Fine particulate matter PM2.5 generated by building demolition increases the malignancy of breast cancer MDA-MB-231 cells

Chun-Wen Cheng
Chung Shan Medical University
Gwo-Tarng Sheu
Chung Shan Medical University
Jing-Shiuan Chou
Chung Shan Medical University
Pei-Han Wang
Chung Shan Medical University
Yu-Chun Cheng
Fu Jen Catholic University
Chane-Yu Lai (cylai@csmu.edu.tw)
Chung Shan Medical University https://orcid.org/0000-0003-4365-8673

Research

Keywords: Demolition, PM2.5, heavy metals, breast cancer, malignancy

Posted Date: February 10th, 2020

DOI: https://doi.org/10.21203/rs.2.23043/v1

Version of Record: A version of this preprint was published at Chemosphere on February 1st, 2021. See the published version at https://doi.org/10.1016/j.chemosphere.2020.129028.

Abstract

Background

$PM_{2.5}$ is associated with increased risk of mortality for a variety of cancers and all subjects, including breast cancer in females, and lung cancer in males. This study investigates the effects of water-extracted $PM_{2.5}$ on a triple-negative breast cancer (TNBC) cell line, MDA-MB-231, by sampling suspended particulates around a building demolition site.

Methods

$PM_{2.5}$ particles were obtained using a high-flow TISCH sampler. Being water-soluble, they were extracted from sampled filters using an ultrasonic oscillator and then freeze-dried. The heavy metal components of soluble $PM_{2.5}$ particle was analyzed by ICP-MS. Cell viability was evaluated by MTT assay for cells that were exposed to $PM_{2.5}$. Wound healing and transwell cell migration and invasion assays were used to measure cell motility and the invasiveness of cancer cells that had been exposed to $PM_{2.5}$ into a chemo-attractant substance. Possible mechanisms of cancer malignancy were analyzed by Western blot analysis.

Results

The results revealed that nearby $PM_{2.5}$ concentrations increased significantly during the deconstruction of buildings, and the Cd, Cu, Pb, Zn and Cr contents of soluble $PM_{2.5}$ also significantly increased. Following exposure to $PM_{2.5}$, the survival rate of breast cancer cells was significantly higher than that of the control group. Soluble $PM_{2.5}$-treated cells also had a higher migration capacity, as determined by wound healing and transwell migration assays. The signaling pathway of FAK/PI3K/AKT proteins was more activated in $PM_{2.5}$-treated cells than the control group. The data show that increased levels of Aurora B and Bcl-2 were associated with cell proliferation. Elevated levels of cathepsins D, β-catenin, N-cadherin, vimentin and MMP-9 were associated with breast cancer cell metastasis

Conclusion

Soluble $PM_{2.5}$ that is generated in building demolition may have a role in the promotion/progression of surviving in TNBC cells, increasing the malignancy of breast cancer. The prevention of environmental $PM_{2.5}$ from deconstruction is strongly recommended.

Introduction

The demolition of a building can produce large amounts of particulate matter (PM), usually seriously degrading ambient air quality in implosion areas (Beck et al. 2003). An extremely high concentration of PM that is generated during the demolition of buildings may be inhaled by field workers and people who live nearby (Farhad Azarmi 2016). The assessment of possible pathogenicity under such environmental

exposure to specific substances with aerodynamic diameters of less than 2.5 μm ($PM_{2.5}$) remains an important issue. Studies of the impact of $PM_{2.5}$ at real-world demolition sites, especially on breast cancer, are still very limited.

The collapse of the New York World Trade Center (WTC) Twin Towers on September 11, 2001, led to an estimated release of 10 million tons of material, exposing more than 300,000 rescue workers and New York City (NYC) residents and local workers to WTC particulate matter (Aldrich et al., 2010; Claudio, 2001; Landrigan, 2001). After the collapse of the WTC building, many neighboring places were stuck in the initial dust/smoke cloud (4 to 8 h) and Lower Manhattan was briefly exposed to $PM_{2.5}$ levels in air in the mg/m^3 (thousands of $\mu g/m^3$) range (Lippmann et al., 2015). The toxicological and physical properties of WTC-PM have been described elsewhere (Lioy et al., 2002; McGee et al., 2003). WTC-PM comprised mostly powered concrete, plastics and other hydrocarbons. WTC-PM was found to be highly alkaline, with pH 9–11 (Lioy et al., 2006; McGee et al., 2003). Exposure to fine ($PM_{2.5}$) and coarse (PM_{53}, > 53 microns) PM has been associated with the development of lung injuries and high sensitive immune responses (Rom et al., 2002; Weiden et al. 2015).

Evidence that causally links air pollution to breast cancer risk remains controversial. A recent study addressed increased breast cancer risk that is associated with environmental air pollutants, including nitrogen dioxide (NO_2), polycyclic aromatic hydrocarbons (PAHs), carbon monoxide, sulfur dioxide, volatile organic compounds and $PM_{2.5}$ (Crouse et al., 2010; Hystad et al., 2015; Parikh and Wei, 2016; Wei et al., 2012). A positive relationship between $PM_{2.5}$ and the risk of death from breast cancer has been mentioned (Tagliabue et al., 2016). Mammographic breast density is a well-established strong risk indicator for breast cancer and women are at higher risk of developing breast cancer because they are exposed to a higher mean of $PM_{2.5}$ concentration (Yaghjyan et al., 2017). However, some works have found no significant correlation between breast cancer and $PM_{2.5}$ (Andersen et al., 2017; Hart et al., 2016; Reding et al., 2015). Interestingly, women who are estrogen receptor-positive (ER+) may develop breast cancer upon prolonged exposure to a xenoestrogenic compound, leading to the tumorigenesis of mammary epithelial cells (Huo et al., 2013). Positive correlations exist between exposure to environmental estrogen-expelling agents and hormone receptor-positive breast cancer risk, and between levels of cadmium compounds to which a person is exposed and risk of hormone receptor-negative tumors (Liu et al., 2015). Approximately 15% of all invasive breast cancers are triple-negative breast cancers (TNBC) that lack estrogen receptor (ER), progesterone receptor (PR), and HER2 (human epidermal growth factor receptor 2) expression, and exhibit a distinct pattern of recurrence with unfavorable outcomes (Dent et al., 2007). To identify the underlying molecular mechanism by which $PM_{2.5}$ acts on TNBC tumor cell malignancies, concentrations of $PM_{2.5}$ were measured during building demolition following the collection of smaller particles than $PM_{2.5}$ as exposure sources. In this investigation, invasive MDA-MB-231 cells were treated with water-soluble extracted $PM_{2.5}$. $PM_{2.5}$-induced cancer characteristics were studied by cell viability and migration assays. The results demonstrated the carcinogenic potential of $PM_{2.5}$ particles in building demolition environments to exacerbate the progression of tumor cells. These findings can improve our understanding of the need for optimal air quality management during building demolition to prevent cancer cell malignancy.

Materials And Methods

Collection of the $PM_{2.5}$ that is generated by building demolition

Airborne particles were obtained using a TISCH high-flow sampler (TE-6070) and a high-volume cascade impactor (TE-231, Tisch Environmental, Cleves, Ohio. USA). Suspended particulates enter the cascade impactor through the first set of parallel slots in the first stage. Particulates with high inertial force that are too large to pass to the next stage are impacted on the quartz fiber filter (Pall, USA) and the smaller particles remain in the air stream and travel to the next stage. The slots become successively smaller and most of the particulates eventually become impacted on one of the collection stages in the filter. Beyond the last stage, the smallest particles will be collected on the backup filter, which will be weighed to determine PM content. The filter was dried at 50 ^{o}C for 24 h and then incubated in a humidifier for 24 h. $PM_{10-2.5}$ (< 10 − 2.5 μm) and $PM_{2.5}$ (< 2.5 μm) were collected using 5.625”x 5.375” and 8”x10” filters, respectively. To evaluate concentrations of PM, collection was carried out in an open area next to a demolition site at a constant flow rate of 1.3 m^3/min for 24 h. PM was collected during demolition from December 23, 2016 to January 13, 2017. For comparison with the demolition-generated PM, airborne PM was collected at the same collection site 14 months later (2018-03-20 to 2018-03-30).

