STEMedicine

RESEARCH ARTICLE

Pinocembrin inhibits migration and invasion of nonsmall cell lung cancer cells by inhibiting STAT3 signaling

Jing Zhang, Zhiqiang Xue, Bin Wang, Jiaxin Wen and Yunxi Wang*

Department of Thoracic Surgery, The First Medical Center of Chinese PLA General Hospital, Beijing, China

Abstract

***Background*:** In cancer treatment, targeting the activation of signal transducer and activator of transcription 3 (STAT3) is crucial as it promotes tumor progression and metastasis through the process of epithelial-mesenchymal transition (EMT). Pinocembrin, a dihydroxyflavanone found naturally in propolis and honey, is known for its antioxidant and vasodilating properties. The objective of this study is to explore the potential of pinocembrin in regulating the STAT3 signaling pathway to inhibit migration and invasion of nonsmall cell lung cancer (NSCLC) cells.

***Methods*:** Hematoxylin staining was used to determine the migration and invasion of A549 cells. The relative expression of EMT-related proteins and invasive proteins in A549 cells was determined by Western blot analysis. STAT3 activity was evaluated using a luciferase assay. Overexpression of STAT3 was utilized to assess the role of pinocembrin in regulating STAT3.

***Results*:** Pinocembrin treatment (50 μM) significantly reduced the number of migrating and invasive cells. Pinocembrin upregulated the protein level of E-cadherin and downregulated the protein levels of N-cadherin and vimentin. Additionally, pinocembrin blocked the phosphorylation and activation of STAT3. Overexpression of STAT3 reversed the inhibitory effects of pinocembrin on cell migration and invasion.

***Conclusion*:** Our study demonstrates that pinocembrin can inhibit the activation of STAT3, which is associated with EMT and thereby attenuate migration and invasion in NSCLC cells.

Keywords: *non-small lung cancer cells*; *STAT3*; *migration*; *invasion*; *EMT*

Received: 7 March 2023; Revised: 16 March 2023; Accepted: 18 March 2023; Published: 24 April 2023

Lung cancer is a leading cause of mortality worldwide (1), with nonsmall cell lung cancer (NSCLC) accounting for approximately 80–85% of cases (2). Unfortunately, about 80% of patients with NSCLC progress to stage IV disease, which carries a significant risk of mortality (3). Despite this, the pathophysiological mechanisms of NSCLC are not fully understood, and there are no effective pharmacological treatments, resulting in prolonged hospital stays and financial burden. While chemotherapy remains a primary management strategy for patients with lung cancer, it carries unavoidable toxicity and detrimental side effects on normal and healthy tissues (4). Therefore, there is an urgent need for an effective and safe targeted therapy to manage this preventable disease. To develop such therapy, a better understanding of the molecular mechanisms and pathophysiological processes of NSCLC is required.

Signal transducer and activator of transcription 3 (STAT3) has been shown to play a critical role in several types of cancer, including brain and intestinal cancer (5–7). In lung cancer, STAT3 activation strongly promotes cancer cell progression and metastasis by transcriptionally regulating genes associated with cell proliferation, angiogenesis, and resistance to apoptosis (8, 9). Studies have found higher expression levels of STAT3 and phosphorylated STAT3 (pSTAT3) in tumors from patients with NSCLC compared to normal patients, with the pSTAT3 expression level being correlated with the survival rate of patients (10). Moreover, studies have demonstrated that inhibitors of c-Scr and JAK could reactivate STAT3 in both NSCLC cell lines and human tumors, leading to the unwanted survival of cancer cells (11, 12). Taken together, these findings suggest that phosphorylation of STAT3 negatively regulates NSCLC proliferation and

 Citation: STEMedicine 2023, 4(2): e173 - http://dx.doi.org/10.37175/stemedicine.v4i2.173

progression, and therefore STAT3 could be considered as a targeted marker for controlling tumor progression and metastasis in NSCLC.

Epithelial-mesenchymal transition (EMT), which is characterized by the transformation of epithelial cells into mesenchymal stem cells leading to the loss of cell polarity and adhesion, ultimately results in cell migration and invasion. E-cadherin is highly expressed in epithelial cells and serves as an EMT marker, whereas N-cadherin and vimentin are highly expressed in mesenchymal stem cells (13, 14). It has been reported that STAT3 activation, along with transcription factors, regulates tumorigenesis that is specifically related to EMT, thereby promoting cell proliferation, metastasis, and invasion (15). Conversely, the inhibition of STAT3 by treating with anti-sense oligonucleotides leads to a reduction in tumor transmission, lung metastasis, and suppression of MMP2 and MMP9, which are well-documented invasive proteins. Although the role of STAT3 in regulating EMT-mediated cell migration and invasion is well documented, the development of a therapeutic approach targeting STAT3 to manage NSCLC remains unaccomplished.

