling [35]. Therefore, we investigated the role of the ERK pathway in PM_{2.5}-induced IL-8 production. PM_{2.5} activated ERK (Fig. 2C), and blocking ERK activation by a chemical inhibitor (U0126) decreased the PM_{2.5}-mediated production of IL-8 (Fig. 2D). These data suggest that the ERK pathway is responsible for IL-8 release in response to PM_{2.5} stimulation in lung epithelial cells.

Senescence and macroautophagy activation in lung epithelial cells were reported to be involved in the pathogenesis of inflammatory lung diseases such as COPD [36]. To investigate the effect of PM_{2.5} on senescence and macroautophagy activation in lung epithelial cells, BEAS-2B cells were treated with PM_{2.5} organic extracts for 24 h, and the expression levels of senescence markers (p16, p21, and p27) and a macroautophagy marker (light chain 3B, LC3B) were evaluated by Western blot analysis. Significant increase in the expression levels of p16, p21, p27, and LC3B were shown (Fig. 3).

The Effect of PM_{2.5} Organic Compounds on Inflammation, Aging, and Macroautophagy Activation in Primary HAECs

To confirm the activation of ERK and increased levels of IL-8 in primary cells, primary HAECs from six healthy control patients with no symptoms of COPD or respiratory diseases were collected using a bronchial brush. The epithelial lineage was verified by immunohistochemical staining. Cultured primary HAECs stained intensely and exclusively for epithelial-specific markers (cytokeratin and E-cadherin). No expression was observed for macrophages and endothelial lineage markers (CD11b and CD31) (data not shown). Verified HAECs were exposed to PM_{2.5} organic extracts (1%) for 24 h. PM_{2.5} (1%) did not affect cell viability of primary HAECs as well as BEAS-2B cells (data not shown). PM_{2.5} activated ERK and induced IL-8 production (Fig. 4A-C). The expression levels of active ERK and IL-8 were significantly higher when the cells were exposed to fall and winter samples than when cells were exposed to spring and summer samples (Fig. 4B-C). Moreover, we observed that PM_{2.5} significantly increased the expression levels of senescence markers (p16, p21, and p27) and activated macroautophagy (Fig. 5A-B). No significant seasonal differences were found in the expression levels of senescence and macroautophagy markers (Fig. 5B).

Analysis of PM_{2.5} constituents correlated with inflammation, aging, and macroautophagy activation

As Fig. 6A shows, the average mass concentration of the twelve PM_{2.5} samples was $83.2 \pm 3.85 \mu\text{g}/\text{m}^3$. Divided seasonally, the highest average PM_{2.5} mass concentration was observed in spring ($149 \pm 11.2 \mu\text{g}/\text{m}^3$), followed by winter ($76.4 \pm 3.41 \mu\text{g}/\text{m}^3$), summer ($59.0 \pm 10.1 \mu\text{g}/\text{m}^3$), and fall ($48.4 \pm 5.97 \mu\text{g}/\text{m}^3$). The average concentrations of OC and EC in the twelve samples were $11.5 \pm 0.34 \mu\text{g}/\text{m}^3$ and $1.32 \pm 0.05 \mu\text{g}/\text{m}^3$, respectively. The seasonal averages of OC and EC concentrations showed similar trend as the seasonal average PM_{2.5} mass concentrations; thus, the average concentrations were the

highest in the spring (OC: $15.3 \pm 0.43 \mu\text{g}/\text{m}^3$, EC: $2.06 \pm 0.05 \mu\text{g}/\text{m}^3$), followed by winter (OC: $13.2 \pm 0.41 \mu\text{g}/\text{m}^3$, EC: $1.38 \pm 0.14 \mu\text{g}/\text{m}^3$), fall (OC: $9.53 \pm 1.25 \mu\text{g}/\text{m}^3$, EC: $1.00 \pm 0.06 \mu\text{g}/\text{m}^3$) and summer (OC: $7.92 \pm 1.51 \mu\text{g}/\text{m}^3$, EC: $0.84 \pm 0.20 \mu\text{g}/\text{m}^3$).

The overall average and seasonal average concentrations of the sum of PAHs, n-alkanes, hopanes and alkylcyclohexanes and isoprenoids were calculated and are presented in Fig. 6B (Table S3). While n-alkanes had the highest average concentrations among organic compounds, the highest average concentration was observed in winter ($96.9 \pm 9.05 \text{ ng}/\text{m}^3$), followed by fall ($80.1 \pm 2.15 \text{ ng}/\text{m}^3$), summer ($79.5 \pm 7.18 \text{ ng}/\text{m}^3$) and spring ($74.4 \pm 1.75 \text{ ng}/\text{m}^3$). The average concentrations of alkylcyclohexanes and isoprenoids were the highest in winter ($92.1 \pm 15.4 \text{ ng}/\text{m}^3$) and the lowest in spring ($23.8 \pm 6.97 \text{ ng}/\text{m}^3$). For PAHs, the average concentration in winter ($34.0 \pm 2.00 \text{ ng}/\text{m}^3$) was the highest, followed by fall ($22.3 \pm 2.65 \text{ ng}/\text{m}^3$), spring ($9.88 \pm 0.44 \text{ ng}/\text{m}^3$), and summer ($7.13 \pm 0.37 \text{ ng}/\text{m}^3$). Unlike other organic compounds, hopanes had the highest average concentration in summer ($1.57 \pm 0.13 \text{ ng}/\text{m}^3$), followed by fall ($1.25 \pm 0.09 \text{ ng}/\text{m}^3$), spring ($1.04 \pm 0.03 \text{ ng}/\text{m}^3$), and winter ($0.69 \pm 0.12 \text{ ng}/\text{m}^3$). The seasonal trends of organic compounds did not follow those of $\text{PM}_{2.5}$ and OC.

The association between $\text{PM}_{2.5}$ organic compounds and IL-8 production was measured by using the Pearson correlation coefficient (r). R values greater than 0.70 with a p-value less than 0.05 were considered highly correlated compounds. $\text{PM}_{2.5}$ mass concentrations, OC and EC had negative or no significant correlations with inflammation, aging, and macroautophagy activation, while several organic compounds showed significant correlations.

The results showed that increases in PAHs and several n-alkanes were highly associated with increases in both ERK activation and IL-8 production (Table S4, Table S5). PAHs such as phenanthrene, anthracene, fluoranthene, pyrene, cyclopenta[cd]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[e]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, picene, benzo[ghi]perylene, and coronene showed a high correlation with the expression levels of active ERK and IL-8. The n-alkanes that had high correlations with active ERK and IL-8 were C27, C30, C31, C32, C33, and C34. Among alkylcyclohexanes and isoprenoids, only dibenzofuran was highly correlated with active ERK and IL-8 (Table S6).