Preparation of water-soluble $PM_{2.5}$ extracts

The sampled $PM_{2.5}$ filter was weighed; cut into small pieces, and then transferred into a 50 ml tube that contained enough double-distilled water for a 30 min sonication. The $PM_{2.5}$ suspension was centrifuged at 13,000 x g for 10 min at 4 ^{o}C and filtered using a 0.22 μm syringe filter. To obtain concentrated PM, the filtered suspension was dried in a vacuum dryer (VIRTIS) at 50 ^{o}C until completely dry. A total of 101.556 mg of $PM_{2.5}$ was estimated for initial sonication and 27.368 mg of $PM_{2.5}$ was recovered and dissolved in 100 mL double-distilled water to perform an in vitro assay. The control for the assay was prepared from a blank quartz fiber filter that was went through all the steps of extraction except for exposure to PM.

Cell culture

The triple-negative breast cancer cell line MDA-MB-231 was purchased from the Bioresources Collection and Research Center (Hsinchu, Taiwan). Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Carlsbad, CA) with 10% fetal bovine serum (FBS, Gibco), 100 IU/mL penicillin G, and 100 g/mL streptomycin in a humidified 37 ^{o}C environment with 5% CO_2.

MTT assay

Human MDA-MB-231 cells were seeded in 24-well plates (3×10^4 cells/well) for 20 h and then treated with 0.5 mL of $PM_{2.5}$ (200, 400 and 600 ∝g/mL) in fresh medium. The MTT assay (Sigma Aldrich, USA) was used to determine relative cell growth after 48 h. Following removal of the culture medium, 200 ∝L of

0.5 mg/mL MTT in fresh medium was added and then incubation was performed for 3 h at 37 ^{o}C. Any remaining crystals were dissolved in 500 µL isopropanol. The absorbance (A) was measured in a microplate reader (Biotek, Winooski, VT, USA) at a wavelength of 570 nm.

Colony formation assay

MDA-MB-231 cells were seeded in a 60 mm dish (5×10^5 cells/well) and treated with 600 µg/mL of $PM_{2.5}$ or a control for 24 h at 37 ^{o}C of incubation. The cells were trypsinized and collected. A total of 1000 cells were seeded in a 60 mm dish and eventually cultured for seven days. The colonies thus formed were fixed with ice-cold methanol for 15 min and then stained with 0.4% crystal violet for 15 min, before being washed in phosphate buffered saline (PBS) solution. Survival fractions were calculated by normalization to the appropriate control group.

Wound healing assay

MDA-MB-231 cells were treated with 600 µg/mL of $PM_{2.5}$ or a control for 24 h and collected as described above. Cells were counted and adjusted to a concentration of 3×10^5 cells/mL. The culture-insert (ibidi 80206, Martinsried, Germany) was loaded into a 60 mm dish; then 100 µL of the prepared cells was added to both chambers to yield a total of 3×10^4 cells. The dish was maintained at 37 °C, 5% CO_2 for 24 h and then the culture-insert was carefully removed. The chambers were then rinsed using PBS solution. The first photograph was taken as 0 h and 1% FBS in fresh medium (4 mL) was added to induce would healing. After 16 h of incubation, the medium was removed and rinsed in PBS, and then the second photograph was taken. Microscopic images of a representative field of the cell-free space were obtained at 0 and 16 h, and the numbers of cells were calculated in ImageJ software (Java 1.8.0_112, imagej.nih.gov).

Transwell migration assay

MDA-MB-231 cells migration was characterized using a transwell migration assay with 24-well hanging-inserts that were fitted with an 8 µm-pore-size membrane (Millicell Cell Culture Inserts Category No. MCEP24H48). A total of 5×10^4 serum free cells (200 µL) were seeded in triplicate in culture medium onto the apical surface of each hanging-insert and placed into wells that contained 10% FBS in culture medium (500 µL). The plate was incubated for 16 h and the lower surface of the insert was fixed with 100% methanol and stained with 0.4% crystal violet for 15 min. Non-migrating cells were removed from the upper surface using a cotton stick and the migrated cells were counted.

Western blot analysis

Proteins (20 ∝g) that had been separated by SDS-PAGE were transferred onto an Immobilon-P membrane that was then subjected to western blotting using a suitable primary antibody against human FAK (Gene Tex), p-FAK(Y925), p38, p-p38, ERK1/2, pERK1/2, JNK, pJNK and vimentin (Cell Signaling Technology, Danvers, MA), PI3K p110, PI3K p85α, Akt1/2/3, N-cadherin, BCL2 and β-catenin (Santa Cruz Biotechnology, Dallas, TX). Anti-Aurora B, -cathepsin D, -MMP9 were purchased from Abcam. The antibody against GAPDH (Cell Signaling Technology) was the endogenous control to which the expression of the proteins of interest was normalized. An appropriate horseradish peroxidase–conjugated secondary antibody was

used to detect each immunoreactive protein and was visualized with an enhanced chemiluminescence assay (Western Blotting Luminol Reagent; Santa Cruz Biotechnology). Band intensity was quantified by densitometry (Digital Protein DNA Imagineware, Huntington Station, NY).

Analysis of metal components of water-extracted $PM_{2.5}$

The vacuum-dried pellet of $PM_{2.5}$ was resuspended with double-distilled water and analyzed by ICP-MS (PerkinElmer/NexION 300X).

Statistical analysis

Data are presented as mean ± standard deviation (S.D.). All cell-based experiments were performed in triplicate for each group of assay test. The statistical significance of difference in results among test groups was determined using Student's t-test. The p-values are represented as *, < 0.05; **, < 0.01; and ***, < 0.001.

Results

Figure 1 Schematic diagram of demolition location and regional monitoring stations around sampling site. A four-floor building was demolished from 2016-12-29 to 2017-01-13. The PM concentration at a demolition site depends strongly on numerous factors, including local and regional PM sources, meteorological conditions and geography. To confirm the concentration of PM in air that was generated by various sources, measurements were made at the building demolition site and the local monitoring stations (station A and station B) before and after the deconstruction process, as indicated in Table 1. The factors were wind speed, temperature and relative humidity (RH). Based on meteorological data from 2016-12-29, 2018-03-20 and 2018-03-21, high-velocity wind might have reduced $PM_{2.5}$ levels in the local air, this finding is consistent with the low $PM_{2.5}$ concentration that was derived from a correlation analysis that was based on 22 months of observation at 68 major cities in seven geographical regions in China (Yang et al., 2017). Wind speed has similarly been negatively correlated with $PM_{2.5}$ level. During the demolition process, the concentration of $PM_{2.5}$ at the collection point significantly exceeded that in the surrounding area (Fig. 2A). Fourteen months later, $PM_{2.5}$ concentrations in the air at the collection point were normally lower than in the local surrounding area (Fig. 2B).

To assess the effect of $PM_{2.5}$ on the progression of breast cancer cells, MDA-MB-231 breast cancer cells were treated with water-extracted $PM_{2.5}$ for 48 h and analyzed using an MTT survival assay (Fig. 3A). The higher $PM_{2.5}$ concentrations resulted in a tumor cell viability of double that of the control group. The proliferation capacity of breast cancer cells was examined using a colony formation assay. After 24 h of exposure to 600 μg/mL $PM_{2.5}$, the number of colonies of the $PM_{2.5}$-treated breast cancer cells significantly exceeded that in the control group after seven days of incubation (Fig. 3B). These data provide strong evidence that water-extracted $PM_{2.5}$ enhances the survival of MDA-MB-231 breast cancer cells.

