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Original Article

Pterostilbene supresses inflammation-induced melanoma metastasis by impeding neutrophil elastase-mediated thrombospondin-1 degradation

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ABSTRACT

Objective: Chronic inflammation plays a fatal role in tumor metastasis. Pterostilbene (PTE) is a natural dimethylated analogue of resveratrol with anticancer and anti-inflammatory activities. This study aimed to investigate the inhibitory effect of PTE on inflammation-associated metastasis and explore the underlying mechanisms.

Methods: Lipopolysaccharide (LPS)-induced lung inflammation and melanoma metastasis models were established in mice. After PTE treatment for four weeks, the organ index, histological changes, proinflammatory cytokines, and the expression and activity of neutrophil elastase (NE), a biomarker of neutrophil influx in the lungs, were analysed. Additionally, direct effects of PTE on NE-induced B16 cell migration were explored in wound healing and Transwell assays, and the expression of thrombospondin-1 (TSP-1) and epithelial-mesenchymal transition (EMT) markers were also detected.

Results: PTE obviously attenuated the LPS-induced metastasis of circulatory B16 cells to lungs by reducing the number of metastatic nodules on the lung surfaces and the lung weight/body weight ratio. PTE treatment also significantly reduced LPS-activated increase levels of tumor necrosis factor (TNF)-α and interleukin (IL)-6 in the lungs of tumor-bearing mice. In addition, increased expression and enzyme activity of NE and decreased expression of TSP-1 were observed, and these were blocked by PTE. *In vitro,* PTE at concentrations without cytotoxicity also markedly suppressed NE-triggered B16 cell migration, prevented NE-induced TSP-1 proteolysis and reversed the expression of vimentin, *N*-cadherin and E-cadherin.

Conclusion: PTE could block inflammation-enhanced tumor metastasis, and the underlying mechanism might be associated with the inhibition of NE-mediated TSP-1 degradation.

1. Introduction

Cancer is a leading cause of death worldwide. Metastasis, which refers to the spread of primary tumor cells to distant organs, is one of the principal reasons for treatment failure and accounts for most cancer-associated mortality (~90%) (Stoletov, Beatty, & Lewis, 2020). Chronic inflammation is a major contributor to tumor metastasis and is currently recognized as a hallmark of cancer and an attractive therapeutic target in cancer (Greten & Grivennikov, 2019; Diakos, Charles, McMillan, & Clarke, 2014; Singh et al., 2019; Hibino et al., 2021).

Multiple immune cell types compose an inflammatory tumor microenvironment to support cancer development and metastasis. Increasing evidence shows that neutrophils contribute to cancer progression, particularly metastasis, by releasing their secretory granules (Wu, Ma, Tan, Zheng, & Liu, 2020). Recently, neutrophil elastase (NE), a neutrophil-specific serine protease, was found to be directly involved in early metastatic events (Deryugina et al., 2020). NE can functionally activate or modify a number of proteins that facilitate epithelial-mesenchymal transition (EMT), degrade extracellular matrix (ECM) proteins and trigger the angiogenic switch, which are all prerequisites for tumor metastasis (Lerman & Hammes, 2018; Sato et al., 2006). Thrombospondin-1 (TSP-1) is a multifunctional matrix glycoprotein with inhibitory activity against NE that can also be degraded by NE. As a potent angiogenesis inhibitor, upregulation of TSP-1 has been suggested to inhibit tumor growth and migration (Ramchandani & Mittal, 2020). However, in a tail vein injection model of melanoma, TSP-1 was reported to be degraded by excessive NE released from lung

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inflammation-recruited neutrophils, thus promoting melanoma metastatic outgrowth in the lung. Genetic ablation of NE protected TSP-1 from proteolysis and suppressed lung metastasis (El Rayes et al., 2015). The NE/TSP-1 axis might be a potential anti-metastatic therapeutic target in the inflammatory tumor microenvironment.

Pterostilbene (PTE) is a natural dimethylated analogue of resveratrol found mainly in blueberries, grapes and narra trees. Previous investigations have found that PTE has similar pharmacological properties to resveratrol, including antioxidant, anti-inflammatory, and anticancer properties (Lin, Leland, Ho, & Pan, 2020; Tsai, Ho, & Chen, 2017), as well as better oral and lipophilic absorption, higher bioavailability and a longer half-life than resveratrol (Remsberg et al., 2008). As a plant phytoalexin polyphenol, PTE inhibits inflammation in a wide range of diseases, including neuroinflammation, dermatitis, pneumonia, pancreatitis, and inflammatory bowel disease (Lin, Leland, Ho, & Pan, 2020). Modulation of neutrophil activity and apoptosis has been introduced as a new strategy attributed to PTE for pharmacological control of chronic inflammatory processes (Jančinová, Perečko, Harmatha, Nosál', & Drábiková, 2012). Moreover, PTE is also a potential anti-carcinogen with potent anticancer activities against various malignancies, and the underlying mechanisms include anti-proliferation, anti-metastasis, anti-angiogenesis, apoptosis induction, and inhibition of cancer stem cells (Ma et al., 2019). However, the effects of PTE on inflammation-induced tumor metastasis have never been elucidated.

