Original Article
IL-17 induces EMT via Stat3 in lung adenocarcinoma

Qi Huang*, Jieli Han*, Jinshuo Fan*, Limin Duan, Mengfei Guo, Zhilei Lv, Guorong Hu, Lian Chen, Feng Wu, Xiaonan Tao, Juanjuan Xu, Yang Jin

*Department of Respiratory Medicine, Key Laboratory of Pulmonary Diseases of Health Ministry, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Avenue, Wuhan 430022, Hubei, China. *Equal contributors.*

Abstract: Epithelial-mesenchymal transition (EMT) plays a vital role in lung inflammatory diseases, including lung cancer. However, the role and mechanism of action of the proinflammatory cytokine IL-17 in EMT in lung adenocarcinoma remain unresolved. In our study, we discovered that the expression of N-cadherin, Vimentin, Snail1, Snail2, and Twist1 was positively correlated with IL-17 expression, while E-cadherin expression was negatively correlated with IL-17 expression in human lung adenocarcinoma tissues. Moreover, we confirmed that IL-17 promoted EMT in A549 and Lewis lung carcinoma (LLC) cells in vitro by upregulating N-cadherin, Vimentin, Snail1, Snail2, and Twist1 expression and downregulating E-cadherin expression. Stat3 was activated in IL-17-treated A549 and LLC cells, and Stat3 inhibition or siRNA knockdown notably reduced IL-17-induced EMT in A549 and LLC cells. Thus, IL-17 promotes EMT in lung adenocarcinoma via Stat3 signaling; these observations suggest that targeting IL-17 and EMT are potential novel therapeutic strategies for lung cancer.

Keywords: IL-17, Stat3, Snail1, Snail2, Twist1, lung adenocarcinoma

Introduction

Lung cancer is the leading cause of cancer death worldwide [1-3]. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancers, and adenocarcinoma is the most common subtype of NSCLC. Outcomes for lung cancer patients remain poor despite substantial advancements in treatment. Currently, the 5-year survival rate for lung cancer patients is approximately 15% [4]. This low survival rate is predominantly attributed to the metastatic tendencies and resistance to various treatments (radiotherapy, chemotherapy, targeted therapy) exhibited by lung cancer. Because lung cancer is often diagnosed at advanced stages and fails to respond to therapy [5], novel strategies that focus on minimizing lung cancer metastasis are urgently needed.

Tumor metastasis is a complex process in which tumor cells from the primary site spread to distant sites. Epithelial-mesenchymal transition (EMT) is a key step in local invasion and distant metastasis and plays an important role in the resistance of lung cancer to targeted treatment [6, 7]. Accumulating data have revealed that cytokines, chemokines, growth factors, and inflammatory mediators derived from both neoplastic and non-neoplastic cells within the tumor microenvironment communicate with oncogene signaling, such as Stat3 signaling, to promote EMT during tumor invasion and metastasis in the context of lung cancer [8, 9].

IL-17 is a newly identified pro-inflammatory cytokine that is primarily produced by activated CD4+ T helper cells (TH17 cells) [10]. Recently, IL-17-producing cells and IL-17/IL-17RA signaling have been widely investigated in the context of human solid tumors [11], including lung cancer [12-14]. Recent reports have indicated that IL-17 promotes EMT profiles in lung inflammatory diseases, such as obliterative bronchiolitis [15], severe asthma [16], pulmonary fibrosis [17], and COPD [18, 19], and stimulates EMT in multiple myeloma [20]. Additionally, studies

Table 1. Sequence of primers used for qRT-PCR amplification

Gene		
Human		
IL-17	CTACAACCGATCCACCTCACC	AGCCCACGGACACCAGTATC
E-CADHERIN	TTCTTCGGAGGAGAGCGG	CAATTTCATCGGGATTGGC
N-CADHERIN	AGAGGCAGAGACTTGCGAAAC	ACACTGGCAAACCTTCACGC
VIMENTIN	CAGGCAAAGCAGGAGTCCAC	TATTCACGAAGGTGACGAGCC
SNAIL 1	TGCCCTCAAGATGCACATCC	GCCGGACTCTTGGTGCTTG
SNAIL 2	AGACCCTGGTTGCTTCAAGG	CAGCCAGATTCCTCATGTTTGT
TWIST 1	GGAGTCCGCAGTCTTACGAG	TCTGGAGGACCTGGTAGAGG
GAPDH	GGTCGGAGTCAACGGATTTG	GGAAGATGGTGATGGGATTTC
Mouse		
E-cadherin	GGGACGGTCAACAACTGCAT	GCAGCTGGCTCAAATCAAAGT
N-cadherin	AATCAGACGGCTAGACGAGAGG	CGCTGCCCTCGTAGTCAAAG
Vimentin	TGGACGTTTCCAAGCCTGAC	CTGTCTCCGGTACTCGTTTGACT
Snail 1	CCTTGTGTCTGCACGACCTGT	CAGCCAGACTCTTGGTGCTTG
Snail 2	GTGCCCTGAAGATGCACATTC	AGATGTGCCCTCAGGTTTGATC
Twist 1	GTCGTACGAGGAGCTGCAGAC	AGCTTGCCATCTTGGAGTCC
Gapdh	TGAAGGGTGGAGCCAAAAG	AGTCTTCTGGGTGGCAGTGAT

have demonstrated that IL-17 facilitates lung cancer progression by, for example, promoting proliferation, angiogenesis [21-23], immune evasion, and metastasis [24, 25].

Resent discoveries have revealed complex interplay between Stat3 signaling and IL-17-producing cells. Stat3 favors the differentiation and expansion of TH17 cells to produce IL-17, which further triggers Stat3 activation to further promote tumor progression in inflammatory tumor microenvironments [11], such as in lung cancer [24, 26]. Although Stat3 is involved in IL-17-induced angiogenesis in many types of cancer, including hepatocellular carcinoma [27] and lung cancer [24], no data have been published on the role of Stat3 in IL-17-modulated EMT in cancers such as lung cancer.

In our study, we explored (i) the relationship between IL-17 and EMT profiles (EMT markers: E-cadherin, N-cadherin and Vimentin; EMT transcription factors (TFs): Snail1, Snail2, Twist1) in tumor tissues from lung adenocarcinoma patients; (ii) the effects of IL-17 on the wound healing and migration of lung adenocarcinoma cells as well as on EMT profiles in vitro; and (iii) the effects of Stat3 knockdown on IL-17-induced EMT profiles in A549 and LLC cells in vitro. These data improve the understanding of the communication between IL-17 and EMT and provide new insights for targeting both inflam-

mation and EMT in lung cancer treatment.

Materials and methods

Clinical samples

Tumor and corresponding peritumoral tissues were obtained from 35 lung adenocarcinoma patients as described previously [28].