Methods

Cell culture

Human lung adenocarcinoma cells (A549 cells, TCHu150, Cell Bank of the Chinese Academy of Sciences, Shanghai, China) and DDP-resistant cells (A549/DDP cells, JL10093, Cell Bank of the Chinese Academy of Sciences) were grown in RPMI-1640 medium (Cyclone, GE Healthcare, UT, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone) and Penicillin/Streptomycin (1:100, Sigma Aldrich, St. Louis, MO, USA) in a humidified atmosphere of 5% CO2 at 37°C. Pinocembrin (S3941, Selleck, Shanghai, China) and TGF-β1 (10804-H08H, Sino Biological, Shanghai, China) were dissolved in dimethyl sulfoxide (DMSO) and RPMI-1640, respectively, and then stored at −80°C.

Western blot analysis

Antibodies used in Western blot analyses included E-Cadherin Rabbit mAb (#3195), N-Cadherin Rabbit mAb (#13116), Vimentin Rabbit mAb (#5741), Stat3 Rabbit mAb (#9139), p-Stat3 Rabbit mAb (#9145), MMP9 Rabbit mAb (#13667), MMP2 Rabbit mAb (#40994), and β-actin mouse mAb (#60008-1-Ig) from Cell Signaling (Shanghai, China) and Proteintech (Beijing, China). Total protein was extracted from A549 and A549/DDP cells, which were treated with or without pinocembrin.

Cell migration and invasion analysis

To assess cell migration and invasion, 24-well Transwell chambers with 8-μm pore size inserts (Costar, Washington, DC, USA) were used. For invasion assays, inserts were coated with 100 μL of a 1:9 dilution of matrigel (BD Biosciences). After 24 h of transfection, 5 × 104 cells were collected, resuspended in 100 μL of medium supplemented with 2% FBS, and added to the upper Transwell chamber. The lower chamber was filled with 600 μL of medium supplemented with 10% FBS. The cells were incubated for 24 h, after which those on the upper membrane were removed with a cotton tip. The cells that had migrated or invaded through the membrane were fixed with polyformaldehyde and stained with hematoxylin. The number of migrated/invaded cells was counted in 10 randomly selected fields under a microscope. The experiments were independently repeated three times.

Luciferase reporter assay

To investigate the effect of STAT3 3′-UTR sequence on gene expression, wild-type (WT-3′-UTR STAT3) (NM_003150) (cat. no. SC218770) and its mutated sequence (MUT-3′-UTR STAT3) (cat. no. CW304411) were cloned downstream of the Renilla luciferase gene in the pMirTarget vector (Origene Technologies, Wuxi, China). Co-transfection of the reporter plasmids or negative control with HEK293T cells was performed using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA). Luciferase activity was measured 48 h after incubation using the Dual-Luciferase Reporter Assay System (cat. no. E1910; Promega, Madison, WI, USA) and GloMax 20/20 luminometer (cat. no. E5311; Promega). HEK293 cells luciferase activity was normalized to Renilla luciferase activity as per the manufacturer's instructions. All experiments were repeated at least three times.

Plasmids and stable cell lines

The full-length STAT3 plasmid, pcDNA3-STAT3, was confirmed via DNA sequencing. To optimize transfection efficiency, pEGFP-C1 (Clontec, Mountain View, CA, USA) was used. Cells were transfected with 2.5 μg/well of plasmids or the control vector pcDNA3 (Thermo Fisher) using Lipofectamine LTX Reagent (10 μL/well) and Plus Reagent (2.5 μL/well) (Thermo Fisher). Following a 6-h incubation in serum- and antibiotic-free medium, the medium was replaced with RPMI 1640 supplemented with 10% FBS. The cells were then cultured for 48 h and subjected to a 2-week selection in medium containing G418 (600 μg/mL).

Statistical analysis

The significant difference was analyzed using a two-tailed *t*-test or one-way ANOVA with Tukey's multiple-comparisons test in GraphPad Prism 7 as described in the figure legends. The data represent the mean ± SEM.

Results

Pinocembrin inhibited A549 cells migration and invasion

To investigate the impact of pinocembrin on the migration and invasion of A549 cells, the cells were exposed to various concentrations of pinocembrin (10, 20, 50, and 100 μM) for a period of 24 h. Treatment with 10 μM pinocembrin did not lead to a significant reduction in cell migration and invasion. However, when treated with 20 μM pinocembrin, the number of migration and invasion cells was significantly reduced compared to the vehicle group ($P < 0.0001$ and $P < 0.05$, respectively), and this effect was further amplified with increasing doses of 50 and 100 μM ($P < 0.0001$ for both) (Fig. 1A and B). There was no significant difference between treatment with 50 and 100 μM pinocembrin. Thus, a concentration of 50 μM pinocembrin was chosen for further analysis.