The metastatic potential of breast cancer is associated with poor prognosis in patients with a short survival time and high recurrence rate (Stovgaard et al., 2018). Therefore, the effect of $PM_{2.5}$ on the motility of MDA-MB-231 cells was determined using a wound healing assay (Figs. 4A and B). Similarly, treatment with 600 μg/mL $PM_{2.5}$ significantly enhanced the motility of MDA-MB-231 cells following incubation for 16 h. The results of transwell migration assays showed that $PM_{2.5}$ promotes the vertical migration capacity of the cells, verifying that water-extracted $PM_{2.5}$ increases the invasiveness of breast cancer cells (Fig. 4C).

During tumor metastasis, cancer cells undergo an epithelial-mesenchymal transition (EMT) in which epithelial tumor parts are converted into aggressive and metastatic ones, which are known as a mesenchymal tumor phenotype with the loss of cell-cell adhesive function (Liao and Yang, 2017; Thiery et al., 2009). Focal adhesion kinase (FAK) is a cytoplasmic non-receptor tyrosine kinase whose phosphorylation at the Tyr397 residue by integrins results in the activation of the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway that promotes tumor cell adhesion and invasion during EMT. The consequence is cancer cell metastasis (Behmoaram et al. 2008) (LoRusso 2016). As expected, after 48 h of treatment with water-extracted $PM_{2.5}$ increased phosphorylation of FAK at Tyr925 (p-FAK-Y925) is correlated with high levels of both PI3K p85α and p110 subunits as well as the overexpression of phosphorylated-AKT (Fig. 5A). In contrast, water-extracted $PM_{2.5}$ that is produced by building demolition has no significant effect on ERK, JNK or p38 MMPK expressions (Fig. 5A).

The characteristics of EMT include reduced intercellular adhesion, acquisition of mesenchymal markers (including vimentin and N-cadherin) and loss of epithelial markers (such as E-cadherin) (Lamouille et al., 2014). Aurora B kinase is one of the major kinases that is responsible for the fidelity of mitosis. An elevated level of Aurora B kinase contributes to chemoresistance and predicts poor prognosis of breast cancer (Zhang et al. 2015). As presented in Fig. 5B, the upregulation of Aurora B kinase and Bcl-2 supports higher viability and proliferation (Fig. 3). $PM_{2.5}$ treatment significantly increased the level of β-catenin, as resulted in the overexpression of N-cadherin, vimentin and MMP-9 proteins. Therefore, Fig. 5C displays a hypothetical model of the induction of TNBC migration by $PM_{2.5}$, which demonstrates that exposure to $PM_{2.5}$ activates the FAK/PI3K/AKT signaling pathway to regulate the process of EMT with the help of cathepsin D, β-catenin, N-cadherin, vimentin and MMP-9, increasing migration.

Growing evidence shows that prolonged exposure to heavy metals is associated with a poor prognosis of cancer. The effects of MAPK and PI3K/AKT signaling pathways on heavy metal-induced carcinogenesis in tumors in the lung have been reported (Ohgami et al., 2015). Accordingly, metal species in water-soluble $PM_{2.5}$ that promote the development of cancer cells by activating the FAK/PI3K/AKT pathway, and thereby, enhance cell proliferation, migration, and metastasis. Inductively coupled plasma mass spectrometry (ICP-MS) analysis revealed that the amounts of the five heavy metals, cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn) and chromium (Cr) were higher in the water-soluble $PM_{2.5}$ extracts that were generated by building demolition than in the post-control sample from the surrounding area (Table 2). The data on the heavy metals were normalized using the environmental factors that are listed in Table 3. Interestingly, our

hypothesis was supported by observations that increased concentrations of Cd and Cu in the water-soluble $PM_{2.5}$ extracts that were obtained from a building demolition site adversely affected colony formation by, and the motility of, MDA-MB-231 cells through activation of the PI3K/AKT pathway.

Discussion And Conclusion

Several investigations have addressed the effect of water-soluble $PM_{2.5}$ on the development of cancer cells, considering the close relationship between human exposure to $PM_{2.5}$ and the risk of circulating $PM_{2.5}$, which promotes lung tumor invasiveness, including the promotion and progression of the tumor during EMT. The effects of building demolition $PM_{2.5}$ on breast cancer cells was also examined. In this study, 1872 m^3 of air was analyzed over nine days of demolition and 101.556 mg of $PM_{2.5}$ was obtained. A total of 27.368 mg $PM_{2.5}$ was obtained after extraction with water, freeze-drying and resuspension. Although some meteorological factors affect $PM_{2.5}$ concentration, building demolition clearly generates a high level of ambient $PM_{2.5}$ in the surroundings. The fact that the level of $PM_{2.5}$ in the cellular model herein exceeds the ambient $PM_{2.5}$ level is a serious unavoidable shortcoming of the model. For example, a 200 μg/mL water-extracted $PM_{2.5}$ is equivalent to 53.88 g/m^3 ambient $PM_{2.5}$, which represents more than ten years of exposure to ambient $PM_{2.5}$ levels following the collapse of the WTC buildings during the 911 event. Notably, although this extraordinary high $PM_{2.5}$ exposure does not occur in the real world, molecular evidence reveals a potentially high risk of cancer malignancy, especially for women with breast cancer, upon exposure to relatively high ambient $PM_{2.5}$ levels.

The underlying cause of death in the majority of breast cancer patients is metastasis (Narod et al., 2015), of which the main characteristic is EMT. The MDA-MB-231 cell line has a high capacity for distant metastasis, and manifestation with malignant mesenchymal features arises from the loss of E-cadherin (Luo et al., 2018). The biochemical data herein reveal that E-cadherin loss the overexpression of N-cadherin and β-catenin screening in cells that had been treated with $PM_{2.5}$. $PM_{2.5}$-treated MDA-MB-231 cells exhibited an increased level of cathepsin D with overexpression of Aurora kinase B, vimentin and MMP-9, which promotes metastasis. The overexpression of cathepsin D enhanced breast cancer cell metastasis by inducing the expression of intercellular cell adhesion molecule-1 (ICAM-1) (Zhang et al. 2018) and has been used as an independent marker of a poor prognosis of breast cancer (Dey et al., 2013; Foekens et al. 1999). Many studies have shown that exposure to $PM_{2.5}$ stimulates lung cancer and epithelium cell motility. Exposure to $PM_{2.5}$ (50 $\mu g/cm^2$ concentrations of $PM_{2.5}$ for 72 h) induces the proliferation and motility of cells of various lung cancer cell lines (Yang et al., 2016). At $PM_{2.5}$ dose ≥ 60 μg/mL, cell apoptosis is evaded, promoting cell proliferation and sustained angiogenesis through the activation of the PI3K-AKT signaling pathway (Zheng et al., 2017). Five components (organic carbon, PAH, Zn, As, V) of $PM_{2.5}$ mostly from traffic emissions were strongly associated with cancer progression, and Zn has a critical role in the activation of PI3K-AKT signal transduction (Zheng et al., 2017; Chen et al., 2013). According to our data and above reports, Zn in water-extracted $PM_{2.5}$ strongly promotes TNBC malignancy. The particles and their solvent extracts had a range of effects on the cell lines, such as the generation of

reactive oxygen species (ROS) and an increase in DNA strand breakage (which is concentration-dependent). Additionally, PM pollution as a result of demolition activity has an adverse effect on the health of people who live close to the demolition sites, especially since exposure to a high dose of $PM_{2.5}$ is associated with FAK/PI3K/AKT signaling activation and enhanced EMT in breast cancer cells. These findings are supported by health assessments that demonstrate that exposure to $PM_{2.5}$ becomes more important when the surroundings are densely built residential areas or sensitive areas, such as schools and hospitals (Farhad &Azarmi, 2016; Azarmi et al., 2016).