PAHs and n-alkanes also showed high correlations with the expression of aging and macroautophagy markers (Table S7, Table S8). The PAHs highly correlated with p27 were pyrene, cyclopenta[cd]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[e]pyrene, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene. The macroautophagy marker, LC3B, was highly correlated with fluoranthene, pyrene, benzo[a]anthracene, benzo[b]fluoranthene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene, and coronene.

Analysis of CMB results correlated with inflammation, aging, and macroautophagy activation

The CMB model using molecular marker was performed to calculate source contributions to OC in PM_{2.5}. Even though only up to 20% of organic compounds can be quantified, these have been applied for source apportionment through CMB [37, 38]. Source contribution estimates and percentages obtained from the CMB model are displayed in Fig. 7 (Table S2). The percent contribution was calculated by dividing the source contribution estimates by the OC concentrations. Four sources were identified as major contributors: vegetative detritus, diesel engines, gasoline motor vehicles, and residential bituminous coal combustion soot.

The source with the highest percent contribution was gasoline motor vehicles (7.8%). The contribution of gasoline motor vehicles in summer (13.5%) was 11.6% higher than that in spring (1.9%). Vegetative detritus, a biogenic source from leaf abrasions [31], had an overall average contribution of 6.0%. The contributions of vegetative detritus in fall (8.6%) and winter (8.4%) were higher than those in spring (3.6%) and summer (2.4%). Residential bituminous coal combustion soot sources had an average contribution to OC of 2.9%. The significantly higher contributions of residential bituminous coal combustion soot in fall (4.1%) and winter (5.9%) than in spring (1.1%) and summer (0.2%) may be due to the higher usage of residential heating during cold seasons. The contribution of diesel engines to the total samples was 2.8%. Although the contributions of diesel engines in spring (4.9%) and summer (3.1%) were higher than those in fall (1.9%) and winter (1.8%), the overall contributions were relatively consistent throughout the seasons. Although the four identified primary sources explained approximately 18% of the total PM_{2.5} source contributions, marked seasonal variations were observed.

Correlations between four major primary contributing sources and ERK activation, IL-8 production, and the expression levels of aging and macroautophagy markers were examined (Fig. 8), and p-ERK, IL-8, p27, and LC3B showed a high correlation with vegetative detritus and residential bituminous coal combustion. Diesel engines and gasoline motor vehicle sources did not show any association. IL-8 release had high correlations with vegetative detritus ($r = 0.84$) and residential bituminous coal combustion soot ($r = 0.85$). Similarly, ERK activation had a high correlation with vegetative detritus ($r = 0.82$) and residential bituminous coal combustion soot ($r = 0.91$). The expression levels of p27 and LC3B had moderately high correlations with vegetative detritus ($r = 0.58$, $r = 0.72$) and residential bituminous coal combustion soot ($r = 0.54$, $r = 0.63$).

Discussion

The constituents and sources of ambient PM_{2.5} organic aerosols inducing adverse health effects are not yet well understood. In the present study, we show that organic extracts of PM_{2.5} collected in Seoul during HCEs induce neutrophilic inflammation, cellular aging, and macroautophagy activation in primary lung epithelial cells. In particular, several organic constituents (i.e., PAHs and n-alkanes), as well as specific sources, including the biomass related source (i.e., vegetative detritus) and residential bituminous coal combustion soot, were found to be highly correlated with increases in inflammation and cell senescence, and macroautophagy activation.

PAHs and n-alkanes were most relevant components to mediate ERK activation-dependent IL-8 production. The PAHs compounds including benzo[a]pyrene, cyclopenta[cd]pyrene, dibenzo[a,h]anthracene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-cd]pyrene were significantly involved in causing IL-8 production. Similar to our results, previous studies have demonstrated that the exposure to PAHs in PM_{2.5}, major components of carbonaceous species significantly induces pro-inflammatory cytokine production [39, 40]. The IL-8 release and ROS generation are known to be mainly related to OC, especially PAHs which are primary organic compounds from heating sources.

The average concentrations of PAHs were higher during cold seasons than that of warm seasons in Seoul and found that PM_{2.5} samples from cold seasons were highly correlated with inflammation. The study which was conducted in Nanjing, China, shows similar seasonal trend. Cold seasons have higher levels of PAHs which mediate lung epithelial cell death and inflammation [40]. While many studies have reported that PM_{2.5} induces the release of several inflammatory cytokines such as IL-1 β , IL-6, TNF- α , organic extracts of PM_{2.5} collected in Seoul specifically induced IL-8 production, which might be due to the difference in chemical composition of PM_{2.5} from different locations and difference in cell-type.

PAHs may be emitted both from natural and anthropogenic sources. However, anthropogenically produced PAHs are predominant [41]. Due to the relationship between temperature and vapor pressure, airborne PAHs are more likely to bound to particulate matter in winter; on the contrary, larger fraction are in gas phase in summer [42, 43]. As PAHs are also produced in the process of incomplete combustion of organic materials [44], high concentrations of PAHs during fall and winter may have affected high contributions of residential bituminous coal combustion soot.

In this study, n-alkanes with high molecular weight such as C₂₇ to C₃₄ were significantly correlated with inflammation. N-alkanes are usually used as marker for sources such as coal combustion, motor vehicle exhaust, and vegetative detritus and are well-known to be related to IL-8 release and ROS generation [45, 40]. In this study, vegetative detritus which is a biogenic source was identified by n-alkanes. Though the average carbon preference index (CPI) [46] of the analyzed samples was 0.8 which indicates the anthropogenic influence of the source.

Many epidemiologic studies have discovered the association between PM_{2.5} sources and mortality [47–49]. In Korea, biomass burning, gasoline, and diesel emission sources were found to be substantially associated with cardiovascular and respiratory mortality [49]. Toxicological studies have found the cytotoxicity and adverse health effects of sources such as combustion and vehicle emission [50–55]. In this study, vegetative detritus and residential bituminous coal combustion sources were found to be highly correlated with inflammation, aging and macroautophagy activation. No significant correlation between vehicle emission sources and inflammation, aging and macroautophagy markers may be resulted from usage of different PM_{2.5} collection method such as particles generated in smog chamber or SRM, cell types and receptor model such as positive matrix factorization from EPA [52, 55, 56].