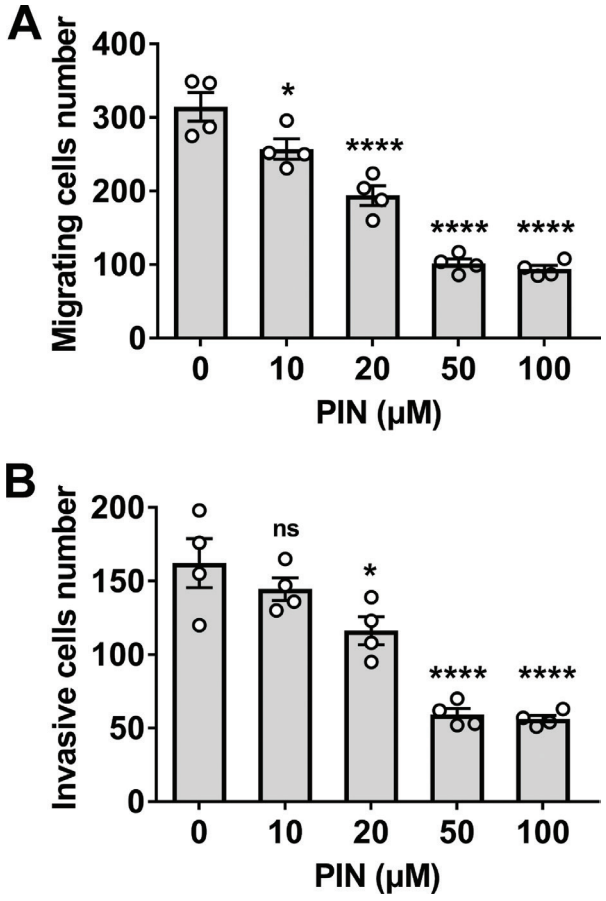

Fig. 1. Effect of pinocembrin on the migration and invasion of A549 cells. A549 cells were treated with pinocembrin (PIN) at the indicated concentration. Cell migration (A) and invasion (B) were analyzed. Migrating and invasive cells were stained with hematoxylin and counted. Four wells per group. The significant difference vs control group was analyzed using one-way ANOVA test. Data represents Mean ± SEM. *$P < 0.05$, ****$P < 0.0001$, ns, not significant.

Pinocembrin altered the expression of EMT-related proteins in A549 cells

In order to determine the effect of pinocembrin on the EMT marks, we evaluated E-Cadherin, N-Cadherin, Vimentin, matrix metalloproteinase-2 (MMP2), and matrix metalloproteinase-9 (MMP9) by western blot. Results showed that treatment with 50 mM pinocembrin significantly increased protein levels of E-Cadherin ($P < 0.001$, Fig. 2A and B) when compared to vehicle treatment. In contrast, the protein levels of N-Cadherin and Vimentin were notably inhibited by the treatment with pinocembrin ($P < 0.0001$, $P < 0.0001$, respectively, Fig. 2A and B). Correspondingly, we found that pinocembrin significantly reduced the protein levels of MMP2 and MMP9 at a dose of 50 μM when compared to the vehicle group ($P < 0.0001$, $P < 0.001$, respectively, Fig. 2A and C). Taken together, our results indicated that pinocembrin reduced A549 proliferation, invasion, and migration.

Pinocembrin inhibited TGF-β1-induced EMT in A549 cells

In order to evaluate the role of pinocembrin in regulating TGF-β1-induced alteration of EMT proteins, A549 cells were pretreated with TGF-β1 along with or without pinocembrin for 24 h. Protein levels of EMT markers were determined by western blot. E-Cadherin was significantly reduced after treatment with TGF-β1. Notably, pinocembrin restored the E-Cadherin protein level to that of the control (vehicle) ($P < 0.0001$, Fig. 3A and B). Corresponding with the E-Cadherin results, our findings showed that N-Cadherin and vimentin were remarkably elevated after treatment with TGF-β1, which were significantly reduced by pinocembrin ($P < 0.0001$, respectively, Fig. 3A, C, and D). These results confirmed that TGF-β1 induces alteration of EMT proteins. More importantly, these findings indicated that pinocembrin significantly inhibited TGF-β1-induced EMT markers, thereby inhibiting cell migration and invasion.

Pinocembrin inhibited the activation of STAT3

The activation of STAT3 has been recognized as a main contributor to lung cancer (16). To further evaluate whether pinocembrin inhibits cell migration and invasion through regulating STAT3, we measured STAT3 activity and protein expressions in A549 cells. We found that the protein expression level of pSTAT3 was significantly reduced after 6 h of treatment with pinocembrin at a dose of 50 μM and maintained until 24 h ($P < 0.0001$, Fig. 4A and B). Furthermore, to evaluate the effect of pinocembrin in activating STAT3 at different doses, A549 cells were treated with 20 or 50 μM of pinocembrin. The results showed that pSTAT3 was remarkably reduced when treated with 20 μM pinocembrin compared to