Scientific research into the effects of airborne particulates that are generated by building demolition since the September 11th event in New York city have shown that the demolition of buildings generates large quantities of PM, which can be inhaled by on-site workers and people who live in the neighborhood. Evidence suggests that long-term exposure to airborne particles triggers cell mutations and increase the risk of breast cancer, but the toxicological mechanisms are unclear. The discovery of estrogen-mimicking compounds in the environment and the synergistic activity of many of these on estrogen receptors have led researchers to hypothesize the role of xenoestrogenic substances in the environment that mimic estrogen in increasing breast cancer risk (Arnold et al., 1996). However, not all breast cancers are responsive to this hormone and its analogs in environmental pollutants. Based on the breast cancer subtypes estrogen receptor (ER) and progesterone receptor (PR) status, ER-positive (ER(+)) and ER-negative (ER(-)) breast cancers have distinctly different risk factors and possibly, therefore, different etiologies (Althuis et al., 2004). Depending on the etiological differences between breast cancer subtypes, ER(+) breast cancers are associated with estrogen-related factors, including early menarche, number of pregnancies, and late-age childbearing (Chen et al., 2013). However, the incidence rate of ER(-) breast cancers in Western populations has been shown to be related to county-level environmental factors, such as pesticide use, toxic air emissions, and pollution from urban activities. In this investigation, MDA-MB-231 invasive, rather than ER(+),breast cancer cells were used to study the effect of water-extracted building demolition $PM_{2.5}$ on ER(-) breast cancer cell progression. Exposure to water-extracted $PM_{2.5}$ enhanced the PI3K/Akt signal transduction pathway in MDA-MB-231 cells, favoring breast cancer cell invasiveness. Moreover, the significantly elevated concentrations of the heavy metals cadmium, copper, lead, zinc and chromium were found in the water-extracted $PM_{2.5}$ during building demolition. Long-term exposure to $PM_{2.5}$ in Italy has been associated with increased risk of death due to breast cancer (Tagliabue et al., 2016). Interestingly, the findings herein are consistent with the previous epidemiological observations of Italian women who live near incinerators, who have been found to have an elevated risk of breast cancer mortality. These women live in areas with a very high total concentration (> 2 ng/m^3) of heavy metals, including lead, cadmium, chromium, cobalt, and copper. The lowest measured ambient concentrations of these heavy metals are of the order of < 0.5 ng/m^3 (Ranzi et al. 2011). Beyond this investigation of the effects of ambient exposure to coarse and fine particle emissions from building demolition sites on breast cancer, identifying genetic factors that respond to ambient air pollution and breast cancer would be very informative (Brody et al. 2007). Polycyclic aromatic hydrocarbons (PAHs), which can cause oxidative stress (Mordukhovich et al. 2010), stimulate cell proliferation and mammary tumors in laboratory animals, leading to neoplasia in breast cancer, warrant particular attention. To the best of our knowledge, this

investigation is the first to highlight the promotion by building demolition $PM_{2.5}$ of breast tumor progression, mediated by PI3K signaling, enhancing the invasion and migration of ER(-) breast cancer cells. This information may contribute to better estimates of ambient air pollution to identify areas that experience disproportionate effects of outdoor air pollution.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was received.

Authors' contributions

C.-Y.L., C.-W.C., G.-T.S. and Y.-C. C. planned work and designed experiments. G.-T.S., C.-Y.L. and C.-W.C. wrote manuscript. J.-S.C. and P.-H.W. collected PM and performed experimental sampling and testing. C.-W.C., G.-T.S. and C.-Y.L. performed statistical analysis. All authors analyzed and discussed the results and commented on the manuscript.

Acknowledgements

We thank the SDI Group, Taiwan, for their assistance with PM sampling.

Authors' information

Chun-Wen Cheng, email: cwcheng@csmu.edu.tw;

Gwo-Tarng Sheu, email: gtsheu@csmu.edu.tw;

Jing-Shiuan Chou, email: car05280@gmail.com;

Pei-Han Wang, email: u9807410@gmail.com;

Yu-Chun Cheng, email: yuchuncheng0817@gmail.com;

Chane-Yu Lai, email: cylai@csmu.edu.tw . Rm. 1215A, No.110, Sec.1, Chien-Kuo N. Rd. Taichung, Taiwan.

References

1. Aldrich TK, Gustave J, Hall CB, Cohen HW, Webber MP, Zeig-Owens R, Cosenza K, Christodoulou V, Glass L, Al-Othman F and others. 2010. Lung function in rescue workers at the World Trade Center after 7 years. N Engl J Med 362(14):1263-72.
2. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. 2004. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. Cancer Epidemiol Biomarkers Prev 13(10):1558-68.
3. Andersen ZJ, Ravnskjaer L, Andersen KK, Loft S, Brandt J, Becker T, Ketzel M, Hertel O, Lynge E, Brauner EV. 2017. Long-term Exposure to Fine Particulate Matter and Breast Cancer Incidence in the Danish Nurse Cohort Study. Cancer Epidemiol Biomarkers Prev 26(3):428-430.
4. Arnold SF, Klotz DM, Collins BM, Vonier PM, Guillette LJ, Jr., McLachlan JA. 1996. Synergistic activation of estrogen receptor with combinations of environmental chemicals. Science 272(5267):1489-92.
5. Azarmi F, Kumar P, Marsh D, Fuller G. 2016. Assessment of the long-term impacts of PM10 and PM2.5 particles from construction works on surrounding areas. Environ Sci Process Impacts 18(2):208-21.
6. Beck CM, Geyh A, Srinivasan A, Breysse PN, Eggleston PA, Buckley TJ. 2003. The impact of a building implosion on airborne particulate matter in an urban community. J Air Waste Manag Assoc 53(10):1256-64.
7. Behmoaram E, Bijian K, Jie S, Xu Y, Darnel A, Bismar TA, Alaoui-Jamali MA. 2008. Focal adhesion kinase-related proline-rich tyrosine kinase 2 and focal adhesion kinase are co-overexpressed in early-stage and invasive ErbB-2-positive breast cancer and cooperate for breast cancer cell tumorigenesis and invasiveness. Am J Pathol 173(5):1540-50.
8. Brody JG, Rudel RA, Michels KB, Moysich KB, Bernstein L, Attfield KR, Gray S. 2007. Environmental pollutants, diet, physical activity, body size, and breast cancer: where do we stand in research to identify opportunities for prevention? Cancer 109(12 Suppl):2627-34.
9. Chen ST, Lin CC, Liu YS, Lin C, Hung PT, Jao CW, Lin PH. 2013. Airborne particulate collected from central Taiwan induces DNA strand breaks, Poly(ADP-ribose) polymerase-1 activation, and estrogen-disrupting activity in human breast carcinoma cell lines. J Environ Sci Health A Tox Hazard Subst Environ Eng 48(2):173-81.
10. Claudio L. 2001. Environmental aftermath. Environ Health Perspect 109(11):A528-36.
11. Crouse DL, Goldberg MS, Ross NA, Chen H, Labreche F. 2010. Postmenopausal breast cancer is associated with exposure to traffic-related air pollution in Montreal, Canada: a case-control study. Environ Health Perspect 118(11):1578-83.

12. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA. 2007. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 13(15 Pt 1):4429-34.
13. Dey N, Barwick BG, Moreno CS, Ordanic-Kodani M, Chen Z, Oprea-Ilies G, Tang W, Catzavelos C, Kerstann KF, Sledge GW, Jr. and others. 2013. Wnt signaling in triple negative breast cancer is associated with metastasis. BMC Cancer 13:537.
14. Farhad Azarmi PK. 2016. Ambient exposure to coarse and fine particle emissions from building demolition. Atmospheric Environment 137:62-79.
15. Foekens JA, Look MP, Bolt-de Vries J, Meijer-van Gelder ME, van Putten WL, Klijn JG. 1999. Cathepsin-D in primary breast cancer: prognostic evaluation involving 2810 patients. Br J Cancer 79(2):300-7.
16. Hart JE, Bertrand KA, DuPre N, James P, Vieira VM, Tamimi RM, Laden F. 2016. Long-term Particulate Matter Exposures during Adulthood and Risk of Breast Cancer Incidence in the Nurses' Health Study II Prospective Cohort. Cancer Epidemiol Biomarkers Prev 25(8):1274-6.
17. Huo Q, Zhang N, Wang X, Jiang L, Ma T, Yang Q. 2013. Effects of ambient particulate matter on human breast cancer: is xenogenesis responsible? PLoS One 8(10):e76609.
18. Hystad P, Villeneuve PJ, Goldberg MS, Crouse DL, Johnson K, Canadian Cancer Registries Epidemiology Research G. 2015. Exposure to traffic-related air pollution and the risk of developing breast cancer among women in eight Canadian provinces: a case-control study. Environ Int 74:240-8.
19. Lamouille S, Xu J, Derynck R. 2014. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 15(3):178-96.
20. Landrigan PJ. 2001. Health consequences of the 11 September 2001 attacks. Environ Health Perspect 109(11):A514-5.
21. Liao TT, Yang MH. 2017. Revisiting epithelial-mesenchymal transition in cancer metastasis: the connection between epithelial plasticity and stemness. Mol Oncol 11(7):792-804.
22. Lioy PJ, Pellizzari E, Prezant D. 2006. The World Trade Center aftermath and its effects on health: understanding and learning through human-exposure science. Environ Sci Technol 40(22):6876-85.
23. Lioy PJ, Weisel CP, Millette JR, Eisenreich S, Vallero D, Offenberg J, Buckley B, Turpin B, Zhong M, Cohen MD and others. 2002. Characterization of the dust/smoke aerosol that settled east of the World Trade Center (WTC) in lower Manhattan after the collapse of the WTC 11 September 2001. Environ Health Perspect 110(7):703-14.
24. Lippmann M, Cohen MD, Chen LC. 2015. Health effects of World Trade Center (WTC) Dust: An unprecedented disaster's inadequate risk management. Crit Rev Toxicol 45(6):492-530.
25. Liu R, Nelson DO, Hurley S, Hertz A, Reynolds P. 2015. Residential exposure to estrogen disrupting hazardous air pollutants and breast cancer risk: the California Teachers Study. Epidemiology 26(3):365-73.
26. LoRusso PM. 2016. Inhibition of the PI3K/AKT/mTOR Pathway in Solid Tumors. J Clin Oncol 34(31):3803-3815.

27. Luo CW, Wu CC, Chang SJ, Chang TM, Chen TY, Chai CY, Chang CL, Hou MF, Pan MR. 2018. CHD4-mediated loss of E-cadherin determines metastatic ability in triple-negative breast cancer cells. Exp Cell Res 363(1):65-72.
28. McGee JK, Chen LC, Cohen MD, Chee GR, Prophete CM, Haykal-Coates N, Wasson SJ, Conner TL, Costa DL, Gavett SH. 2003. Chemical analysis of World Trade Center fine particulate matter for use in toxicologic assessment. Environ Health Perspect 111(7):972-80.
29. Mordukhovich I, Rossner P, Jr., Terry MB, Santella R, Zhang YJ, Hibshoosh H, Memeo L, Mansukhani M, Long CM, Garbowski G and others. 2010. Associations between polycyclic aromatic hydrocarbon-related exposures and p53 mutations in breast tumors. Environ Health Perspect 118(4):511-8.
30. Narod SA, Iqbal J, Giannakeas V, Sopik V, Sun P. 2015. Breast Cancer Mortality After a Diagnosis of Ductal Carcinoma In Situ. JAMA Oncol 1(7):888-96.
31. Ohgami N, Yamanoshita O, Thang ND, Yajima I, Nakano C, Wenting W, Ohnuma S, Kato M. 2015. Carcinogenic risk of chromium, copper and arsenic in CCA-treated wood. Environ Pollut 206:456-60.
32. Parikh PV, Wei Y. 2016. PAHs and PM2.5 emissions and female breast cancer incidence in metro Atlanta and rural Georgia. Int J Environ Health Res 26(4):458-66.
33. Ranzi A, Fano V, Erspamer L, Lauriola P, Perucci CA, Forastiere F. 2011. Mortality and morbidity among people living close to incinerators: a cohort study based on dispersion modeling for exposure assessment. Environ Health 10:22.
34. Reding KW, Young MT, Szpiro AA, Han CJ, DeRoo LA, Weinberg C, Kaufman JD, Sandler DP. 2015. Breast Cancer Risk in Relation to Ambient Air Pollution Exposure at Residences in the Sister Study Cohort. Cancer Epidemiol Biomarkers Prev 24(12):1907-9.
35. Rom WN, Weiden M, Garcia R, Yie TA, Vathesatogkit P, Tse DB, McGuinness G, Roggli V, Prezant D. 2002. Acute eosinophilic pneumonia in a New York City firefighter exposed to World Trade Center dust. Am J Respir Crit Care Med 166(6):797-800.
36. Stovgaard ES, Nielsen D, Hogdall E, Balslev E. 2018. Triple negative breast cancer - prognostic role of immune-related factors: a systematic review. Acta Oncol 57(1):74-82.
37. Tagliabue G, Borgini A, Tittarelli A, van Donkelaar A, Martin RV, Bertoldi M, Fabiano S, Maghini A, Codazzi T, Scaburri A and others. 2016. Atmospheric fine particulate matter and breast cancer mortality: a population-based cohort study. BMJ Open 6(11):e012580.
38. Thiery JP, Acloque H, Huang RY, Nieto MA. 2009. Epithelial-mesenchymal transitions in development and disease. Cell 139(5):871-90.
39. Wei Y, Davis J, Bina WF. 2012. Ambient air pollution is associated with the increased incidence of breast cancer in US. Int J Environ Health Res 22(1):12-21.
40. Weiden MD, Kwon S, Caraher E, Berger KI, Reibman J, Rom WN, Prezant DJ, Nolan A. 2015. Biomarkers of World Trade Center Particulate Matter Exposure: Physiology of Distal Airway and Blood Biomarkers that Predict FEV(1) Decline. Semin Respir Crit Care Med 36(3):323-33.
41. Yaghjyan L, Arao R, Brokamp C, O'Meara ES, Sprague BL, Ghita G, Ryan P. 2017. Association between air pollution and mammographic breast density in the Breast Cancer Surveilance Consortium. Breast

Cancer Res 19(1):36.

42. Yang B, Chen D, Zhao H, Xiao C. 2016. The effects for PM2.5 exposure on non-small-cell lung cancer induced motility and proliferation. Springerplus 5(1):2059.
43. Yang Q, Yuan Q, Li T, Shen H, Zhang L. 2017. The Relationships between PM2.5 and Meteorological Factors in China: Seasonal and Regional Variations. Int J Environ Res Public Health 14(12).
44. Zhang C, Zhang M, Song S. 2018. Cathepsin D enhances breast cancer invasion and metastasis through promoting hepsin ubiquitin-proteasome degradation. Cancer Lett 438:105-115.
45. Zhang Y, Jiang C, Li H, Lv F, Li X, Qian X, Fu L, Xu B, Guo X. 2015. Elevated Aurora B expression contributes to chemoresistance and poor prognosis in breast cancer. Int J Clin Exp Pathol 8(1):751-7.
46. Zheng L, Liu S, Zhuang G, Xu J, Liu Q, Zhang X, Deng C, Guo Z, Zhao W, Liu T and others. 2017. Signal Transductions of BEAS-2B Cells in Response to Carcinogenic PM2.5 Exposure Based on a Microfluidic System. Anal Chem 89(10):5413-5421.

Tables

Table 1. Sampling duration and monitoring sites with $PM_{2.5}$ concentrations and meteorological data.