Conclusions

Organic extracts of PM_{2.5} collected in Seoul, South Korea, during HCEs induced inflammation, cellular aging, and macroautophagy activation in primary lung epithelial cells. The average mass concentrations, OC and EC had no significant correlations with PM_{2.5} effects. Both PAHs and n-alkanes were the most relevant components of PM_{2.5} for inflammation, aging and macroautophagy activation. The findings support the idea that the chemical constituents of PM_{2.5} are more important than the level of PM_{2.5} mass concentrations and even low concentration of PM_{2.5} may have adverse impacts on the public health [6, 57, 58].

To our knowledge, this is the first study to assess the effects of organic compounds of seasonal ambient PM_{2.5} collected in Seoul on inflammation, cellular aging, and macroautophagy in primary lung epithelial cells. Our results may be used as a reference for the implementation of PM_{2.5} reduction policy based on chemical constituents and sources which cause adverse health effects. The limitation of this study was that we did not consider other chemical constituents which may have affected lung epithelial cells. Therefore, further studies which analyze other chemical constituents of PM_{2.5} with larger number of samples for detailed source apportionment are needed.

Methods

Sampling Site and Collection Procedure

PM_{2.5} samples were collected on the rooftop of the Graduate School of Public Health building (37.58°N, 127.00°E) at Seoul National University in Seoul, Korea. Samples were collected for twenty-four-hour by using a high-volume air sampler and a low-volume air sampler equipped with filter pack (URG-2000-30FG, URG, USA) and cyclone (URG-2000-30EH, URG, USA). A high-volume air sampler loaded with quartz microfiber filters (Whatman™, UK) collected PM_{2.5} with a flow rate of 40 cfm, and the collected filters were used for organic extraction. A low-volume air sampler was loaded with Teflon filters (PTFE membrane, Pall Corporation, USA) to measure mass concentrations and with quartz filters (Quartz microfiber filter, Pall Corporation, USA) to quantify the concentrations of OC and EC. The PM_{2.5} mass concentration was measured with a semimicro balance (accuracy of 0.01 mg) (CP225D, Sartorius), and twelve samples that were collected during HCEs between May 2016 and January 2017 were selected. Three HCE samples from each season were selected by the Korean national air quality standards of PM_{2.5} which is 24 h average concentration of 35 µg/m³. Thus, a total of twelve HCE samples were used for this study.

Organic Extraction of the Collected PM_{2.5} Samples

Quartz filters were baked in a furnace at 450°C for 24 h, and the collected filters were stored at -20°C before use. Samples were punched by a stainless cutter, and two of the punched filters (4 cm x 4 cm) were used for extraction. Solvent mixture of dichloromethane:methanol (3:1, v/v) was used for sample

extractions with ultrasonic bath. The extracted samples were concentrated to 10 ml by a Turbovap II (Zymark Co., USA) with N₂ gas and 0.2 μm Acrodisc Syringe Filters (Pall Corporation, USA) were used for filtration. The filtered samples were then concentrated to 1 ml with a Turbovap II and Reacti-Therm (Thermo Fisher Scientific, USA) under a gentle stream of N₂ gas and were stored at -20°C. The concentrated samples were used for organic compound analysis and *in vitro* experiments.

Cells

Normal human bronchial epithelial cells (BEAS-2B from ATCC, Manassas, VA, USA) were maintained in defined keratinocyte-SFM (Gibco by Thermo Fisher Scientific, Waltham, MA, USA) at 37°C under 5% CO₂. Normal primary HAECs were obtained after review and approval by the Seoul National University Hospital Institutional Review Board (SNUH IRB number: H-1602-108-742). Primary HAECs were isolated and grown as previously described and is detailed in supporting information Materials and Methods [28].

Cell Viability

MTT and LDH release assays were performed as previously described [29] and is detailed in supporting information Materials and Methods.

Protein Extraction and Western Blot Analysis

Protein extraction and Western blot analysis were performed as previously described (Lee et al., 2017) and is detailed in supporting information Materials and Methods.

Multiplex Bead Assay

The levels of cytokines in cell culture media were determined using a Bio-Plex Pro™ Cytokine Assay Kit (Bio-Rad, Hercules, CA) according to the manufacturer's instructions.

GC/MS Analysis and OC/EC Analysis

Gas chromatography-mass spectrometry (7080B/5977B, Agilent Technologies, Inc., USA) was employed to quantify 52 organic compounds in each extract. The analyzed species included 23 species of PAHs, 17 species of n-alkanes, 7 species of hopanes and 5 species of alkylcyclohexanes and isoprenoids.

The samples collected in the low-volume sampler were punched (1.5 cm x 1.0 cm) to analyze major components of carbon species which are OC and EC. OC and EC were analyzed with a carbon aerosol analyzer (Sunset Laboratory Inc., USA). Thermal/optical transmittance (TOT) method was used for the data quantification.

Source apportionment of organic compounds in PM_{2.5} using CMB model

Source apportionment of the OC fraction of PM_{2.5} was performed by using a chemical mass balance (CMB v8.2) provided by the U.S. Environmental Protection Agency (EPA). The CMB air quality model is one of the receptor models that has been widely used to identify sources and quantify source

contributions [30]. The concentrations of organic compounds, OC, and EC in twelve samples were used as ambient data in addition to the speciated source profile data (Table S1). The optimal set of source profiles contained four sources which are vegetative detritus [31], residential bituminous coal combustion soot [32], diesel engines [33], and gasoline motor vehicles [33].

Abbreviations

PM_{2.5}: Fine particulate matter; COPD: Chronic Obstructive Pulmonary Disease; SRM: Standard Reference Materials; HAECs: Primary Human Airway Epithelial Cells; OC: Organic Carbon; EC: Elemental Carbon; PAHs: Polycyclic Aromatic Hydrocarbons; HCEs: High Concentration Events; GC/MS: Gas Chromatography-Mass Spectrometry; CMB: Chemical Mass Balance; EPA: Environmental Protection Agency; LDH: Lactate Dehydrogenase; V.C: Vehicle Control; IL-1b: Interleukin-1b; IL-8: Interleukin-8; TNF-a: Tumor Necrosis Factor-a; bFGF: Basic Fibroblast Growth Factor; VEGF: Vascular Endothelial Growth Factor; MAP: Mitogen-activated protein; ERK: Extracellular signal-Regulated Kinase;

Declarations

Acknowledgments

This study was supported by BK21 PLUS (no. 22A20130012682), the National Strategic Project-Fine particle (NRF-2017M3D8A1092019), and NRF-2019R1A2C2007484 of the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT and grant **03-2019-0430** from Seoul National University Hospital.