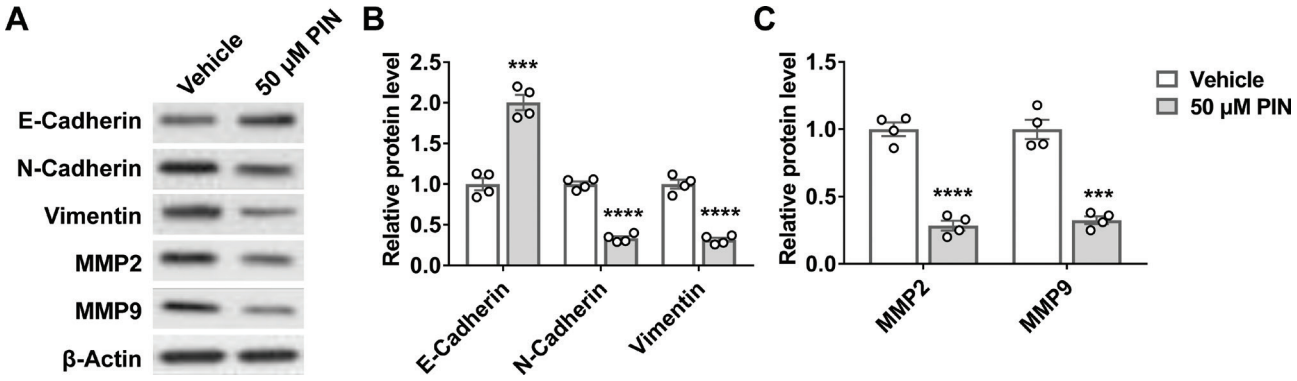

Fig. 2. Pinocembrin altered the expression of epithelial-mesenchymal transition related proteins in A549 cells. A549 cells were treated with 50 μM pinocembrin (PIN) for 24 h, and the protein levels of epithelial-mesenchymal transition proteins were determined by western blot. Four wells per group. The significant difference vs control group was analyzed using two-tailed *t*-test. ***$P < 0.001$, ****$P < 0.0001$.

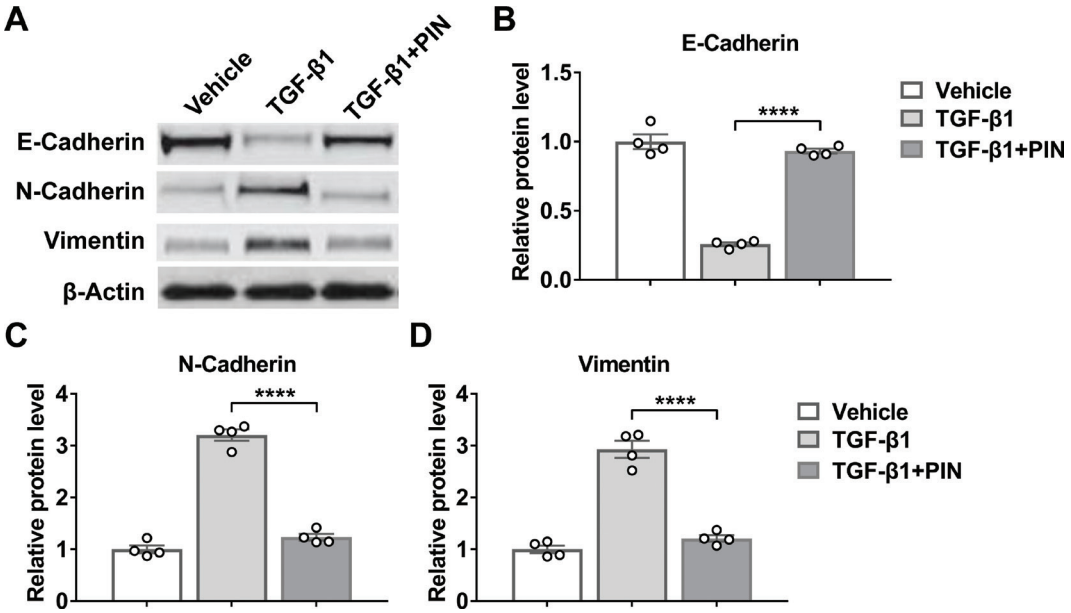

Fig. 3. Pinocembrin inhibited TGF-β1-induced EMT in A549 cells. A549 cells were treated with 10 ng/ml TGF-β1 and with or without 50 μM pinocembrin (PIN) for 24 h, and the protein levels of E-Cadherin (A and B), N-Cadherin (A and C), and Vimentin (A and D) were determined by western blot. Four wells per group. The significant difference was analyzed using one-way ANOVA test. ****$P < 0.0001$.

the vehicle ($P < 0.05$), and a greater decrease was observed when treated with 50 μM pinocembrin ($P < 0.0001$, Fig. 4C and D). In parallel, the activity of pSTAT3 was significantly inhibited by pinocembrin at a dose of 20 μM when compared to the vehicle ($P < 0.001$) and was further reduced by the treatment with pinocembrin at a dose of 50 μM ($P < 0.0001$, Fig. 4E). Taken together, these findings suggest that pinocembrin inhibits cell migration and invasion potentially by inhibiting the phosphorylation and activation of STAT3.

Overexpression of STAT3 reversed the effect of pinocembrin on A549 cells

To further confirm the regulatory role of pinocembrin in STAT3 activation, EMT proteins were determined in A549 cells overexpressed with STAT3. Overexpression of STAT3 significantly reversed the number of migrating and invasive cells to that of the control ($P < 0.0001$, respectively, Fig. 5A and B). EMT markers, N-Cadherin, Vimentin, and MMP9 were strikingly upregulated in cells overexpressed with STAT3 compared with transfection