In this study, we combined lipopolysaccharide (LPS)-induced lung inflammation with mouse metastasis models to investigate the inhibitory effect of PTE on pulmonary inflammation-induced melanoma metastasis and identified the underlying mechanisms through the NE/TSP-1 pathway.

2. Materials and methods

2.1. Chemicals and antibodies

NE purified from human sputum was purchased from Elastin Products (Owensville, MO, USA). PTE, LPS (Escherichia coli055: B5), dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). ELISA kits for TNF-α and IL-6 were purchased from Multisciences Biotech Co., Ltd. (Hangzhou, China). RPMI-1640 medium and foetal bovine serum (FBS) were obtained from Gibco (Grand Island, NY, USA). The BCA Protein Assay Kit was acquired from Beyotime Institute of Biotechnology (Haimen, China). Primary antibodies against vimentin, E-cadherin, *N*-cadherin, and TSP-1 and secondary antibodies were purchased from Cell Signaling Technology (Boston, MA, USA).

2.2. LPS-induced pulmonary inflammation and melanoma metastasis model

Male C57BL/6J mice aged six weeks were purchased from Shanghai Super B&K Laboratory Animal Co., Ltd. (certificate: SCXK2018-0006). The mice were housed in pathogen-free sterile isolators maintained at 25 °C under a 12-h light/dark cycle. Sterilized food and water were available ad libitum. All the animal experiments were approved by the Animal Welfare and Research Ethics Committee of the Changzhou No.2 People's Hospital. After 7 d of acclimation, the mice were anaesthetized using isoflurane, and LPS at a concentration of 0.25 mg/mL in a 50-μL volume was administered intranasally on 0, 3rd, and 7th days as described in a previous study (El Rayes et al., 2015). Three days after the last LPS treatment, B16 cells (2×10^5) were injected via the tail vein to simulate tumor metastasis. The experimental mice were divided into four groups, which were the control group, model group, low-dose PTE group and high-dose PTE group, with 10 mice in each group. Mice were given intraperitoneally injection of either corn oil or PTE (25 and 50 mg/kg) once daily for four weeks (Fig. 1A). Subsequently, the mice were sacrificed, and lung metastatic foci were identified. Four lung samples from each group were fixed with 10% formaldehyde and embedded in paraffin medium for haematoxylin-eosin (HE) and immunohistochemical (IHC) staining, and the other six lung samples from each group were homogenized at 4 °C and frozen at − 80 °C until use. TNF-α and IL-6 concentrations in the lung tissue supernatant were detected using ELISA according to the manufacturer's instructions.

2.3. Measurement of NE activity

NE activity was determined as described previously (Ishii et al., 2010). Briefly, the supernatant of the lung homogenate (20 μL) was incubated with NE substrate (*N*-methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide) solution (500 μmol/L, 80 μL) in a 96-well plate. After incubation at 37 °C for 24 h, the liberated amount of *p*-nitroanilide was measured at 405 nm on a microplate reader (Thermo Fisher, Shanghai, China). A standard curve was also plotted, and NE activity was corrected with the protein concentration.

2.4. HE and IHC staining

Lung tissue specimens were sectioned into 5-μm slices and then stained with H&E for histological analysis. Immunohistochemical analysis was used to detect NE expression. After epitope retrieval and blocking of endogenous peroxidase activity, the slides were incubated with anti-NE overnight at 4 °C and then with secondary antibodies according to the protocol supplied with the ABC Staining System kit (Boster, Wuhan, China). Images were captured using an Olympus microscope (Tokyo, Japan).

2.5. Cell culture and treatment

Mouse B16-F10 melanoma cells purchased from American Type Culture Collection (ATCC) were cultured in RPMI-1640 medium containing 10% FBS at 37 °C in a humidified incubator with 5% CO_2. The cells were treated with different concentrations of NE and/or PTE for 24 h and then evaluated as described later.

2.6. Cell viability

Cell viability was determined using the MTT assay. B16 cells were seeded at a density of 5×10^3 cells/well in 96-well plates. After incubation with NE and/or PTE for 24 h, 10 μL of MTT solution (5 mg/mL) was added to each well, and the cells were incubated at 37 °C for an additional 4 h. Subsequently, the medium in each well was replaced with 200 μL DMSO to dissolve the formazan, and the absorbance was measured at 490 nm on a microplate reader. The effect of NE and/or PTE on cell viability is expressed as a percentage relative to control values.