Date of Sampling	Building demolition $PM_{2.5}$ ($\mu g/m^3$)	Wuri station $PM_{2.5}$ ($\mu g/m^3$)	Changhua station $PM_{2.5}$ ($\mu g/m^3$)	Wind speed (m/sec)	Wind direction	Average temperature (°C)	Relative humidity (RH, %)
2016.12.23	47.40	42.58	37.37	2.92	North	20.3	58.1
2016.12.24	73.77	45.83	27.09	1.99	North	20.6	67.0
2016.12.25	66.42	39.21	35.04	2.00	East-North	21.6	77.0
2016.12.29	30.29	14.58	10.91	4.15	East-North-East	15.7	66.3
2016.12.30	54.66	28.30	19.79	2.01	North	18.4	70.3
2016.12.31	49.74	24.21	22.41	1.88	North	19.9	78.2
2017.01.01	78.05	40.83	29.41	1.88	North	20.8	80.0
2017.01.08	48.41	21.75	34.29	2.28	North	20.7	74.3
2017.01.13	39.55	15.75	56.50	3.13	North	16.1	76.5
2018.03.20	12.93	15.10	9.86	7.28	North	19.7	81.8
2018.03.21	14.80	16.50	17.00	7.65	North	17.1	59.3
2018.03.22	24.40	27.48	30.86	4.56	North	18.4	60.8
2018.03.23	22.71	27.88	28.29	3.97	North	21.0	48.5
2018.03.26	43.14	35.79	30.57	3.91	North	22.3	74.4
2018.03.27	41.24	40.04	40.43	5.07	North	22.3	78.8
2018.03.28	35.06	37.25	44.71	6.35	North	23.2	81.4
2018.03.29	49.58	58.95	67.57	3.22	North	23.7	81.3
2018.03.30	27.79	36.00	35.43	3.68	North	25.0	69.4

Table 2. Components of heavy metals identified by ICP-MS.

		Cd	Cu	Hg	Pb	Ni	As	Zn	Cr	V
Control	mg/L	N.D.	2.38	N.D.	1.2	N.D.	N.D.	49.0	4.30	N.D.
$PM_{2.5}$	mg/L	61.9	1780	N.D.	927	160	111	10800	307	110
Post-Ctl	mg/L	32.9	1400	N.D.	655	189	112	7040	133	445

Table 3. Concentrations of heavy metals in $PM_{2.5}$ collected at building demolition site.

		Cd	Cu	Hg	Pb	Ni	As	Zn	Cr	V
$PM_{2.5}$	ng/L	0.367	10.565	N.D.	5.502	0.95	0.659	64.103	1.822	0.65
Post-Ctl	ng/L	0.195	8.31	N.D.	3.888	1.122	0.665	41.785	0.789	2.641

Figures

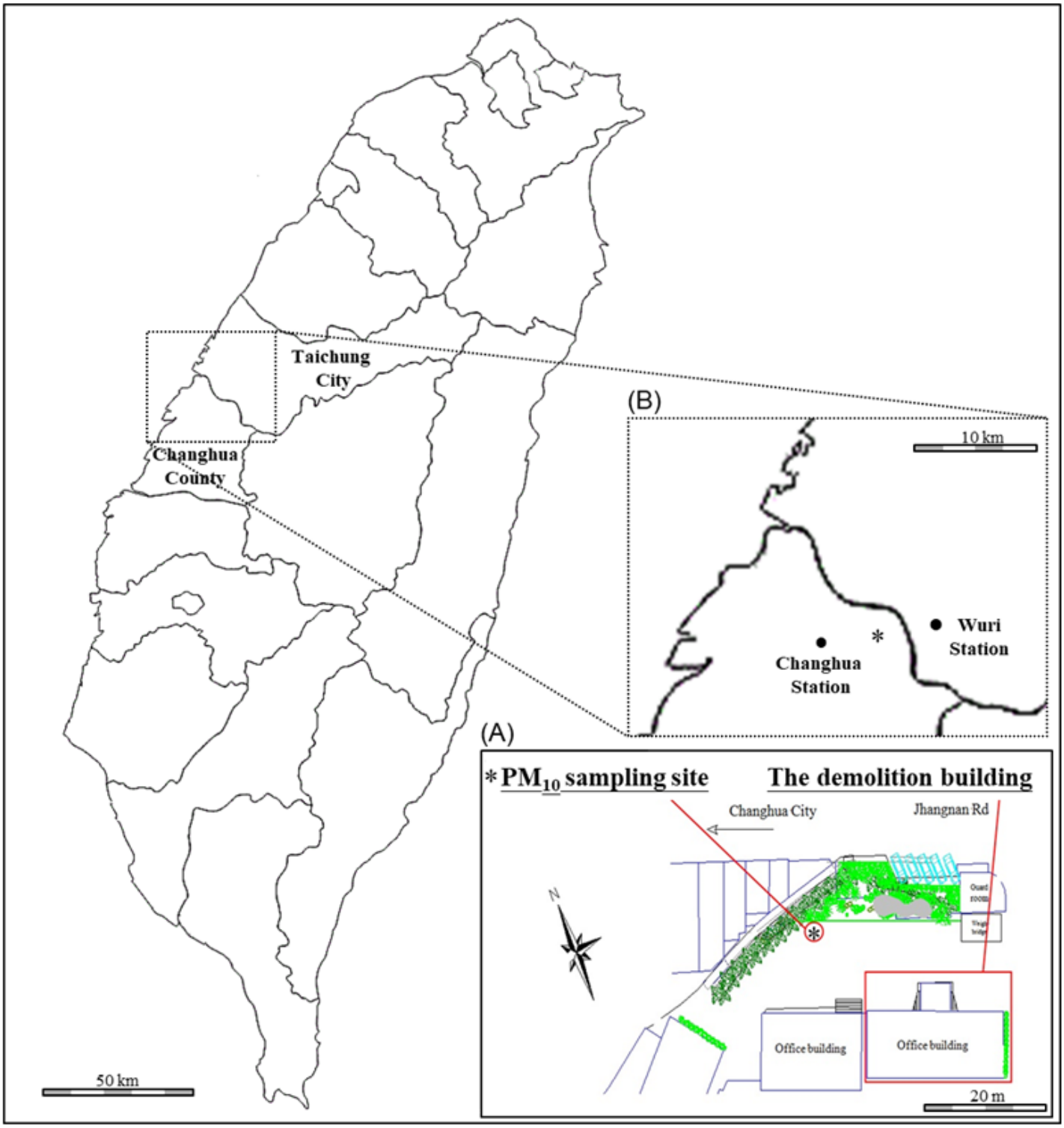

Figure 1

Site map showing location of demolition building sampling site and local monitor stations. (A) Open circle indicates high-flow sampler and open square indicates demolition building location. (B) Monitoring Wuri station (close circle) is to the northeast and monitoring Changhua station (close circle) is to the southwest of the demolition site (close star).