Cell lines

The A549 human lung adenocarcinoma cell line (ATCC #CCL-185) and the Lewis lung carcinoma (LLC) cell line (ATCC #CRL-1642) were purchased from ATCC (Manassas, VA, USA) and cultured according to the provided guidelines. Recombinant human and mouse IL-17 were obtained from PeproTech (Rocky Hill, NJ). The Stat3 inhibitor (WP1066) was acquired from Santa Cruz Biotechnology.

Wound healing assay

A549 and LLC cells (3×10^5 cells/well) were seeded in six-well plates and incubated overnight. After cells had reached 80-90% confluence, the culture medium was removed, and a denuded area of constant width was created by mechanical scraping with a sterile 200-µl pipette tip. Cell debris was removed, and the monolayer was washed three times with serum-free medium. The cells were cultured for an additional 24 h, and wound closure was monitored and photographed under a microscope. Results are expressed as migration indices.

Cell migration assays

Migratory profiles were evaluated using Costar 24-well plates containing polycarbonate filter inserts with 8-mm pores. A549 and LLCs (3×10^5 cells/well) were added to the upper inserts and resuspended in serum-free medium; 500 µl of medium containing 10% FBS with or without IL-17 and/or WP1066 were added to the corresponding lower chambers. After 24 h, non-migrating cells were removed, and the
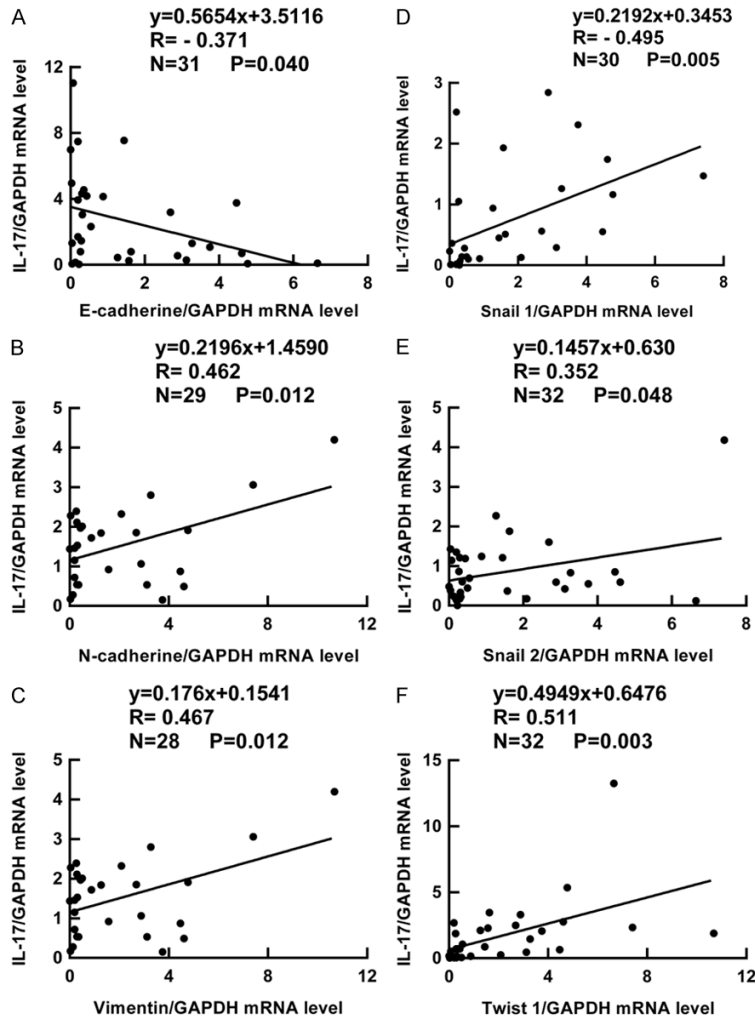
Figure 1. Correlation between IL-17 and EMT markers in human lung adenocarcinoma tissues. IL-17, E-cadherin, N-cadherin, Vimentin, Snail1, Snail2, and Twist1 expression was determined in 35 human lung adenocarcinoma tissues by qRT-PCR. (A-F) Pearson correlations analysis were used to compare the mRNA expression of IL-17 and E-cadherin (A), N-cadherin (B), Vimentin (C), Snail1 (D), Snail2 (E), or Twist1 (F) in lung adenocarcinoma tissues.

at a final concentration of 80 nm for 48 h according to the manufacturer's protocol.

Quantitative real-time PCR

A549 and LLC cells (3×10^5 cells/well) were seeded in 12-well plates and incubated overnight. For inhibitory assays, the cells were stimulated with human or mouse IL-17 (100 ng/ml) in the presence of the Stat3 inhibitor (30 µM) or medium for an additional 6 h. For RNA interference experiments, A549 and LLC cells (1×10^5 cells/well) were transfected with siRNA for 48 h and then treated with human or mouse IL-17 (100 ng/ml), respectively, for an additional 6 h. Then, total RNA was extracted. Reverse transcription and real-time quantitative PCR (qRT-PCR) were performed as previously described [26], and the mRNA levels of target genes were normalized to those of Gapdh. The primer sequences used for PCR are presented in **Table 1**.

Western blotting

A549 and LLC cells (1×10^6 cells/well) were seeded in six-well plates and incubated overnight. Cell lysates were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked in 5% BSA containing 0.05% Tween-20 and probed with the appropriate primary antibodies overnight at 4°C. The membranes were washed three times for 10 min each and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Membranes were then washed as above and visualized by enhanced chemiluminescence. All the antibodies were purchased from CST (P-Stat3: #9138; Stat3: #14801; EMT antibody sampler kit: #9782; Gapdh: #5174) except for anti-human and anti-mouse N-Cadherin (13769-1-AP,

underside of the insert was stained with crystal violet. Cells that had migrated into the lower chamber medium were collected and counted. Migration was calculated in terms of total cells on the lower membrane surface.

RNA interference

The Stat3 and control siRNAs (human STAT3-siRNA, sc-29493; mouse Stat3-siRNA, sc-29494; human and mouse control-siRNA, sc-siRNA, sc-37007) were obtained from Santa Cruz Biotechnology. siRNAs were transfected into A549 and LLC cells with the siRNA Reagent System (sc-29528, Santa Cruz Biotechnology)
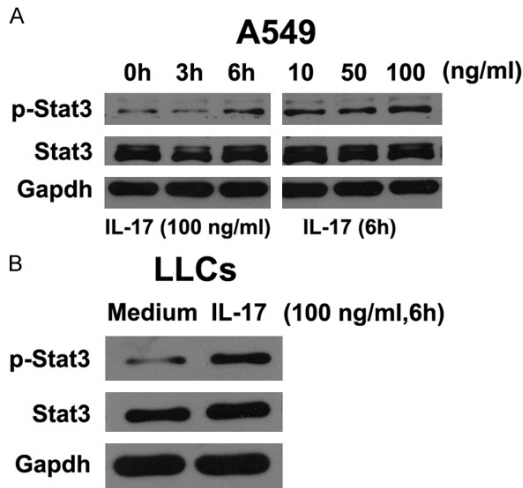
Figure 2. The effect of IL-17 on Stat3 signaling in A549 and LLC cells in vitro. A. A549 cells (5×10^5 cells/well) were treated with or without IL-17 at 100 ng/ml for the indicated time (0, 3, 6 h) or at different concentrations (0, 50, 100 ng/ml) for 6 h. The protein levels of p-Stat3 and Stat3 were determined by western blotting. B. LLC cells (5×10^5 cells/well) were treated without or with 100 ng/ml IL-17 for 6 h; p-Stat3 and Stat3 protein levels were determined by western blotting. Target gene expression levels were normalized to those of the Gapdh housekeeping gene, and the data from two independent experiments are presented.