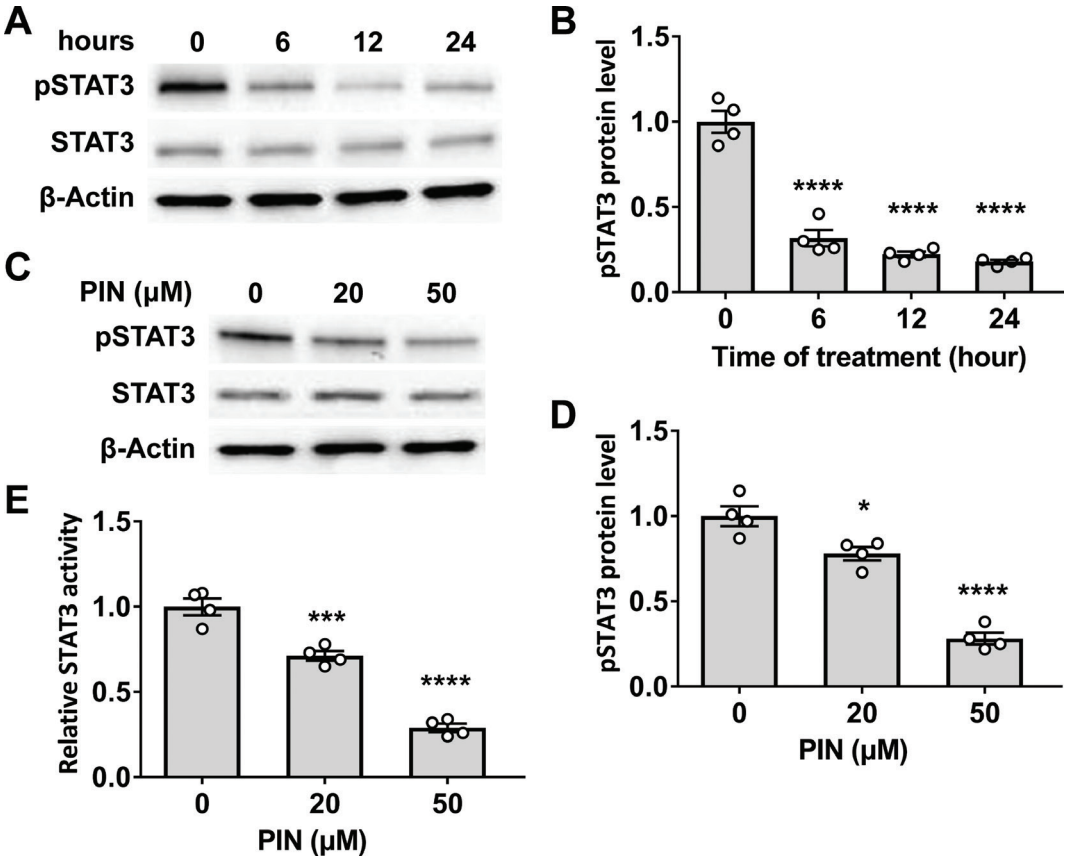

Fig. 4. Pinocembrin inhibited the activation of STAT3. (A–B) A549 cells were treated with 50 μM pinocembrin (PIN) for the indicated time, and the protein levels of pSTAT3 and STAT3 were determined by western blot. (C–E) A549 cells were treated with pinocembrin (PIN) at the indicated concentration for 12 h, and the protein levels of pSTAT3 and STAT3 were determined by western blot (C and D), and the activity of pSTAT3 was analyzed by luciferase assay (E). Four wells per group. The significant difference was analyzed using one-way ANOVA test. *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$.

with vector ($P < 0.0001$, respectively, Fig. 5C–F). These results confirmed that pinocembrin reduced cell migration and invasion, which may be through inhibiting the activation of STAT3 signaling.

Discussion

NSCLC contributes to 80–85% of lung cancer and is associated with a high risk of mortality (1). The development of an effective and safe reagent for the treatment of NSCLC is required to improve NSCLC outcomes. In this study, we demonstrated that pinocembrin, a dihydroxyflavanone found in propolis and honey, attenuated NSCLC cell migration and invasion by inhibiting the activation of STAT3 signaling.

Pinocembrin has been reported to reduce the migration and invasion of ovarian and colorectal cancer cells (17, 18). In this study, we revealed that pinocembrin inhibited the migration and invasion of A549 cells at a dose of 20 μM. This inhibitory effect was significant at doses of 50 and 100 μM. Several studies have reported that EMT markers, including E-cadherin, N-cadherin, and vimentin, are associated with cell adhesion and proliferation in NSCLC (13). Accordingly, the protein level of E-cadherin was significantly upregulated, whereas the protein levels of N-cadherin and vimentin were downregulated by pinocembrin. These results suggest that pinocembrin significantly reduced A549 cell adhesion and proliferation, thereby inhibiting EMT-mediated cell migration and invasion. MMP2 and MMP9 are known to play a role in promoting tumor metastasis and are highly expressed in NSCLC (19, 20). We found that pinocembrin significantly reduced the elevated levels of MMP2 and MMP9 in A549 cells, indicating an inhibitory effect of pinocembrin on NSCLC metastasis and invasion. E-cadherin, N-cadherin, vimentin, MMP2, and MMP9 are recognized as key factors in predicting the clinical outcome of patients with NSCLC (21, 22). Taken together, our results suggest that pinocembrin could potentially serve as a therapeutic strategy for the treatment of NSCLC in clinical settings.