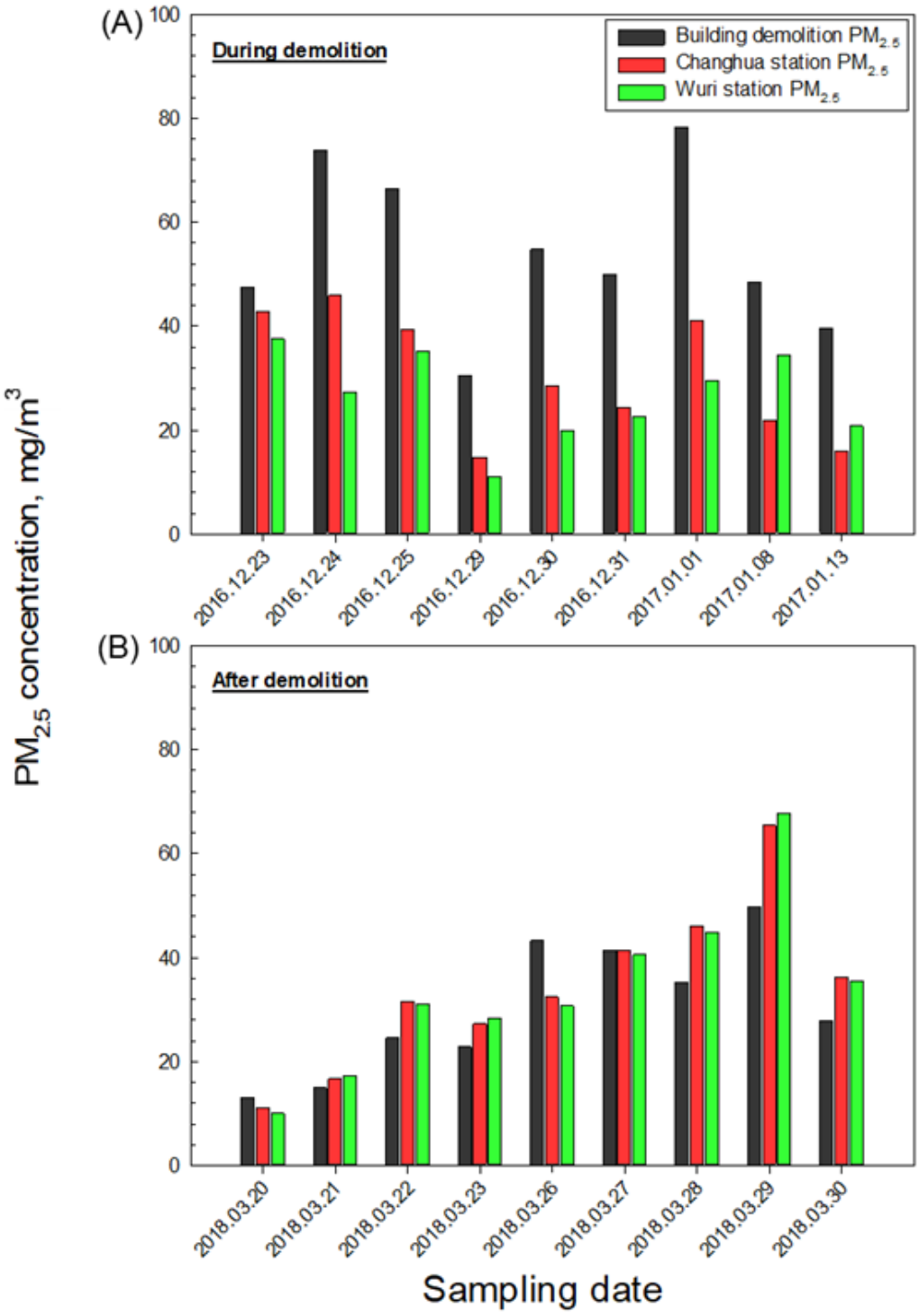

Figure 2

Comparison of PM2.5 concentrations at demolition site and local area. (A) PM2.5 concentrations at demolition site and in local area during demolition process. (B) PM2.5 concentrations at demolition site and in local area post-demolition.

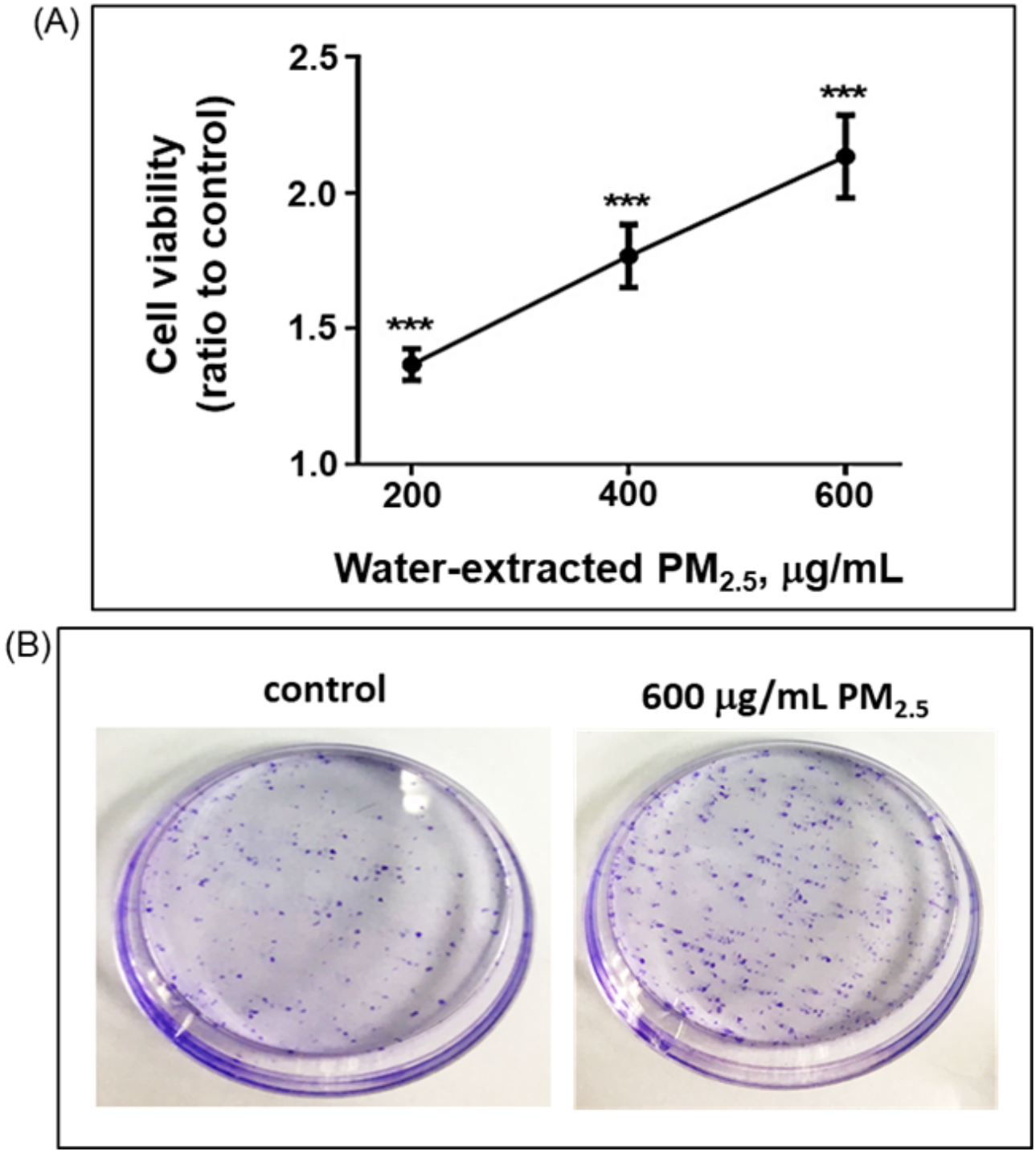

Figure 3

Effects of PM2.5 on viability and proliferation of MDA-MB-231 cells. (A) Viabilities of cells were evaluated by MTT assay after 48 h of exposure to PM2.5 and the detected absorbance was normalized to control group as the ratio of cell viability. (B) Representative photographs of colony formation assay by cells that were treated with PM2.5 for 24 h and then incubated for 7 days.