Proteintech Group, Inc.) and anti-Twist 1 (ab175430, Abcam).

Statistics

The data are presented as the mean ± SEM. T-tests for independent events were used to evaluate the significance of differences. Data in different groups were compared by Kruskal-Wallis one-way analysis of variance. Correlations were used to analyze the relationships in human lung cancer tissue. Statistical analyses were conducted using SPSS statistical software, version 16.0 (Chicago, IL); two-tailed results with $p < 0.05$ were regarded as significant.

Results

Correlation between IL-17 and EMT markers in human lung adenocarcinoma tissues

IL-17 and EMT have been reported to promote lung cancer metastasis; thus, we explored the relationship between IL-17 and EMT marker expression in human lung adenocarcinoma tis-

sues using qRT-PCR. Interestingly, our results clearly demonstrated that IL-17 expression was positively correlated with N-cadherin, Vimentin, Snail1, Snail2, and Twist1 expression and negatively correlated with E-cadherin expression in human lung adenocarcinoma tissues (**Figure 1A-F**), suggesting that IL-17 may participate in EMT in human lung adenocarcinoma.

Stat3 is activated in IL-17-treated A549 and LLC cells in vitro

Our in vitro western blotting results indicated that IL-17 treatment led to a significant increase in Stat3 phosphorylation at different time points and concentrations in A549 cells (**Figure 2A**). Moreover, Stat3 was also activated in LLC cells by 100 ng/ml IL-17 at 6 h (**Figure 2B**). For subsequent experiments, 100 ng/ml IL-17 was utilized.

Stat3 inhibitor is required for IL-17-mediated wound healing in A549 and LLC cells in vitro

To evaluate the effect and mechanism of action of IL-17 in A549 and LLC cells in vitro, wound healing assays were performed. IL-17 markedly and persistently promoted A549 and LLC cell wound closure at several time points (**Figure 3A-C**). Furthermore, we noted that the Stat3 inhibitor significantly abrogated IL-17-induced A549 and LLC wound healing (**Figure 3A-C**).

Stat3 inhibitor is required for IL-17-mediated A549 and LLC cell migration in vitro

The metastatic properties of IL-17-treated A549 and LLC cells were further validated by migration assays. As expected, the two cell lines displayed greater migratory activity after IL-17 treatment compared with control medium (**Figure 4A-D**). Moreover, the Stat3 inhibitor decreased the IL-17-induced migration of the two cell lines (**Figure 4A-D**).

Stat3 knockdown decreases IL-17-induced EMT in A549 and LLC cells in vitro

To further determine whether IL-17 enhances EMT properties in A549 and LLC cells, we used qRT-PCR and western blotting to assess EMT markers after treatment with or without IL-17. Our data indicated that E-cadherin mRNA expression was decreased and that N-cadherin, Vimentin, Snail1, Snail2 and Twist1 mRNA expression levels were increased in IL-17-
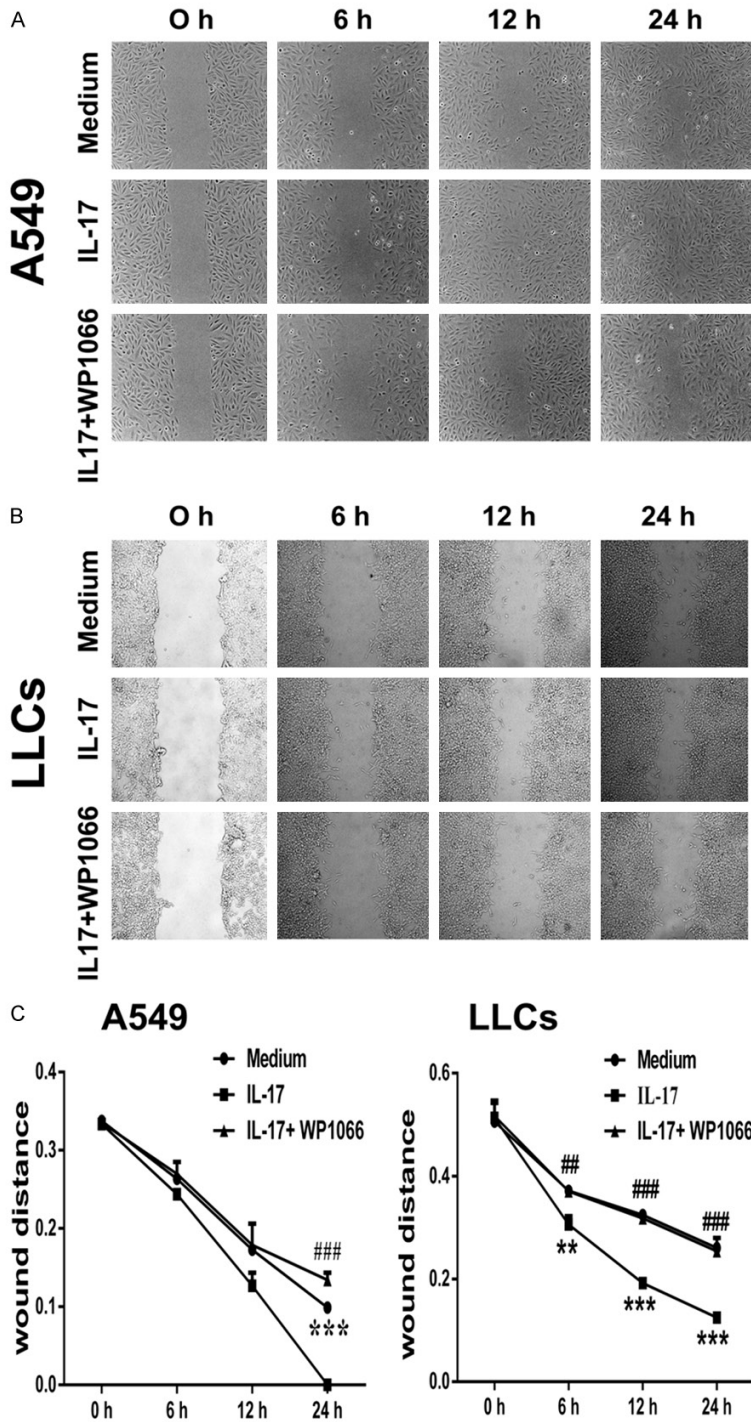
Figure 3. Stat3 inhibitor reduced IL-17-induced wound healing by A549 and LLCs. A549 and LLCs were incubated with medium alone, IL-17, or IL-17 plus the Stat3 inhibitor for 24 h (IL-17, 100 ng/ml; inhibitor, 30 μM). Microscopic photography was performed at several time points (0, 6, 12, 24 h) after wound generation on a confluent monolayer of A549 and LLCs (magnification, ×200). A and C. Graphs show the wound closure over time by A549 cells. B and C. Graphs show the wound closure over time by LLCs. Data are presented as the mean ± SEM of three independent experiments. The comparisons were evaluated by Kruskal-Wallis one-way analysis of variance on ranks. * and #, IL-17 compared with medium and the Stat3 inhibitor, respectively. * and #, p < 0.05; ** and ##, p < 0.01; *** and ###, p < 0.001.