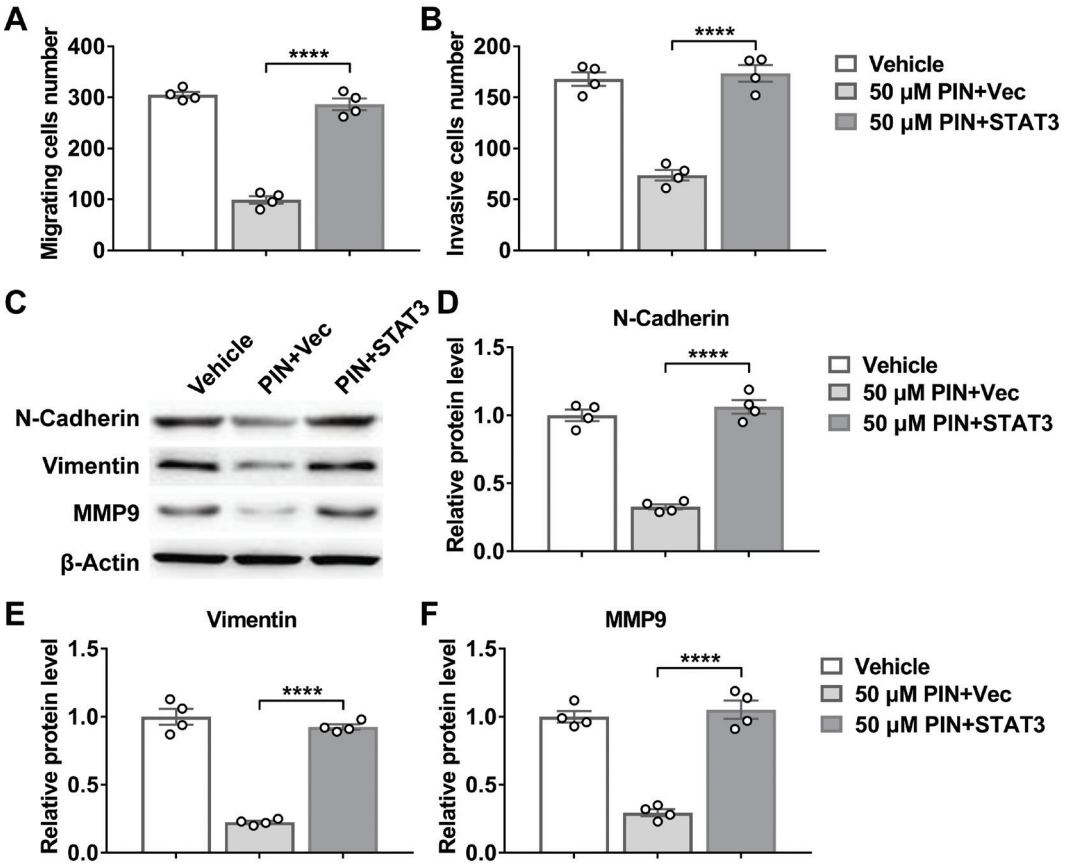

Fig. 5. Overexpression of STAT3 reversed the effect of PIN on A549 cells. A549 cells were transfected with vector or plasmid coding STAT3 and treated with 50 μM pinocembrin (PIN). Cell migration (A) and invasion (B) were analyzed. And the protein levels of N-Cadherin (C and D), Vimentin (C and E) and MMP9 (C and F) were determined by western blot. Four wells per group. The significant difference was analyzed using one-way ANOVA test. ****$P < 0.0001$.

EMT is a reversible process of loss of cell adhesion, resulting in cell migration and invasion. TGF-β1-induced EMT is characterized by the downregulation of E-cadherin and upregulation of N-cadherin and vimentin (23, 24). In agreement with published work, our results showed that pinocembrin remarkably reversed the alteration of protein expressions caused by TGF-β1 treatment in A549 cells.

STAT3 plays a pivotal role in EMT transformation and tumor metastasis. A few studies have shown that STAT3 is continuously activated in tumor cells, including breast cancer and prostate cancer (25– 27). In parallel, we found that STAT3 was highly phosphorylated in A549 cells without treatment. In contrast, the protein level of pSTAT3 was significantly reduced by treatment with pinocembrin. Consistently, the activity of STAT3 showed a striking decrease in the pinocembrin group in A549 cells. To link STAT3 activation to EMT, A549 cells were overexpressed with STAT3. Consistent with previous results, we found that the protein expressions of EMT-related proteins, including N-cadherin, Vimentin, and MMP9, were significantly reduced in A549 cells treated with pinocembrin. Notably, overexpression of STAT3 reversed the protective effect of pinocembrin, and the protein levels of N-cadherin, Vimentin, and MMP9 returned to the level of control. Studies have demonstrated that constant STAT3 activation induces the process of EMT, which could occur through STAT3 nuclear translocation and upregulation of Twist-1 in breast cancer (27). A recent study showed that a traditional Chinese medicine, Fuzheng Kang-Ai, effectively inhibits lung cancer cell metastasis by blocking the STAT3/MMP9 pathway (28). Xie et al. showed that STAT3 upregulates the transcription of MMP2 by directly binding to the MMP2 promoter in melanoma cells (29). Blocking STAT3 activation inhibits melanoma cell invasion and metastasis, highlighting STAT3 as a novel target to manage tumor invasion and metastasis. Here, our findings showed a clear correlation between the activation of STAT3 and EMT-related proteins, indicating that targeting STAT3 could be an effective approach for inhibiting tumor migration and invasion, especially in NSCLC. Our results provide

evidence that pinocembrin inhibits NSCLC migration and invasion mechanistically by inhibiting the phosphorylation and activation of STAT3, which promotes EMT transformation in NSCLC.