2.7. Wound-healing assay

A wound-healing assay was used to evaluate cell migration. Briefly, B16 cells (2×10^5 cells/well) were seeded in a 6-well plate. When the cells reached 90%−95% confluence, the centre of the cell monolayers was scraped using a sterile 200-μLpipette tip. After washing three times with phosphate-buffered saline (PBS), the cells were incubated in FBS-free medium containing NE and/or PTE for 24 h. The wounds were photographed at 0 h and 24 h with a Nikon inverted microscope (Nikon, Tokyo, Japan). The rate of cell

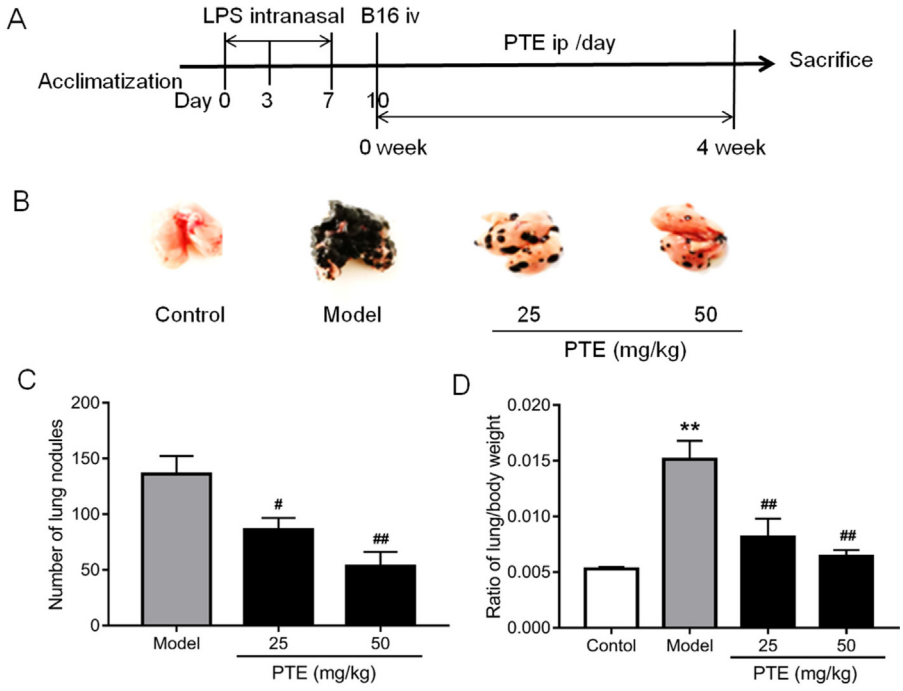

Fig. 1. PTE inhibited pulmonary inflammation-induced melanoma metastasis in mice (mean ± SEM, n = 10). (A) Schematic depiction of the experimental procedure. (B) Representative images of the lungs in each group. (C) Nnumber of lung metastases foci in each group. (D) Ratio of lung/body weight in each group. $^{**}P < 0.01$ *vs* control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* model group.

migration was evaluated based on the rate of wound closure using Image-Pro Plus 6.0 software.

2.8. Transwell migration assay

Cell migration assays were carried out using a transwell chamber (Corning Costar, Cambridge, Massachusetts). B16 cells resuspended in serum-free medium (1×10^5 cells/200 μL) in the presence or absence of PTE were added to the upper chambers of each well. The bottom chambers were filled with 500 μL RPMI 1640 medium supplemented with 10% FBS and 10 nmol/L NE. After 12 h of incubation at 37 °C, cells in the upper surface of the chamber were gently wiped with a cotton swab, and the cells that migrated to the lower surface were fixed with 4% formaldehyde and stained with 0.1% crystal violet. Then, the migrated cells were counted under a Nikon inverted microscope (Nikon, Tokyo, Japan).

2.9. Western blot analysis

Total protein was extracted using RIPA lysis buffer, and the concentration of proteins was quantified using the BCA protein assay kit. Then, equal amounts of proteins were subjected to 10% SDS–PAGE and transferred to polyvinylidene difluoride membranes (Millipore Corp., MA, USA). After blocking, the membranes were incubated with primary antibodies against vimentin, E-cadherin, *N*-cadherin and TSP-1 overnight at 4 °C. After washing with TBST, they were incubated with their respective secondary antibodies at room temperature for 1 h. The immunocomplexes were visualized using the enhanced chemiluminescence method. The signal densities on the blots were digitally scanned for subsequent analysis with normalization to β-actin.

2.10. Statistical analysis

Experiments were repeated a minimum of three times. The data are expressed as the mean ± SEM and were assessed by either two-tailed Student's *t*-test or one-way analysis of variance (ANOVA), followed by post hoc Dunnett's test. Differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. PTE reduced pulmonary inflammation-induced melanoma metastasis

The inflammatory microenvironment fosters tumor growth and metastasis. PTE has both anti-inflammatory and anticancer activities. To determine whether PTE has an inhibitory effect on inflammation-associated tumor metastasis, a mouse model was established by combining LPS-induced pulmonary inflammation with a melanoma metastatic burden following i.v. administration (El Rayes et al., 2015). As shown in Fig. 1B and C, after B16 cell implantation for 4 weeks, macroscopically visible metastatic foci were observed on the lung surfaces in the model group, while PTE treatment obviously decreased the numbers of metastatic nodules. Concomitantly, the lung/body weight ratio was markedly increased in the model group but significantly decreased in the PTE-treated groups (Fig. 1D). Taken together, the results showed that PTE had an inhibitory effect on pulmonary inflammation-enhanced metastasis.