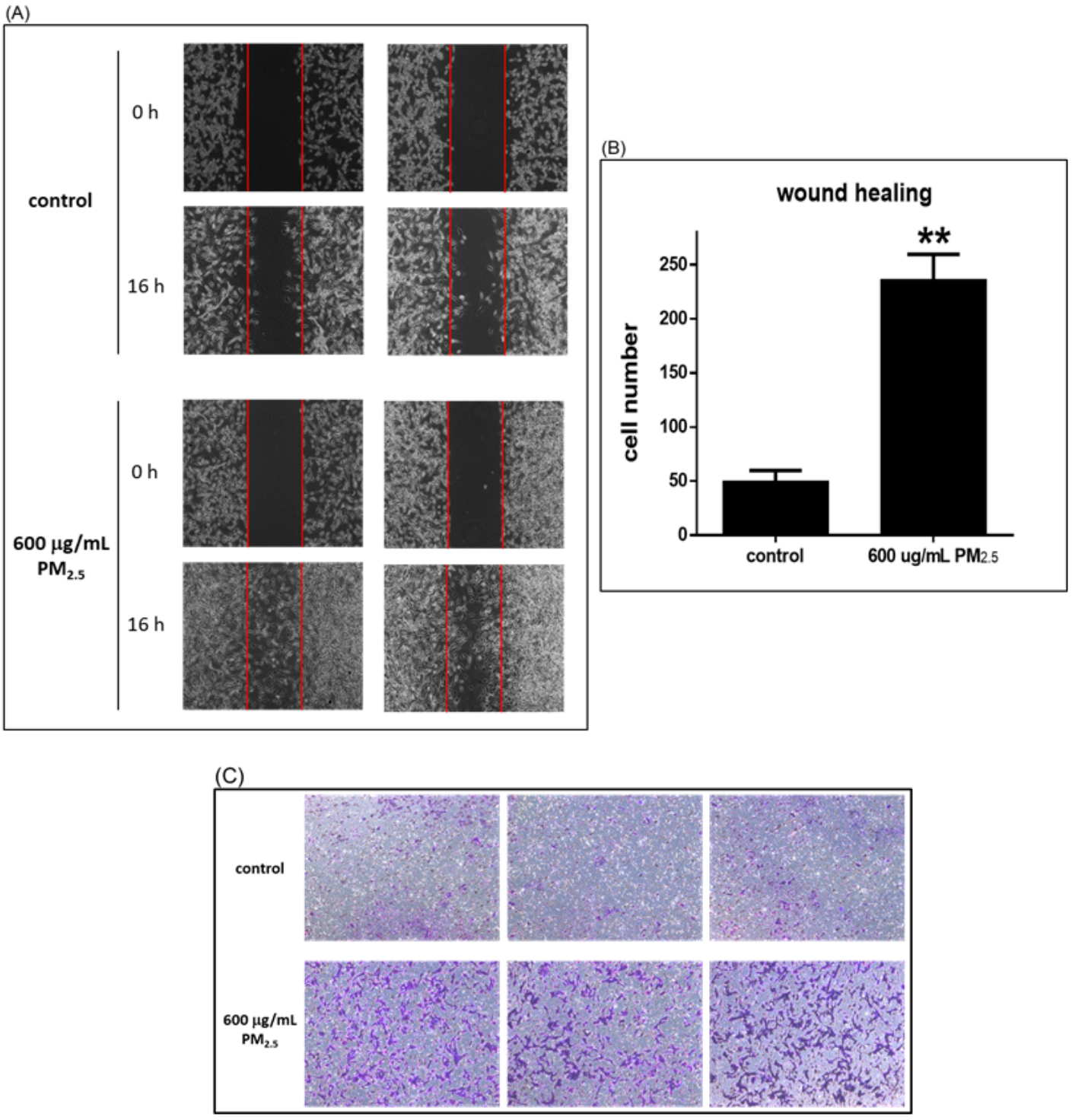

Figure 4

Effects of PM2.5 on migration of MDA-MB-231 cells. (A) PM2.5-treated cells were analyzed using wound healing assay and the representative photographs are displayed. (B) Cells that migrated across the solid line were counted. (C) Representative photographs of transwell migration assay are shown. Cells were treated with PM2.5 for 24 h and allowed to migrate for 16 h before staining.

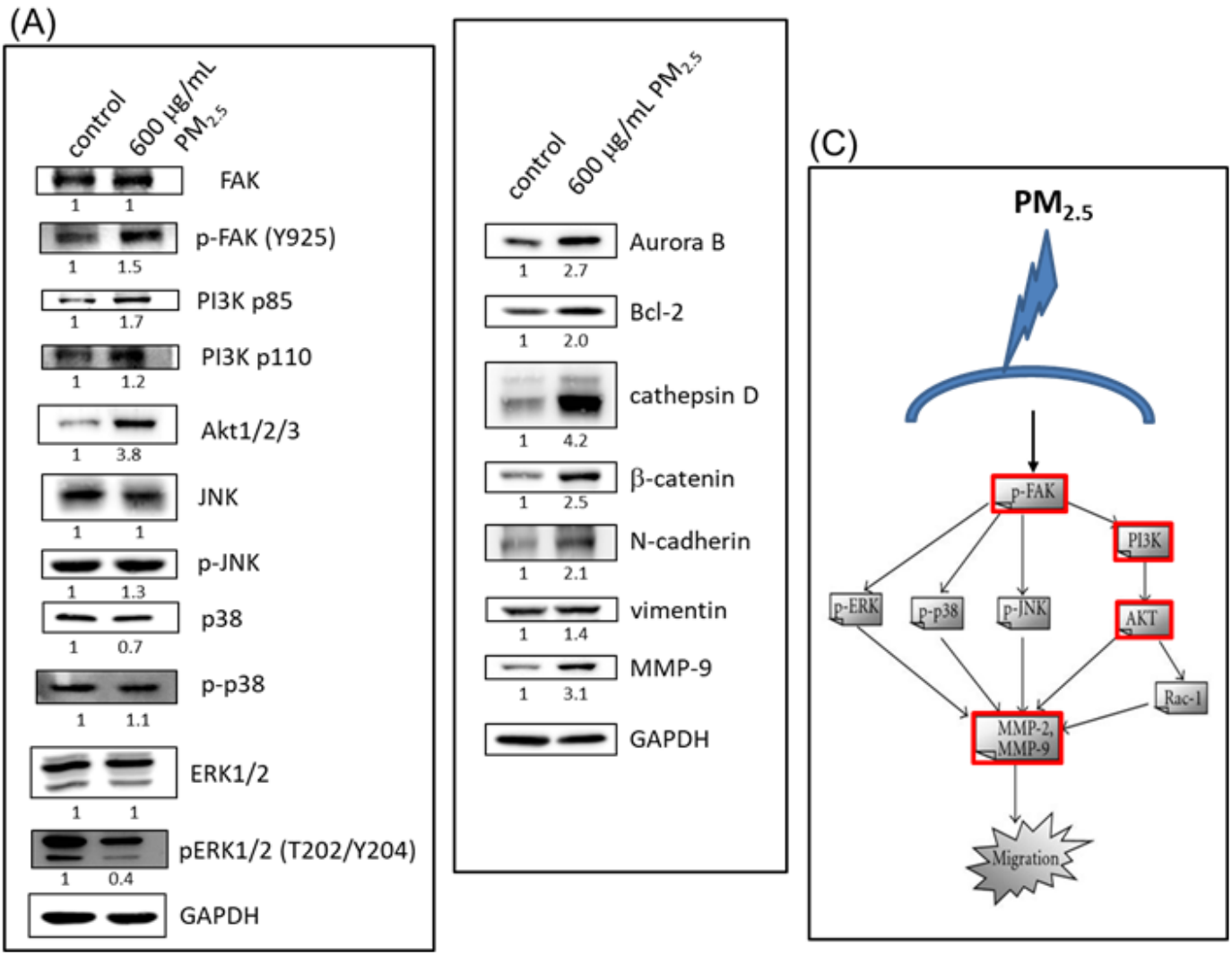

Figure 5

Association of PM2.5 with signaling pathways and regulation of migration of MDA-MB-231 cells. (A) Expression of various signal protein kinases that may be activated by PM2.5, analyzed by western blot. (B) Expression of migration markers, analyzed by western blot. (C) Summary of effects of PM2.5 on TNBC migration.