Authors' contributions

JH and CGY supervised study design; JP, KHL, HK, and JW performed experiments; JP and KHL wrote the first draft of the manuscript; JP, KHL, JH, CHL, SMY, CGY contributed to interpretation of the data; All authors read and approved the final manuscript.

Availability of data and materials

Source profiles which were used for CMB models and the CMB results are provided. In addition, average concentrations of analyzed chemical species and detailed correlation tables are provided in the supporting information. Further datasets are available from the authors on reasonable request.

Ethics approval and consent to participate

This study was approval by the Institutional Review Board of the Seoul National University Hospital (SNUH IRB number: H-1602-108-742).

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

1. BK21 PLUS (no. 22A20130012682) from the National Research Foundation (NRF) funded by the Ministry of Science and ICT (MSICT) of South Korea.
2. The National Strategic Project-Fine particle (NRF-2017M3D8A1092019) from the NRF funded by the MSICT
3. NRF-2019R1A2C2007484 from the NRF funded by the MSICT
4. Grant **03-2019-0430** from the Seoul National University Hospital.

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Figures

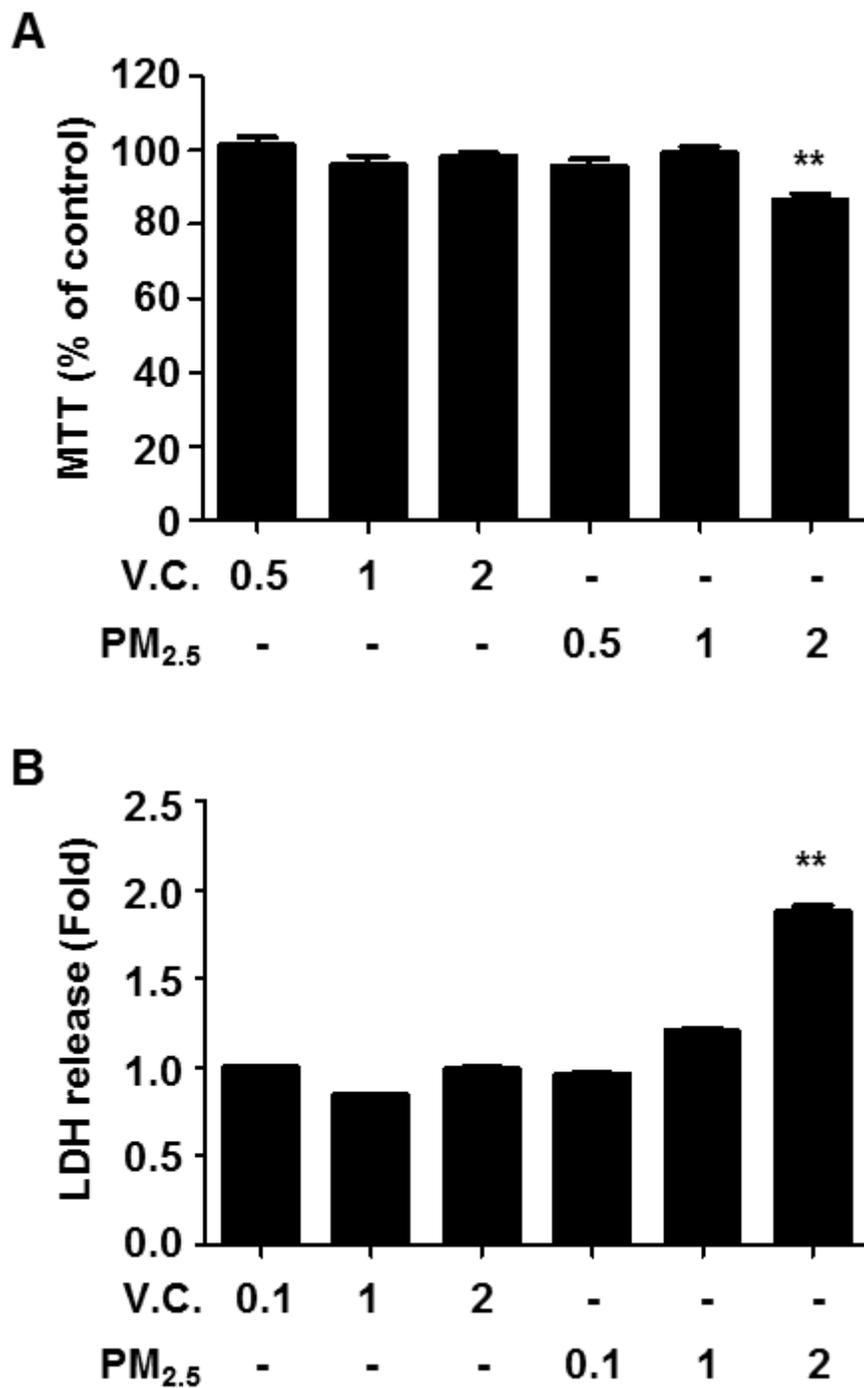


Figure 1

The effects of PM_{2.5} on cell viability in lung epithelial cells. BEAS-2B cells were exposed to V.C. or PM_{2.5} organic compounds (0.1, 0.5, 1, 2%) for 24 h. MTT (A) and LDH release assays (B) were performed. Data are presented as the mean \pm SD. ** $p < 0.05$.