3.2. PTE inhibited LPS-induced inflammation in lungs of tumor-bearing mice

Lung inflammation induced by intranasal LPS administration was characterized by increased immune cell influx and proinflammatory cytokine production. In the LPS-induced pulmonary metastasis mouse model, pulmonary inflammation manifested as both pulmonary interstitial oedema and drastic recruitment of inflammatory cells in the peribronchial regions (Fig. 2A). However, these pathological features were significantly improved following treatment with PTE. In addition, PTE also significantly reduced the

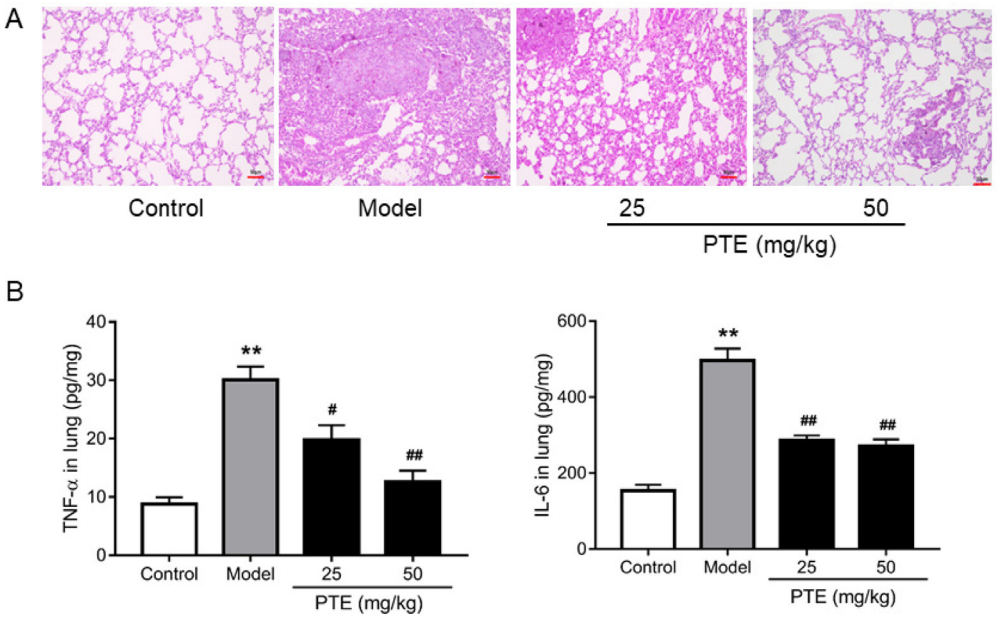

Fig. 2. PTE attenuated LPS-induced inflammation in the lungs of tumor-bearing mice. (A) Representative HE staining images of the lungs (×200, Scalbar = 50 μm). (B) Levels of TNF-α and IL-6 in the lungs (mean ± SEM, n = 6). $^{**}P < 0.01$ *vs* control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* model group.

levels of the proinflammatory cytokines TNF-α and IL-6 in the lung tissue (Fig. 2B). These results indicated that PTE could attenuate the inflammatory microenvironment, which consequently blocked the LPS-induced enhancement of lung metastasis of B16 cells introduced via the tail vein.

3.3. PTE prevented NE-mediated degradation of TSP-1 in lungs of tumor-bearing mice

Among the immune cells infiltrating the tumor microenvironment, neutrophils were recently speculated to modify tumor growth and invasiveness. The specific serine proteinase NE was reported to mediate inflammation-induced metastasis by degrading TSP-1 (El Rayes et al., 2015). Given that TSP-1 is a potent inhibitor of tumor growth and metastasis, we speculated that PTE might inhibit neutrophil recruitment, modulate NE activity and further protect TSP-1 from proteolysis, thus inhibiting metastasis. To evaluate neutrophil influx into the inflamed lungs of tumor-bearing mice, the expression and activity of NE were detected. As shown in Fig. 3A, NE was strongly expressed in lungs of tumor-bearing mice, while lung sections from the PTE treatment groups showed less NE deposition and lower NE activity (Fig. 3B). Furthermore, we monitored TSP-1 expression by immunoblotting. Consistent with the enhanced expression and activity of NE, inflamed lungs showed significant loss of TSP-1 expression, whereas this change was reversed in the PTE-treated groups (Fig. 3C). Taken together, these results indicated that PTE exerted antimetastatic effects by blocking excessive neutrophil influx and interfering with NE-associated degradation of TSP-1.