The excessive activation of STAT3 contributing to tumor migration, metastasis, and invasion is well recognized as a novel approach for the management of NSCLC progression and metastasis. The excessive activation of STAT3, contributing to tumor migration, metastasis, and invasion, is well recognized as a novel approach for the management of NSCLC progression and metastasis. Dephosphorylating STAT3 or blocking its activation has shown to exhibit an antitumor effect in lung cancer in vivo and in vitro. The mechanisms of STAT3 activation-induced EMT program are complicated and still need further research. Pinocembrin is a flavonoid compound naturally found in propolis, honey, flowers, red wine, and fruits. Pinocembrin has been approved by the China Food and Drug Administration (CFDA) for the treatment of cerebral ischemia and is undergoing phase II clinical trials. Studies have shown that pinocembrin, as a strong antioxidant, has properties including antiapoptosis, anti-inflammation, and vasodilation. A recent study showed that pinocembrin effectively downregulates invasive protein MMP2 and EMT marker N-cadherin in colorectal carcinoma by upregulating LACTB (18). Moreover, pinocembrin has been reported to inhibit the proliferation and migration of ovarian carcinoma by suppressing the mRNA expressions of N-cadherin and GABAB (17). However, the role of pinocembrin in lung carcinoma remains unclear. In the present study, we demonstrated that pinocembrin inhibited NSCLC migration and invasion by blocking the phosphorylation of STAT3, thereby downregulating invasive proteins and EMT-related proteins.

Conclusion

Pinocembrin inhibits lung cancer cell migration and invasion by upregulating E-cadherin and downregulating EMT markers, N-cadherin and vimentin, and invasive proteins such as MMP2 and MMP9. Our results show that pinocembrin inhibits the activation of STAT3 in the lung cancer cells, thereby exhibiting antitumor effect.

Acknowledgments

None.

Conflict of interest and funding

The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

Data availability statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71(3): 209–49. doi: 10.3322/caac.21660
2. Islami F, Torre LA, Jemal A. Global trends of lung cancer mortality and smoking prevalence. Transl Lung Cancer Res 2015; 4(4): 327–38.
3. Viale PH. The American Cancer Society's facts & figures: 2020 edition. J Adv Pract Oncol 2020; 11(2): 135–6. doi: 10.6004/jadpro.2020.11.2.1
4. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346(2): 92–8. doi: 10.1056/NEJMoa011954
5. de la Iglesia N, Konopka G, Puram SV, Chan JA, Bachoo RM, You MJ, et al. Identification of a PTEN-regulated STAT3 brain tumor suppressor pathway. Genes Dev 2008; 22(4): 449–62. doi: 10.1101/gad.1606508
6. Lee J, Kim JC, Lee SE, Quinley C, Kim H, Herdman S, et al. Signal transducer and activator of transcription 3 (STAT3) protein suppresses adenoma-to-carcinoma transition in Apcmin/+ mice via regulation of Snail-1 (SNAI) protein stability. J Biol Chem 2012; 287(22): 18182–9. doi: 10.1074/jbc.M111.328831
7. Musteanu M, Blaas L, Mair M, Schlederer M, Bilban M, Tauber S, et al. Stat3 is a negative regulator of intestinal tumor progression in Apc(Min) mice. Gastroenterology 2010; 138(3): 1003–11.e1–5. doi: 10.1053/j.gastro.2009.11.049
8. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, et al. Stat3 as an oncogene. Cell 1999; 98(3): 295–303. doi: 10.1016/S0092-8674(00)81959-5
9. Haura EB, Zheng Z, Song L, Cantor A, Bepler G. Activated epidermal growth factor receptor-Stat-3 signaling promotes tumor survival in vivo in non-small cell lung cancer. Clin Cancer Res 2005; 11(23): 8288–94. doi: 10.1158/1078-0432.CCR-05-0827
10. Zhao X, Sun X, Li XL. Expression and clinical significance of STAT3, P-STAT3, and VEGF-C in small cell lung cancer. Asian Pac J Cancer Prev 2012; 13(6): 2873–7. doi: 10.7314/APJCP.2012.13.6.2873
11. Byers LA, Sen B, Saigal B, Diao L, Wang J, Nanjundan M, et al. Reciprocal regulation of c-Src and STAT3 in non-small cell lung cancer. Clin Cancer Res 2009; 15(22): 6852–61. doi: 10.1158/1078-0432.CCR-09-0767
12. Tong M, Wang J, Jiang N, Pan H, Li D. Correlation between p-STAT3 overexpression and prognosis in lung cancer: a systematic review and meta-analysis. PLoS One 2017; 12(8): e0182282. doi: 10.1371/journal.pone.0182282
13. Tsoukalas N, Aravantinou-Fatorou E, Tolia M, Giaginis C, Galanopoulos M, Kiakou M, et al. Epithelial-mesenchymal transition in non small-cell lung cancer. Anticancer Res 2017; 37(4): 1773–8. doi: 10.21873/anticanres.11510
14. Chen X, Guan X, Zhang H, Xie X, Wang H, Long J, et al. DAL-1 attenuates epithelial-to mesenchymal transition in lung cancer. J Exp Clin Cancer Res 2015; 34(1): 3. doi: 10.1186/s13046-014-0117-2
15. Zhang X, Liu G, Kang Y, Dong Z, Qian Q, Ma X. N-cadherin expression is associated with acquisition of EMT phenotype and with enhanced invasion in erlotinib-resistant lung cancer cell lines. PLoS One 2013; 8(3): e57692. doi: 10.1371/journal.pone.0057692