treated A549 and LLC cells relative to control cells (**Figure 5A**). These findings were further confirmed by western blotting (**Figure 5B**), demonstrating that IL-17 enhanced EMT in A549 and LLC cells in vitro.

Subsequently, to determine if Stat3 knockdown is involved in IL-17-induced EMT on lung adenocarcinoma, the cells were pretreated with a Stat3 inhibitor (WP1066) or Stat3 siRNA before IL-17 stimulation and EMT profiles were determined by were evaluated by qRT-PCR and western blotting.

Importantly, our findings indicated that the Stat3 inhibitor significantly suppressed the IL-17-induced EMT profile in A549 and LLC cells at the mRNA and protein levels (**Figure 5A**, **5B**). Furthermore, after transfection with Stat3 siRNA for 48 h, the cells displayed an effective Stat3 knockdown (~90%) and markedly reduced Stat3 activation by qRT-PCR and western blotting (**Figure 6A**, **6B**). Next, Stat3 siRNA had a similar effect in the two cell lines; it considerably weakened the IL-17-mediated EMT profile compared to control siRNA (**Figure 7A**, **7B**). In summary, Stat3 was involved in the IL-17-mediated EMT profile in lung adenocarcinoma in vitro.

Discussion

Previous lung cancer studies, including ours, have demonstrated that high expression of IL-17 and/or IL-17RA signaling correlate with TNM stage [24, 28] and poor prognosis [22] in NSCLC patients and promote metastasis by enhancing angiogenesis [21]
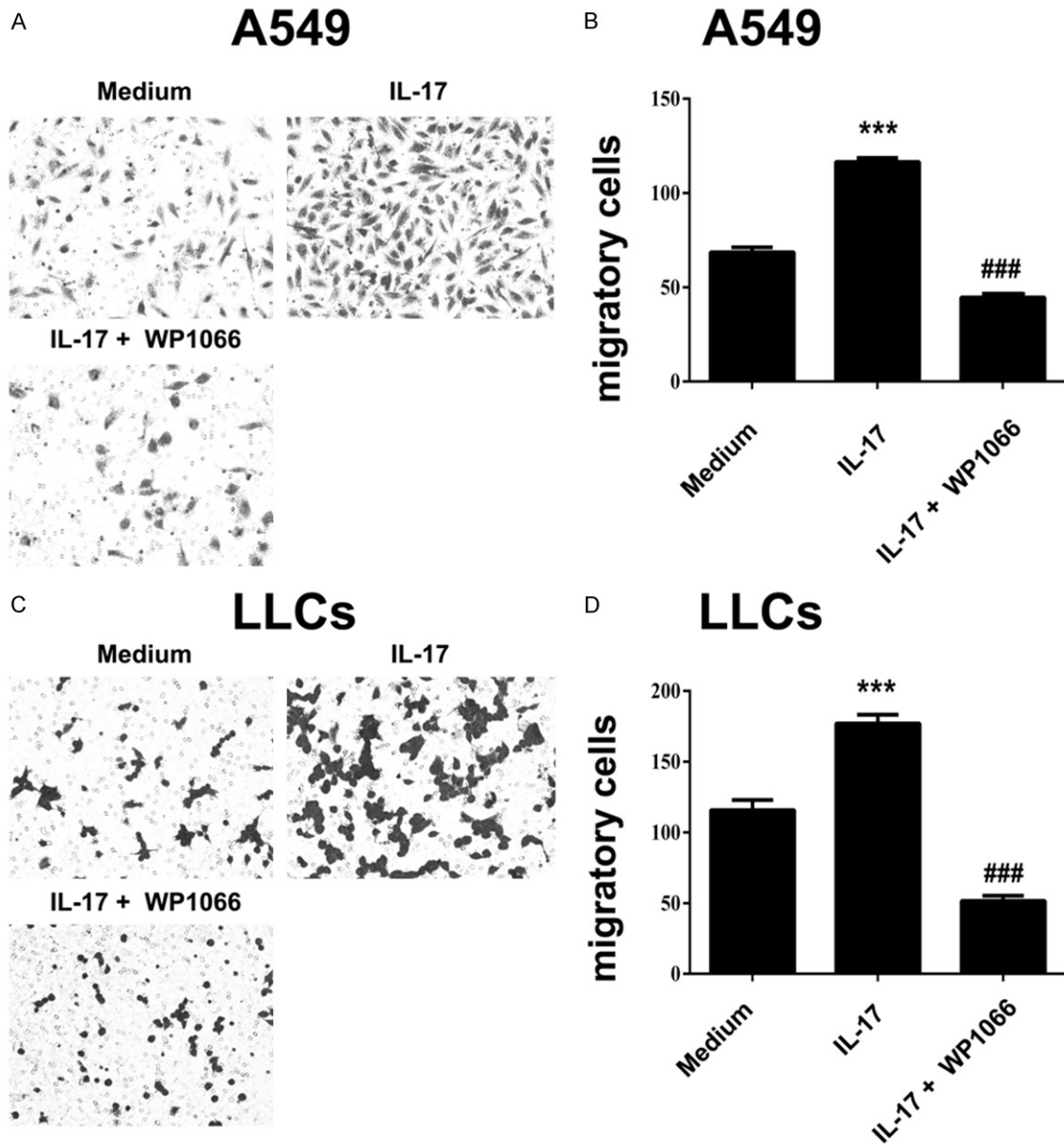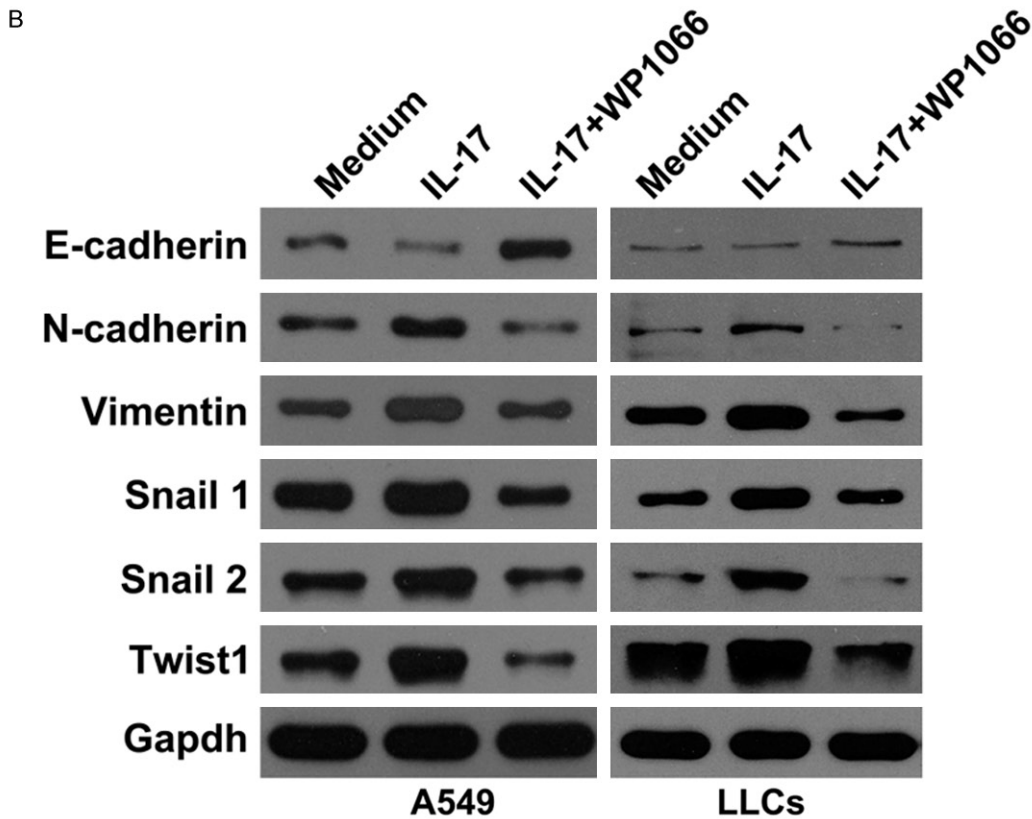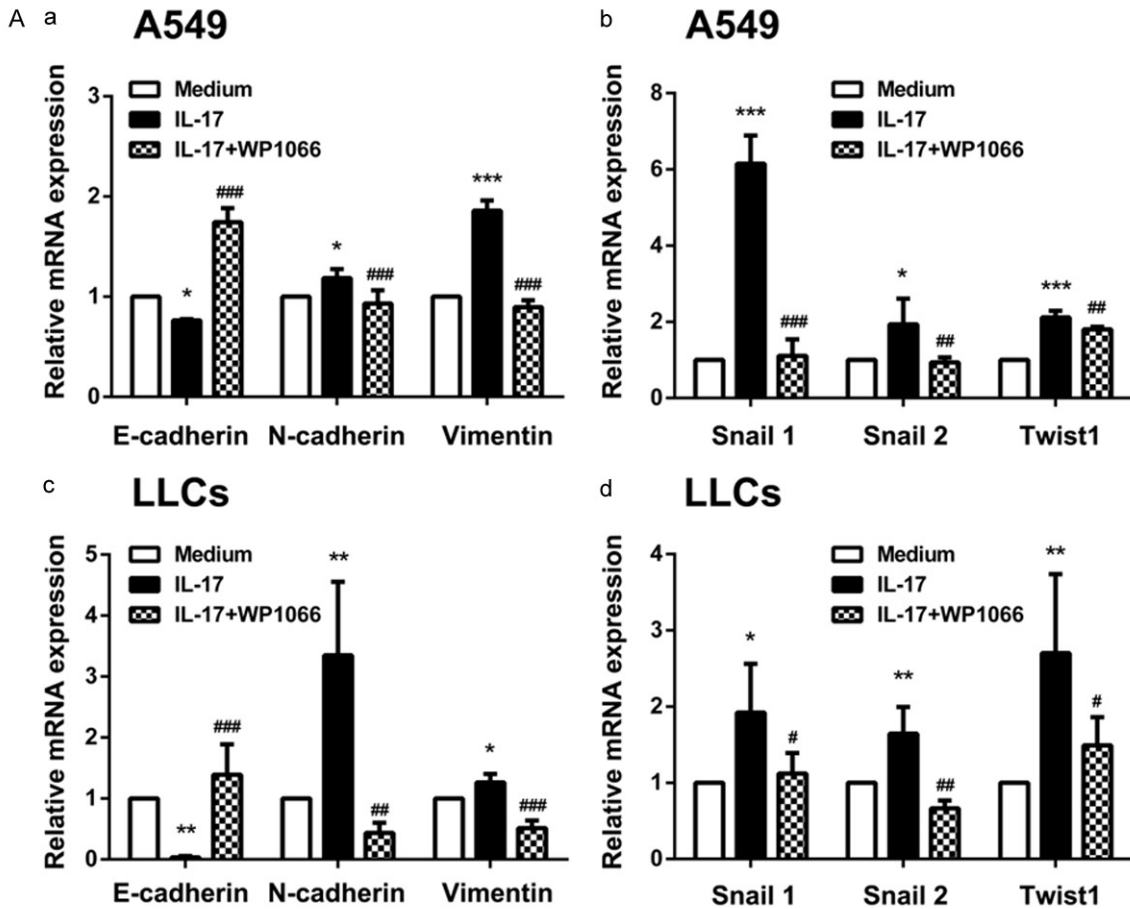
Figure 4. Stat3 inhibitor impaired IL-17-induced migration of A549 and LLC cells. A and C. Images of migratory A549 and LLC cells on the bottom surface of the transwell membrane (magnification, ×200). B and D. Graphs depict the number of migratory A549 and LLC cells. Data are presented as the mean ± SEM of three independent experiments. The comparisons were analyzed by Kruskal-Wallis one-way analysis of variance. * and #, IL-17 compared with medium and the Stat3 inhibitor, respectively. * and #, $p < 0.05$; ** and ##, $p < 0.01$; *** and ###, $p < 0.001$.