3.4. PTE inhibited NE-induced B16 cell migration

Based on the results indicating that PTE has a significant ability to inhibit LPS-driven tumor metastasis by targeting the NE/TSP-1 axis *in vivo*, we further tested the direct effects of PTE on NE-induced migration of B16 cells *in vitro*. As illustrated in Fig. 4, the cytotoxicity of NE and PTE with or without NE was first evaluated in B16 cells. The MTT results showed that NE at concentrations lower than 20 nmol/L had no effects on cell viability (Fig. 4A). Thus, 10 nmol/L NE was selected as the intervention condition. Without NE, PTE at concentrations from 0.01 to 1 mmol/L showed no cytotoxic effects (Fig. 4B). In the presence of 10 nmol/L NE, PTE (0.1, 0.3, 1 mmol/L) also had no effects on cell viability (Fig. 4C). To further explore the effects of PTE with or without NE on cell migration, we conducted both wound healing and transwell assays (Fig. 5). In the wound healing assay, the healing of the simulated wound by B16 cells was significantly enhanced by the presence of 10 nmol/L NE; however, cotreatment with PTE (1 mmol/L) abolished the mobility of the cancer cells (Fig. 5A and B). In the transwell assay, an increasing number of B16 cells moved from the top chamber to the bottom chamber after exposure to NE, while treatment with PTE also abolished this invasive activity (Fig. 5A and C). These results indicated that PTE has a substantial inhibitory effect on NE-induced B16 cell migration, which was consistent with the *in vivo* results.

3.5. PTE inhibits NE-induced regulation of EMT and degradation of TSP-1

The migration activity of cancer cells is mainly dependent on EMT (Friedl & Wolf, 2003). To determine whether PTE regulates the NE-stimulated EMT process, the expression of EMT-related molecules was evaluated by Western blot. The results showed that the expression of vimentin and *N*-cadherin was significantly increased after NE exposure, whereas PTE treatment reversed these changes (Fig. 6). The level of E-cadherin was reduced upon NE stimulation, while PTE returned E-cadherin to normal levels. Additionally, TSP-1 expression was also detected. The results showed that TSP-1 expression was significantly decreased after treatment with NE, and this effect was ameliorated after PTE addition. Together, these results demonstrated that PTE strongly attenuated the NE-induced promotion of metastatic tumor activity by blocking the EMT process and TSP-1 degradation.

4. Discussion

Melanoma is a lethal malignant tumor, and its incidence has increased steadily worldwide (Schadendorf et al., 2018). This can-

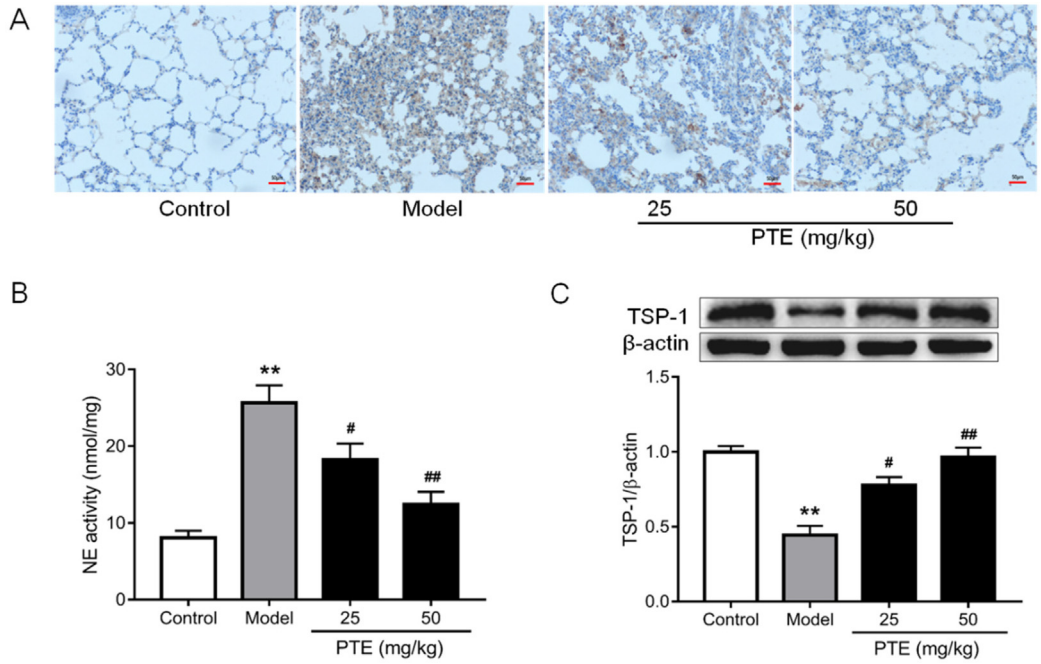

Fig. 3. PTE inhibited neutrophil infiltration and NE-mediated degradation of TSP-1 in lungs of tumor-bearing mice (mean ± SEM, n = 6). (A) Representative immunohistochemistry images of NE in lungs (×200, Scalbar = 50 μm); (B) NE activity; (C) Protein expression of TSP-1 in lungs. $^{**}P < 0.01$ *vs* control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* model group.