16. Dutta P, Sabri N, Li J, Li WX. Role of STAT3 in lung cancer. JAKSTAT 2014; 3(4): e999503. doi: 10.1080/21623996.2014.999503
17. Gao J, Lin S, Gao Y, Zou X, Zhu J, Chen M, et al. Pinocembrin inhibits the proliferation and migration and promotes the apoptosis of ovarian cancer cells through down-regulating the mRNA levels of N-cadherin and GABAB receptor. Biomed Pharmacother 2019; 120: 109505. doi: 10.1016/j.biopha.2019.109505
18. Jiang L, Yang Y, Feng H, Zhou Q, Liu Y. Pinocembrin inhibits the proliferation, migration, invasiveness, and epithelial-mesenchymal transition of colorectal cancer cells by regulating LACTB. Cancer Biother Radiopharm 2022; 37(7): 527–36. doi: 10.1089/cbr.2020.4052
19. Pinto CA, Carvalho PE, Antonangelo L, Garippo A, Da Silva AG, Soares F, et al. Morphometric evaluation of tumor matrix metalloproteinase 9 predicts survival after surgical resection of adenocarcinoma of the lung. Clin Cancer Res 2003; 9(8): 3098–104.
20. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002; 295(5564): 2387–92. doi: 10.1126/science.1067100
21. Iniesta P, Moran A, De Juan C, Gomez A, Hernando F, Garcia-Aranda C, et al. Biological and clinical significance of MMP-2, MMP-9, TIMP-1 and TIMP-2 in non-small cell lung cancer. Oncol Rep 2007; 17(1): 217–23. doi: 10.3892/or.17.1.217
22. Sohal SS, Mahmood MQ, Walters EH. Clinical significance of epithelial mesenchymal transition (EMT) in chronic obstructive pulmonary disease (COPD): potential target for prevention of airway fibrosis and lung cancer. Clin Transl Med 2014; 3(1): 33. doi: 10.1186/s40169-014-0033-2
23. Liu RY, Zeng Y, Lei Z, Wang L, Yang H, Liu Z, et al. JAK/STAT3 signaling is required for TGF-beta-induced epithelial-mesenchymal transition in lung cancer cells. Int J Oncol 2014; 44(5): 1643–51. doi: 10.3892/ijo.2014.2310
24. Baek SH, Ko JH, Lee JH, Kim C, Lee H, Nam D, et al. Ginkgolic acid inhibits invasion and migration and TGF-beta-induced EMT of lung cancer cells through PI3K/Akt/mTOR inactivation. J Cell Physiol 2017; 232(2): 346–54. doi: 10.1002/jcp.25426
25. Li B, Huang C. Regulation of EMT by STAT3 in gastrointestinal cancer (Review). Int J Oncol 2017; 50(3): 753–67. doi: 10.3892/ijo.2017.3846
26. D'Amico S, Shi J, Martin BL, Crawford HC, Petrenko O, Reich NC. STAT3 is a master regulator of epithelial identity and KRAS-driven tumorigenesis. Genes Dev 2018; 32(17–18): 1175–87. doi: 10.1101/gad.311852.118
27. Kim MS, Lee HS, Kim YJ, Lee DY, Kang SG, Jin W. MEST induces twist-1-mediated EMT through STAT3 activation in breast cancers. Cell Death Differ 2019; 26(12): 2594–606. doi: 10.1038/s41418-019-0322-9
28. Li L, Wang S, Yang X, Long S, Xiao S, Wu W, et al. Traditional Chinese medicine, Fuzheng KangAi decoction, inhibits metastasis of lung cancer cells through the STAT3/MMP9 pathway. Mol Med Rep 2017; 16(3): 2461–8. doi: 10.3892/mmr.2017.6905
29. Xie TX, Wei D, Liu M, Gao AC, Ali-Osman F, Sawaya R, et al. Stat3 activation regulates the expression of matrix metalloproteinase-2 and tumor invasion and metastasis. Oncogene 2004; 23(20): 3550–60. doi: 10.1038/sj.onc.1207383

***Yunxi Wang**
Department of Thoracic Surgery
The First Medical Center of Chinese PLA General Hospital
28 Fuxing Road
100853 Beijing, China
Email: wangyx301@sina.com