and lymphangiogenesis [22]. Our results are the first to clearly show that EMT marker expression levels were positively correlated with IL-17 expression in lung adenocarcinoma patient tissues. IL-17 also triggers EMT profiles in multiple myeloma and lung inflammatory diseases. A prior study indicated that IL-17 promotes EMT by repressing E-cadherin and inducing Vimentin, Snail and Slug in multiple myeloma [20]. In addition, several previous studies have shown that IL-17 induces EMT in airway epithelial cells, 16HBE cells, and alveolar epithelial cells via TGF-β-dependent and/or TGF-β-independent mechanisms in obliterative bronchiolitis [15], severe asthma [16], and pulmonary fibrosis [17]. Importantly, lung inflammatory diseases and COPD [18, 19], as well as lung cancer [29], share common pathogenic mechanisms,

Figure 5. Stat3 inhibitor down-regulated IL-17-induced EMT in A549 and LLC cells in vitro. A549 and LLC cells were incubated with medium alone, IL-17, or IL-17 plus the Stat3 inhibitor for 6 or 48 h (IL-17, 100 ng/ml; inhibitor, 30 μM). The mRNA and protein expression levels of EMT markers were determined by qRT-PCR and western blotting, respectively. A. (a and c) E-cadherin, N-cadherin, and Vimentin mRNA production in A549 and LLC cells. A. (b and d) Snail1, Snail2, and Twist1 mRNA production in A549 and LLC cells. B. E-cadherin, N-cadherin, Vimentin, Snail1, Snail2, and Twist1 protein levels in A549 and LLC cells. Target gene expression was normalized to that of the Gapdh housekeeping gene. Expression values were then calculated based on the $2^{-\Delta\Delta Ct}$ method. Data are presented as the mean ± SEM of three independent experiments. The comparisons were analyzed by Kruskal-Wallis one-way analysis of variance. * and #, IL-17 compared with medium and Stat3 inhibitor, respectively. * and #, p<0.05; ** and ##, p<0.01; *** and ###, p<0.001.
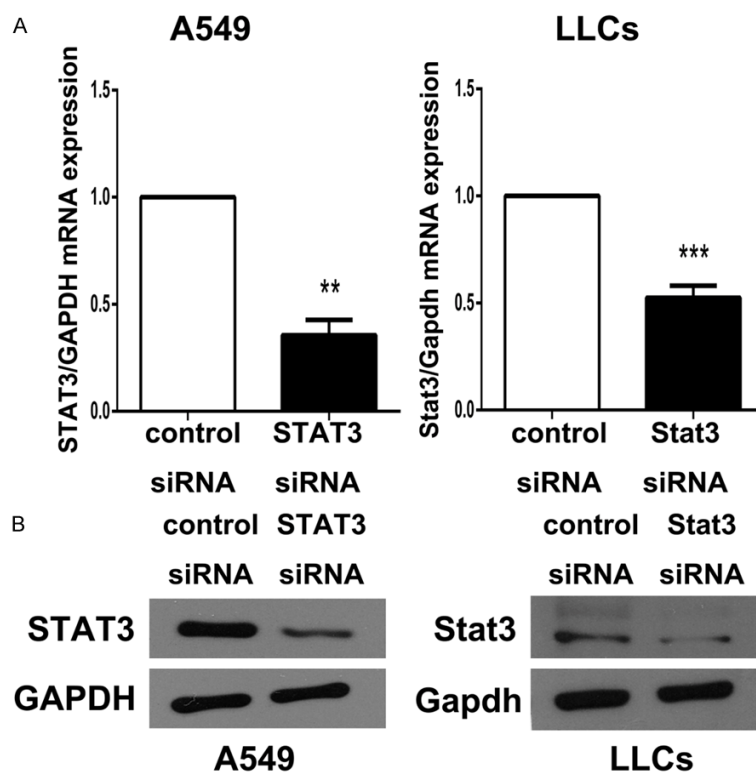

Figure 6. Stat3 knockdown by siRNA in A549 and LLCs. A549 and LLCs (2×10^5 cells/well) were transfected with siRNA for 48 h, and Stat3 mRNA and Stat3 protein expression was determined by qRT-PCR (A) and western blotting (B).
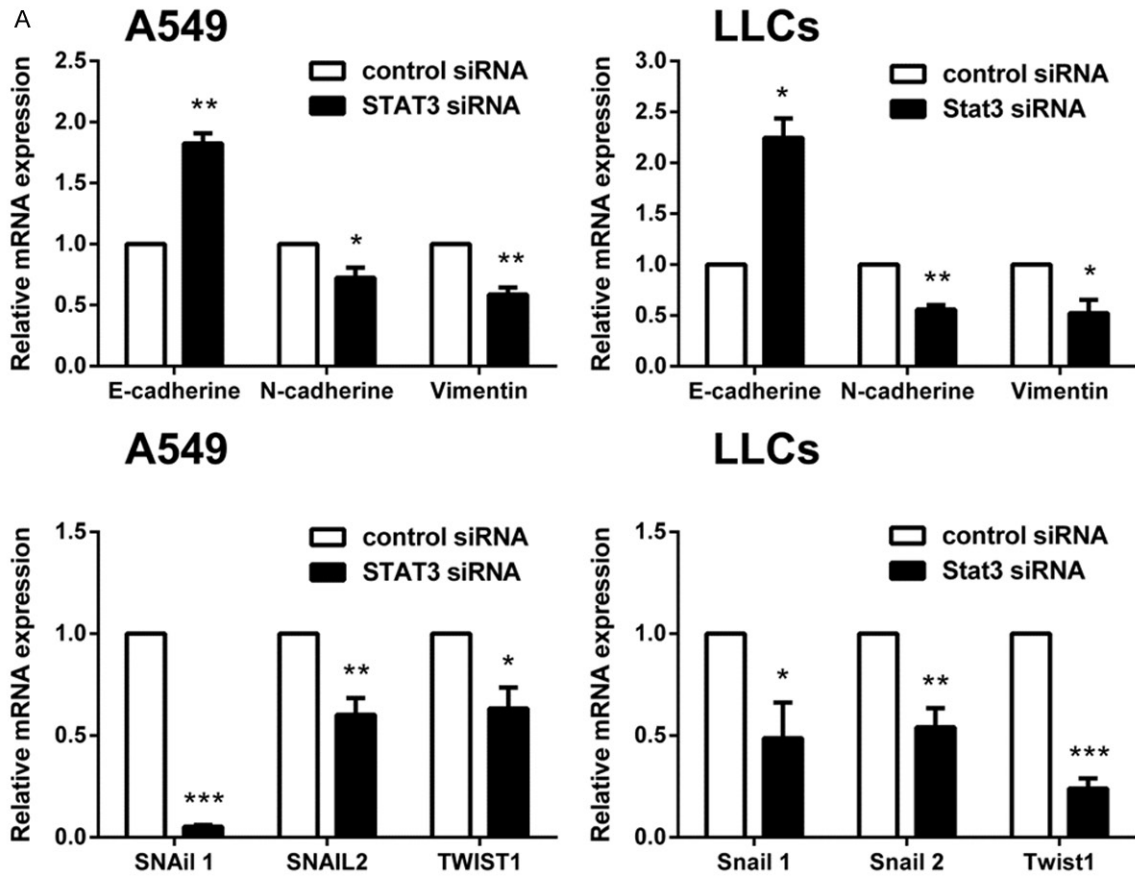
EMT profiles are directly modulated by EMT TFs (Snail family, Twist1) or indirectly modulated by the Stat3 pathway in many malignancies [9, 31-33], including lung cancer [6, 34, 35]. Stat3 signaling is a common mechanism through which IL-17 mediates tumor progression [24, 27, 29], including that of lung cancer [24]. Interestingly, we found that Stat3 was activated by IL-17 and that Stat3 knockdown significantly suppressed IL-17-mediated A549 and LLC wound healing and migration; these results were somewhat similar to those of a previous study implicating Stat3 in IL-17-mediated A549 migration [24].