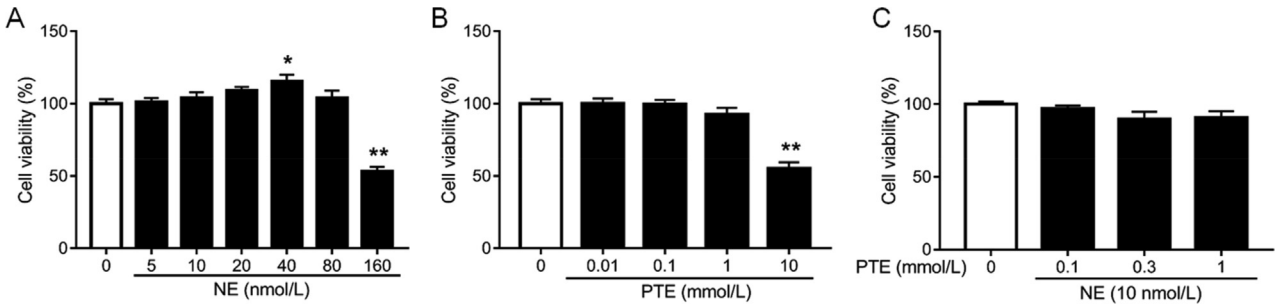

Fig. 4. Effects of NE and/or PTE on cell viability (mean ± SEM, n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* control group.

cer has a predilection for metastasis early in disease progression, which is one of the main reasons for treatment failure (Bedrosian et al., 2000). Inflammation is an important promoter of melanoma metastasis (Melnikova & Bar-Eli, 2009). Mediators involved in the inflammatory microenvironment of distant organs represent a target for therapeutic strategies against metastatic melanoma. PTE, a natural dimethylated analogue of resveratrol, was shown to exhibit anti-inflammatory and anticancer effects. However, the actions of PTE on inflammation-induced melanoma metastasis and the detailed mechanisms responsible for these activities are still unclear. Herein, using lung inflammation combined with an experimental tumor metastasis model, we found that PTE treatment obviously decreased the incidence of circulating melanoma cell metastasis to lungs with LPS-induced inflammation by reducing the expression of proinflammatory mediators, inhibiting inflammatory neutrophil infiltration and preventing NE-mediated degradation of TSP-1 in lung tissue. The *in vitro* assays further confirmed that B16 cell migration, TSP-1 degradation and the expression of EMT-related molecules were activated in the presence of NE and attenuated by PTE.

The lung is a frequent metastatic site for diverse extrapulmonary malignancies because it is susceptible to inflammatory injuries (Altorki et al., 2019; Budczies et al., 2015). Clinically, metastasis to the lung is common in melanoma patients, and the lung is often the first site of visceral metastasis (Damsky, Rosenbaum, & Bosenberg, 2010). Approximately-one-tenth of patients with melanoma will develop lung metastases at some point during their illness (Harpole, Johnson, Wolfe, George, & Seigler, 1992). The strong association between inflammation and melanoma lung metastasis has been explored previously (El Rayes et al., 2015; Yan, Cai, & Xu, 2013; Hyun et al., 2020). LPS-induced lung-specific inflammation promoted not only metastasis of orthotopic primary tumors but also the metastatic burden following tail vein injection (Yan, Cai, & Xu, 2013). The latter is well accepted and addresses how an inflammatory environment in distant organs affects tumor extravasation, seeding, colonization and growth at secondary tissue sites. Studies have shown that PTE could alleviate the LPS-induced lung inflammatory response and inhibit the metastatic growth of melanoma (Zhang et al., 2021; Ferrer et al., 2005). Here, we demonstrated the antimetastatic effect of PTE on inflammation-induced melanoma lung metastasis.

The LPS-induced lung inflammation model is not new. Intranasal LPS administration can create a local inflammatory environment in the lung by triggering proinflammatory cytokine