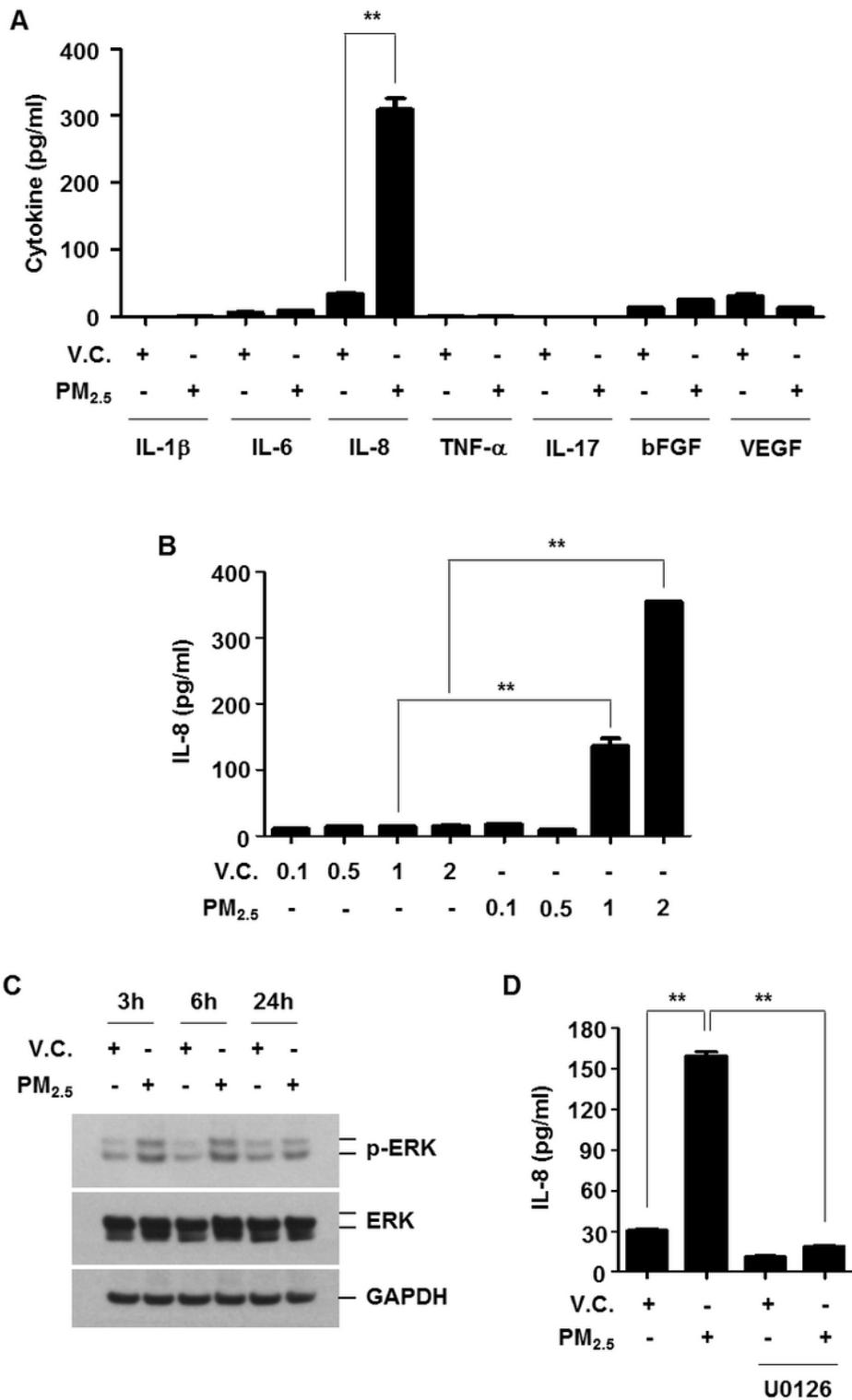


Figure 2

The effects of PM_{2.5} on cytokine production in BEAS-2B cells. (A) BEAS-2B cells were treated with V.C. or PM_{2.5} organic compounds (1%) for 24 h. (B) Cells were incubated with various concentrations (0.1, 0.5, 1, 2%) of V.C. or PM_{2.5} for 24 h. The levels of cytokines (IL-1β, IL-6, IL-8, TNF-α, IL-17, bFGF, and VEGF) in culture media were measured by a multiplex bead assay. Data are presented as the mean ± SD. **p < 0.05. (C) BEAS-2B cells were treated with V.C. or PM_{2.5} (1%) for the indicated times. Total cellular extracts were

subjected to Western blot analysis for p-ERK, ERK, and GAPDH. (D) Cells were pretreated with U0126 (4 μ M) for 2 h and then stimulated with V.C. or PM2.5 organic compounds (1%) for 24 h in the presence or absence of U0126. The level of IL-8 in media was measured by a multiplex bead assay. Data are presented as the mean \pm SD. ** $p < 0.05$.

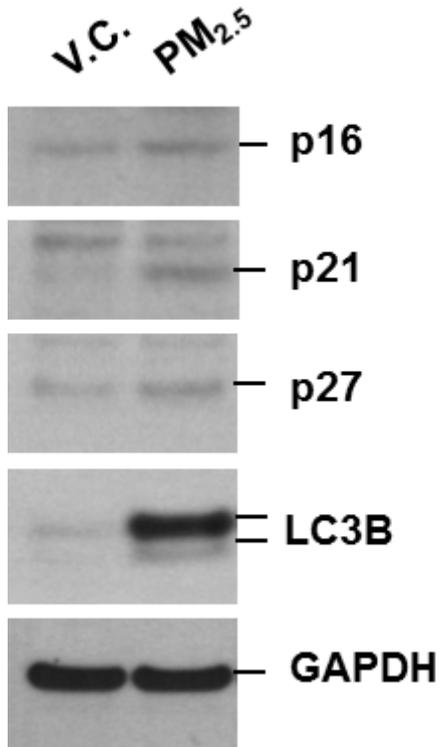


Figure 3

The effects of PM2.5 on the expression of aging and macroautophagy markers in BEAS-2B cells. Cells were exposed to V.C. or PM2.5 organic compounds (1%) for 24 h. Total cell lysates were extracted and then subjected to Western blot analysis for p16, p21, p27, LC3B, and GAPDH.