Next, we further explored whether Stat3 is also involved in IL-17-induced EMT in vitro and revealed that Stat3 knockdown notably inhibited the IL-17-mediated increases in N-cadh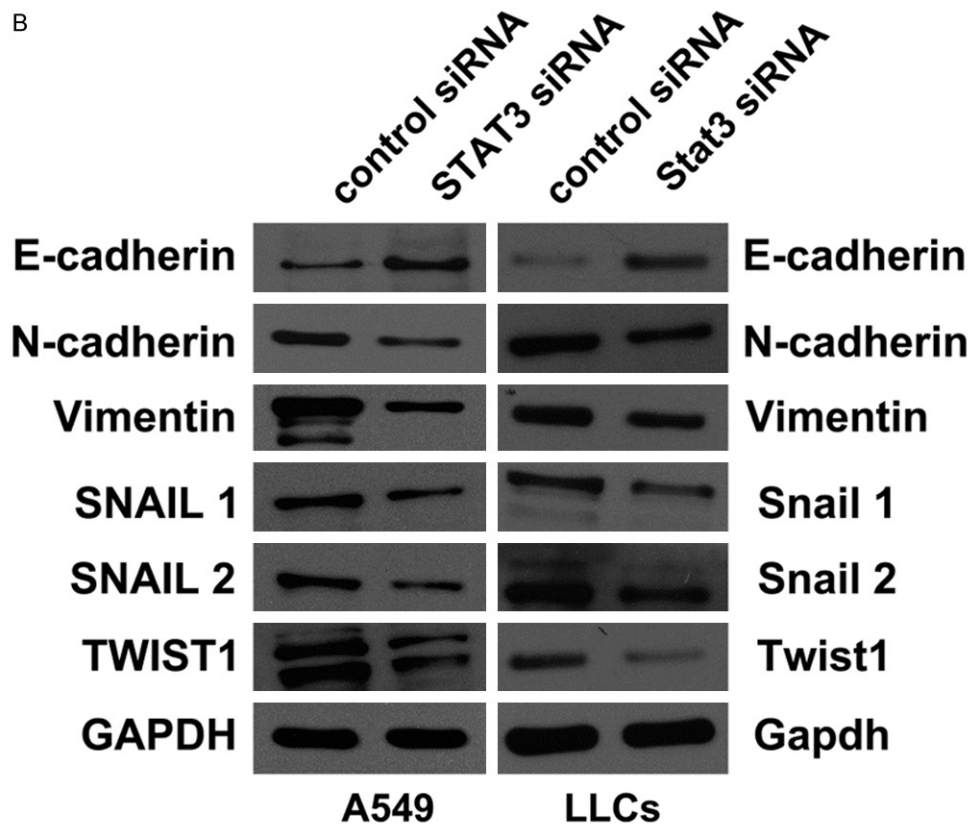erin, Vimentin and EMT TFs (Snail1, Snail2, Twist1) and reversed the IL-17-induced decrease in E-cadherin expression in A549 and LLC cells. Compared with the work by Gu et al. [29], our data innovatively showed that IL-17 exerted a similar effect in the mouse lung adenocarcinoma cell line, LLC. Moreover, we confirmed these results in A549 cells and showed that IL-17 also induced N-cadherin in A549 cells. Thus, we concluded that IL-17 promotes EMT in lung adenocarcinoma via Stat3 signaling.

It is well established that inflammatory cytokines drive the excessive activation of Stat3 signaling to facilitate EMT in many types of cancer [9], including lung cancer [36]. For example,

including EMT. Our prior discoveries in lung adenocarcinoma patient tissues and previous evidence connecting IL-17 and EMT in other diseases led us to further explore the effects and corresponding mechanism of action of IL-17 on EMT profiles in lung adenocarcinoma in vitro.

As reported previously, IL-17 decreases E-cadherin and increases Vimentin in A549 cells via the NF-κB-mediated upregulation of ZEB1 but not of Snail1, Snail2, or Twist1 [29]. Although NF-κB signaling is required for IL-17-mediated effects in many inflammatory and immune diseases, IL-17 is a weak NF-κB activator [30]. Accumulating evidence suggests that

A

A549

LLCs

A549

LLCs

B

Figure 7. Stat3 siRNA reversed IL-17-induced EMT in A549 and LLCs in vitro. Next, A549 (5×10^5 cells/well) were transfected with siRNA for 48 h and then treated with human or mouse IL-17 (100 ng/ml), respectively, for additional 6 or 48 h. The mRNA and protein expression levels of EMT markers were determined by qRT-PCR and western blotting, respectively. A. E-cadherin, N-cadherin, Vimentin, Snail1, Snail2, and Twist1 mRNA production in A549 and LLCs. B. E-cadherin, N-cadherin, Vimentin, Snail1, Snail2, and Twist1 protein levels in A549 and LLCs. Target gene expression was normalized to that of the Gapdh housekeeping gene. Expression values were then calculated based on the $2^{-\Delta\Delta Ct}$ method. Data were analyzed using the T test, and the results are presented as the mean ± SEM of three independent experiments. *, Stat3 siRNA group compared with the control siRNA group. *p < 0.05; **p < 0.01; ***p < 0.001.

it has been frequently reported that Stat3 is required for IL-6-induced EMT in lung cancer [31, 37-39]. As described previously, the IL-6/Stat3 axis favors the differentiation of TH17 cells to produce IL-17, which further triggers these cells to promote tumor progression in the inflammatory microenvironment [11], such as is present for lung cancer [24, 26]. In turn, IL-17 facilitates the progression of many types of cancer [24, 27, 29], including lung cancer [24], via Stat3 signaling. All these data may imply that EMT represents one outcome of the interplay between IL-17-producing cells and IL-6/Stat3 signaling in lung cancer, but this remains to be fully elucidated.