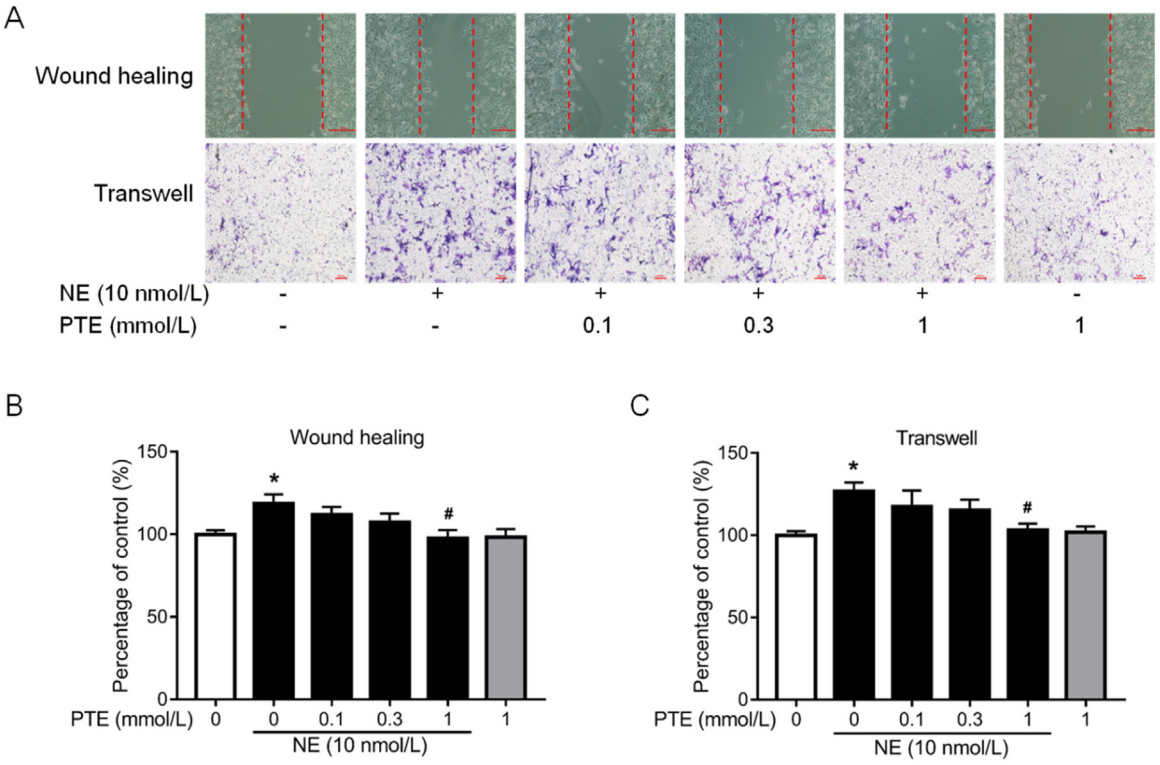

Fig. 5. PTE inhibited NE-induced B16 cell migration (mean ± SEM, $n = 3$) by wound healing (A: Scalbar = 500 μm, B) and transwell assays (A: Scalbar = 500 μm, C). $^{*}P < 0.05$ *vs* control group; $^{\#}P < 0.05$ *vs* NE-treated group.

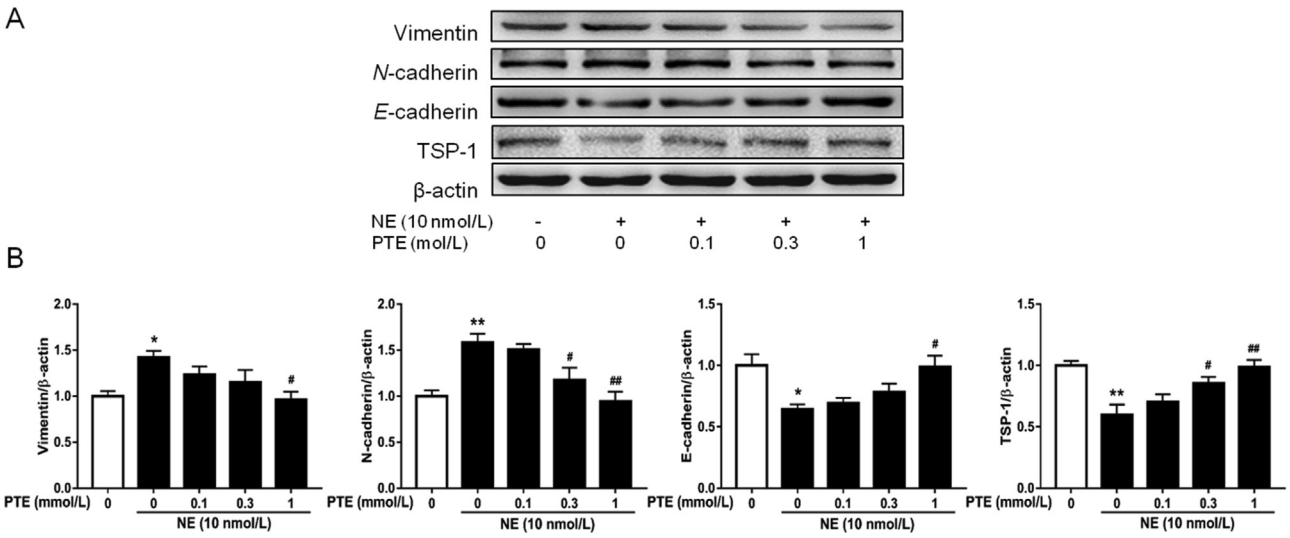

Fig. 6. Effects of PTE on NE-stimulated expression of EMT markers and TSP-1 (mean ± SEM, $n = 3$). (A) Representative Western blot images of vimentin, *N*-cadherin, E-cadherin and TSP-1 protein expression. (B) Quantitative analysis of the corresponding bands. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* NE-treated group.