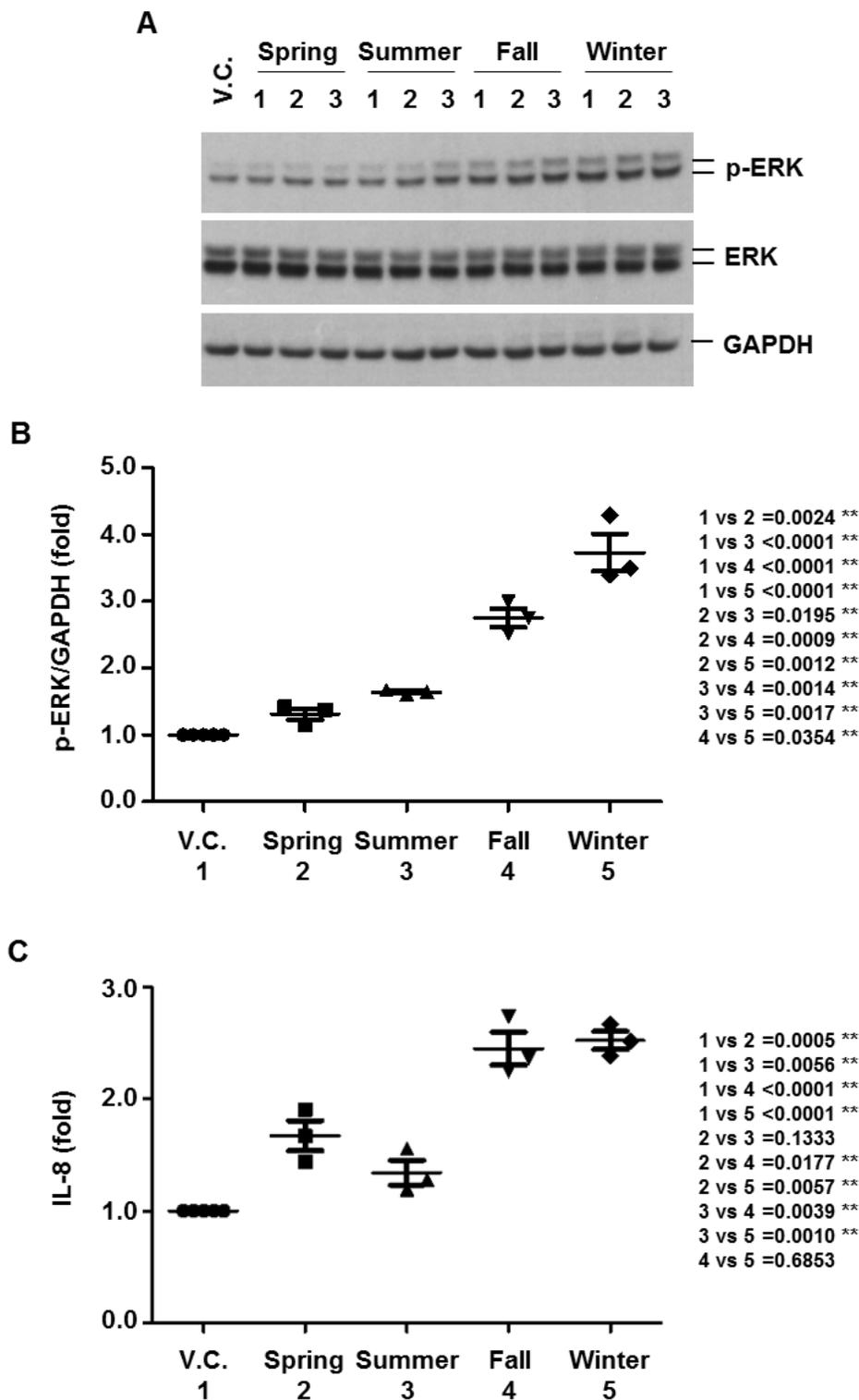


Figure 4

The effects of PM_{2.5} on ERK activation and IL-8 production in primary HAECs. Primary HAECs (n=6) were exposed to V.C. or PM_{2.5} organic compounds (1%) for 24 h. Total cell lysates were extracted and then subjected to Western blot analysis for p-ERK, ERK, and GAPDH (A). Densitometry analysis using Scion image software for p-ERK. Blots were normalized to GAPDH expression (B). The IL-8 concentration in the

cell culture media was measured using a multiplex bead assay (n=5) (C). Data are presented as the mean \pm SE. **p<0.05.

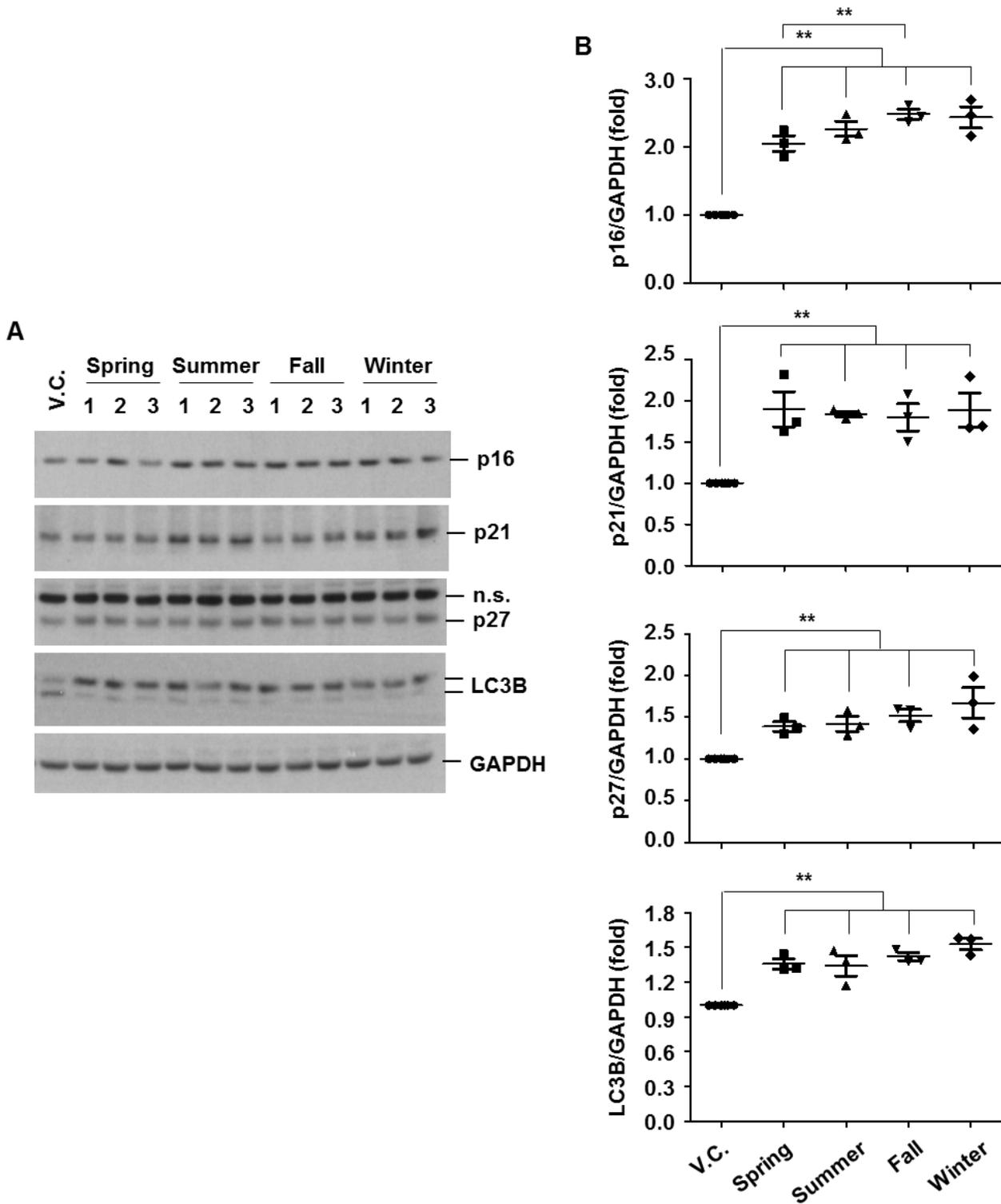


Figure 5

The effects of PM2.5 on the expression of aging and autophagy markers in primary HAECs. Primary HAECs (n=6) were exposed to V.C. or PM2.5 organic compounds (1%) for 24 h. Total cell lysates were

extracted and then subjected to Western blot analysis for p16, p21, p27, LC3B, and GAPDH (A). Densitometry analysis using Scion image software (B). Data are presented as the mean \pm SE. $**p < 0.05$.