production, such as TNF-α, IL-6, and IL-1β (El Rayes et al., 2015). These inflammatory mediators play an important role in the chemoattractant-directed migration, adhesion and accumulation of melanoma at the site of inflammation (Melnikova & Bar-Eli, 2009). Our results showed that during the progression from inflammation to melanoma metastatic growth in the lung, the levels of TNF-α and IL-6 were significantly increased, which is consistent with the results of previous studies. However, treatment with PTE obviously reduced proinflammatory mediator production, which might contribute to the inhibition of melanoma lung metastasis.

Altered cytokines in LPS-inflamed lungs play important roles not only in recruiting tumor cells but also in immune cells. Among these immune cells, the functions of neutrophils in cancer have been largely overlooked until recently (Coffelt, Wellenstein, & de Visser, 2016). Under normal physiological conditions, neutrophils are the "first responders" to participate in the inflammatory response against infection or tissue damage. However, under chronic inflammatory conditions, heterogeneous neutrophils have emerged as key contributors to cancer growth and metastasis (Altorki et al., 2019; Coffelt, Wellenstein, & de Visser, 2016; Liang & Ferrara, 2016). Accumulating evidence indicates that NE released

from activated neutrophils acts as a main stimulatory factor in tumor metastasis, since its genetic deletion or pharmacological inhibition markedly reduces tumor metastatic potential in some preclinical studies (Lerman & Hammes, 2018). Because high levels of NE protein and NE activity have been demonstrated almost exclusively in neutrophil influx into inflamed tissues, they were determined in the study. Our results confirmed that the enhanced metastasis was consistent with excessive neutrophil influx, activation and degranulation, whereas PTE effectively reversed these changes. Although PTE has been reported to possess antimetastatic potential against B16 cells *in vitro* (Ferrer et al., 2005), the role of PTE in NE-induced migration of B16 cells is still unclear. The direct induction of B16 migration by NE and the inhibition of NE-induced migration by PTE were also observed in wound healing and transwell assays. We found that NE treatment (10 nmol/L) enhanced the migration of B16 cells without promoting proliferation. However, PTE at concentrations without cytotoxicity effectively inhibited the NE-induced aggressive properties of B16 cells. These observations suggested that PTE could inhibit NE enzyme activity and therefore NE-induced metastasis.

As NE is the key neutrophil-derived protease that promotes tumor progression, NE-mediated ECM degradation and remodelling mainly contribute to tumor cell migration and invasion (Swierczak, Mouchemore, Hamilton, & Anderson, 2015). TSP-1, a secreted ECM protein in the tumor microenvironment, is critical for cancer growth and spreading (Stenina-Adognravi, Muppala, & Gajeton, 2018). The contribution of TSP-1 to lung metastasis suppression has been demonstrated, while NE-induced TSP-1 proteolysis abolished the antimetastatic effect of TSP-1 (El Rayes et al., 2015; Catena et al., 2013). In our study, decreased expression of TSP-1 was observed both in lungs with LPS-induced inflammation and in NE-stimulated B16 cells, while PTE protected TSP-1 from degradation by NE, consistent with the inhibition of metastasis and migration. In addition, NE is also known to trigger EMT in tumor cells. Grosse-Steffen *et al* demonstrated that NE can contribute to EMT by degrading E-cadherin and enhancing vimentin in pancreatic cancer cells (Grosse-Steffen et al., 2012). In ovarian cancer cells, NE also altered tumor cells towards a mesenchymal and migratory phenotype by downregulating the expression of E-cadherin (Mayer et al., 2016) In the present study, mesenchymal markers (vimentin and *N*-cadherin) were increased, while epithelial markers (E-cadherin) were decreased after NE treatment, whereas PTE reversed these changes to suppress EMT. These data indicated that the antimetastatic effect of PTE was related to the blockage of NE-mediated TSP-1 degradation and EMT.

5. Conclusion

In summary, the present study demonstrated that PTE could effectively suppress pulmonary inflammation-promoted melanoma metastasis and block NE-induced B16 cells migration. The underlying mechanism may be related to limiting neutrophil infiltration, inhibiting pulmonary inflammation response, and modulating NE release and NE-mediated TSP-1 degradation as well as EMT process. These results provide a novel antimetastatic mechanism of PTE, which might extend its antitumoral application. However, detailed molecules mechanisms that regulate NE/TSP-1 pathway by PTE still warrant further investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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