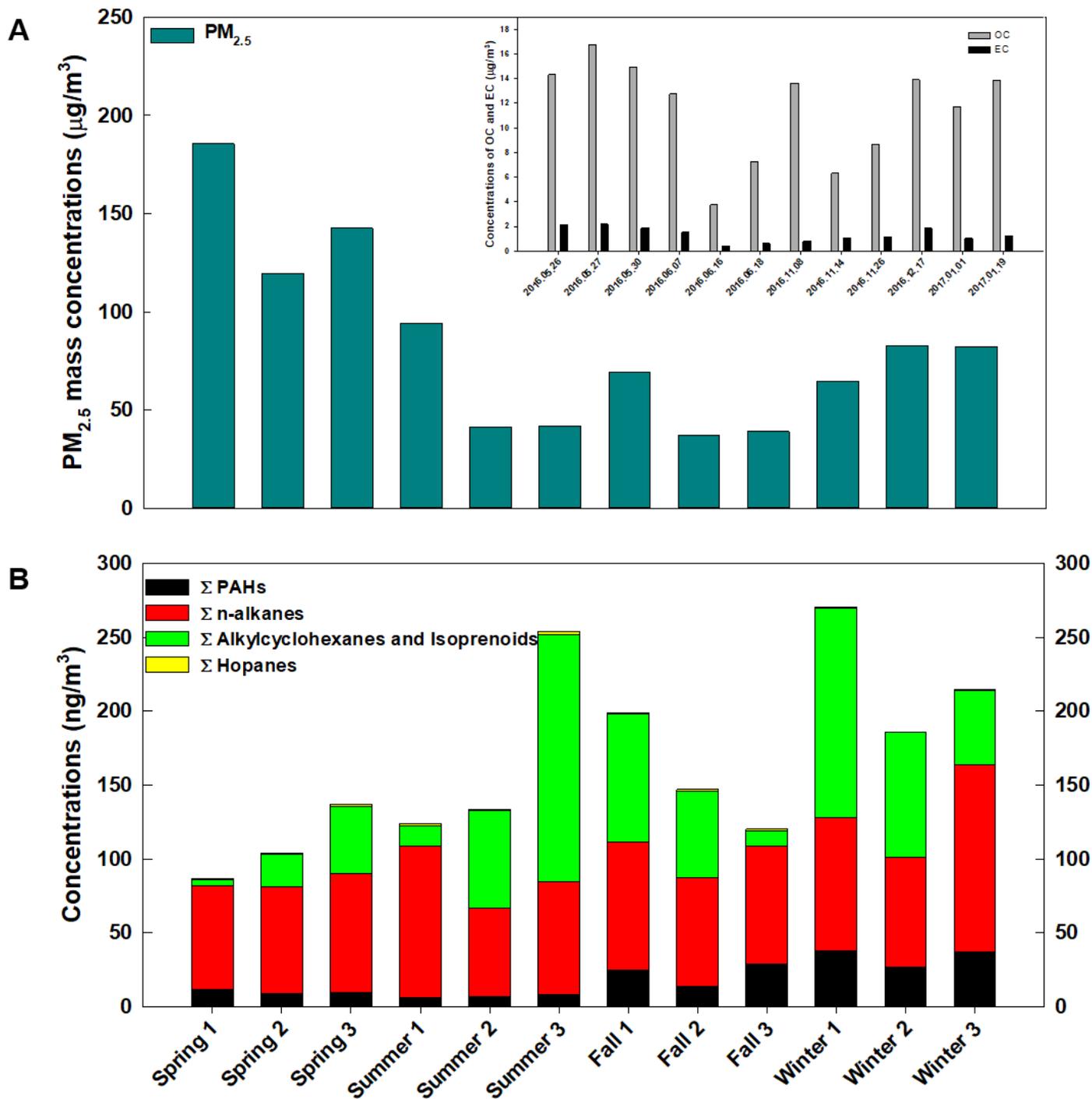


Figure 6

PM_{2.5} mass concentrations and concentrations of organic compounds. (A) PM_{2.5} mass concentrations and OC and EC concentrations in twelve samples. (B) Concentrations of organic compounds, including PAHs, n-alkanes, alkylcyclohexanes and isoprenoids, and hopanes.

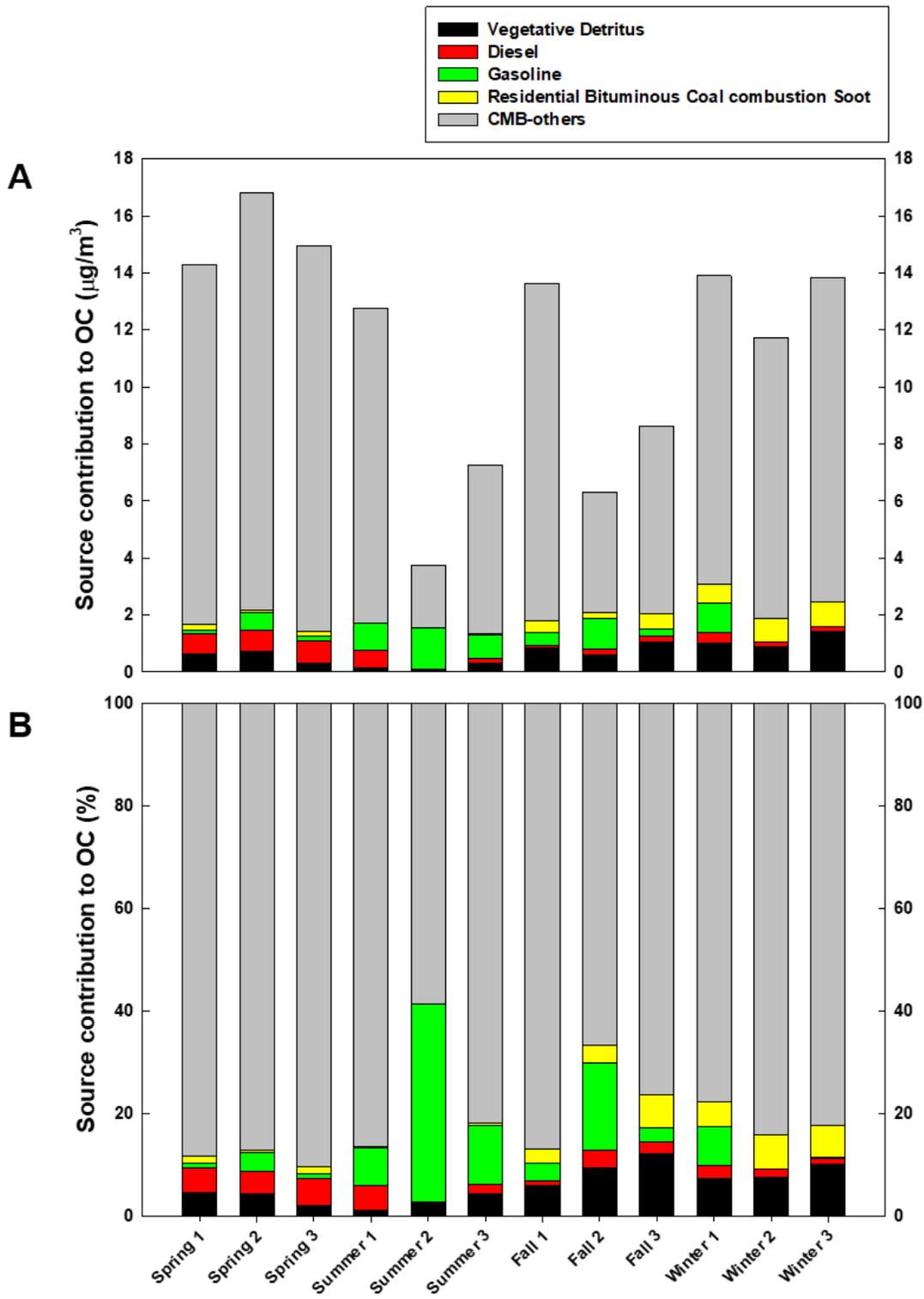


Figure 7

Results of the molecular marker of CMB source apportionments for the twelve samples. (A) Source contribution estimates of the four sources. (B) Percent contributions to OC of the four sources.

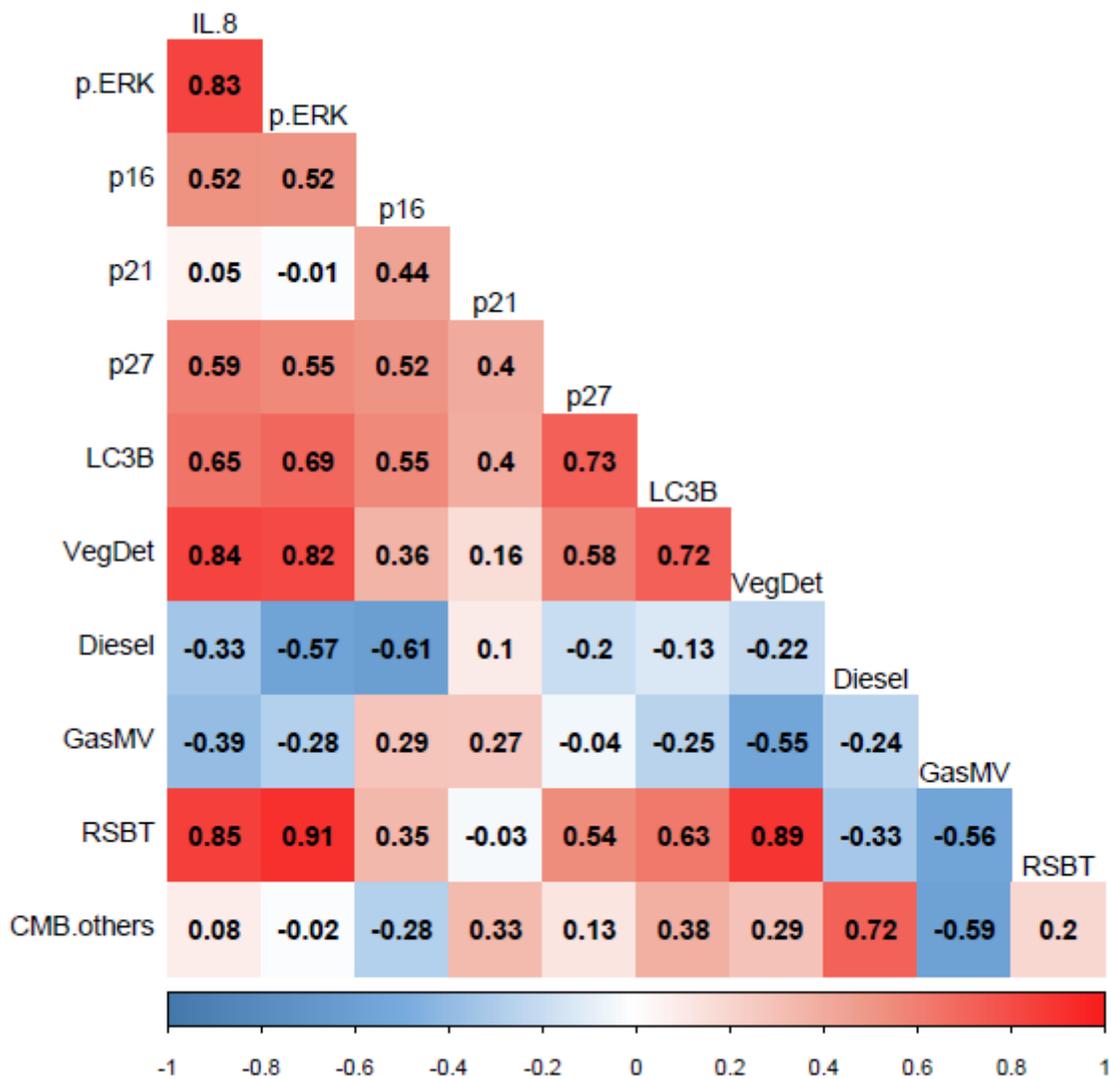


Figure 8

Correlation matrix between four sources and ERK activation, IL-8 production, and the expression levels of aging/macroautophagy markers (VegDet: vegetative detritus, GasMV: gasoline motor vehicles, RSBT: residential bituminous coal combustion).

Supplementary Files

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