In conclusion, Stat3 is activated as part of an inflammatory repair process that promotes EMT in both COPD and lung cancer [9, 18, 19, 40]. In addition, IL-17 signaling frequently induces EMT in both lung inflammatory diseases and lung cancer. Therefore, targeting IL-17 and EMT may represent a potential strategies for preventing early molecular events in lung inflammatory diseases to decrease or delay the development of lung cancer. Our investigation sheds new light on inflammation and EMT in lung cancer and thereby contributes to possibilities for preventing lung tumorigenesis at earlier stages.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Yang Jin, Department of Respiratory Medicine, Key Laboratory of Pulmonary Diseases of Health Ministry, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Avenue, Wuhan 430022, Hubei, China. Tel: 86-13554361146; Fax: 86-027-85755457; E-mail: whuhjy@126.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] K.D. Miller, A. Banavali, D. Pyle, K. Fincham, C. Miller, B. Nadler. Global cancer epidemiology and the cancer divide, in: K.D. Miller (Ed.) Glob. Persp. Cancer Inciden. Care Exp., Praeger, Santa Barbara, CA, 2015, pp. 5.

[3] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.

[4] Hensing T, Chawla A, Batra R, Salgia R. A personalized treatment for lung cancer: molecular pathways, targeted therapies, and genomic characterization. Adv Exp Med Biol 2014; 799: 85-117.

[5] Wood SL, Pernemalm M, Crosbie PA, Whetton AD. The role of the tumor-microenvironment in lung cancer-metastasis and its relationship to potential therapeutic targets. Cancer Treat Rev 2014; 40: 558-66.

[6] Soltermann A, Tischler V, Arbogast S, Braun J, Probst-Hensch N, Weder W, Moch H, Kristiansen G. Prognostic significance of epithelial-mesenchymal and mesenchymal-epithelial transition protein expression in non-small cell lung cancer. Clin Cancer Res 2008; 14: 7430-7.

[7] Reka AK, Chen G, Jones RC, Amunugama R, Kim S, Karnovsky A, Standiford TJ, Beer DG, Omenn GS, Keshamouni VG. Epithelial-mesenchymal transition-associated secretory pheno-

type predicts survival in lung cancer patients. Carcinogenesis 2014; 35: 1292-300.

[8] Engels EA. Inflammation in the development of lung cancer: epidemiological evidence. Expert Rev Anticancer Ther 2008; 8: 605-15.

[9] Wendt MK, Balanis N, Carlin CR, Schiemann WP. STAT3 and epithelial-mesenchymal transitions in carcinomas. JAKSTAT 2014; 3: e28975.

[10] Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17 family and IL-17 receptors. Cytokine Growth Factor Rev 2003; 14: 155-74.

[11] Murugaiyan G, Saha B. Protumor vs antitumor functions of IL-17. J Immunol 2009; 183: 4169-75.

[12] Neurath MF, Finotto S. The emerging role of T cell cytokines in non-small cell lung cancer. Cytokine Growth Factor Rev 2012; 23: 315-22.

[13] Reppert S, Koch S, Finotto S. IL-17A is a central regulator of lung tumor growth. Oncoimmunology 2012; 1: 783-785.

[14] Chang SH, Mirabolfathinejad SG, Katta H, Cumpian AM, Gong L, Caetano MS, Moghaddam SJ, Dong C. T helper 17 cells play a critical pathogenic role in lung cancer. Proc Natl Acad Sci U S A 2014; 111: 5664-9.

[15] Vittal R, Fan L, Greenspan DS, Mickler EA, Gopalakrishnan B, Gu H, Benson HL, Zhang C, Burlingham W, Cummings OW, Wilkes DS. IL-17 induces type V collagen overexpression and EMT via TGF-beta- dependent pathways in obliterative bronchiolitis. Am J Physiol Lung Cell Mol Physiol 2013; 304: L401-14.

[16] Ji X, Li J, Xu L, Wang W, Luo M, Luo S, Ma L, Li K, Gong S, He L, Zhang Z, Yang P, Zhou Z, Xiang X, Wang CY. IL4 and IL-17A provide a Th2/Th17-polarized inflammatory milieu in favor of TGF-beta 1 to induce bronchial epithelial-mesenchymal transition (EMT). Int J Clin Exp Pathol 2013; 6: 1481-92.

[17] Mi S, Li Z, Yang HZ, Liu H, Wang JP, Ma YG, Wang XX, Liu HZ, Sun W, Hu ZW. Blocking IL-17A promotes the resolution of pulmonary inflammation and fibrosis via TGF-beta1-dependent and -independent mechanisms. J Immunol 2011; 187: 3003-14.

[18] Young RP, Hopkins RJ. How the genetics of lung cancer may overlap with COPD. Respirology 2011; 16: 1047-55.

[19] Yang IA, Relan V, Wright CM, Davidson MR, Sriram KB, Savarimuthu Francis SM, Clarke BE, Duhig EE, Bowman RV, Fong KM. Common pathogenic mechanisms and pathways in the development of COPD and lung cancer. Expert Opin Ther Targets 2011; 15: 439-56.

[20] Sun Y, Pan J, Mao S, Jin J. IL-17/miR-192/IL-17Rs regulatory feedback loop facilitates multiple myeloma progression. PLoS One 2014; 9: e114647.

[21] Numasaki M, Watanabe M, Suzuki T, Takahashi H, Nakamura A, McAllister F, Hishinuma T, Goto J, Lotze MT, Kolls JK, Sasaki H. IL-17 enhances the net angiogenic activity and in vivo growth of human non-small cell lung cancer in SCID mice through promoting CXCR-2-dependent angiogenesis. J Immunol 2005; 175: 6177-89.

[22] Chen X, Wan J, Liu J, Xie W, Diao X, Xu J, Zhu B, Chen Z. Increased IL-17-producing cells correlate with poor survival and lymphangiogenesis in NSCLC patients. Lung Cancer 2010; 69: 348-54.

[23] Chen X, Xie Q, Cheng X, Diao X, Cheng Y, Liu J, Xie W, Chen Z, Zhu B. Role of interleukin-17 in lymphangiogenesis in non-small-cell lung cancer: Enhanced production of vascular endothelial growth factor C in non-small-cell lung carcinoma cells. Cancer Sci 2010; 101: 2384-90.

[24] Li Q, Han Y, Fei G, Guo Z, Ren T, Liu Z. IL-17 promoted metastasis of non-small-cell lung cancer cells. Immunol. Immunol Lett 2012; 148: 144-50.

[25] Chung AS, Wu X, Zhuang G, Ngu H, Kasman I, Zhang J, Vernes JM, Jiang Z, Meng YG, Peale FV, Ouyang W, Ferrara N. An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. Nat Med 2013; 19: 1114-23.

[26] Ye ZJ, Zhou Q, Gu YY, Qin SM, Ma WL, Xin JB, Tao XN, Shi HZ. Generation and differentiation of IL-17-producing CD4+ T cells in malignant pleural effusion. J Immunol 2010; 185: 6348-54.

[27] Gu FM, Li QL, Gao Q, Jiang JH, Zhu K, Huang XY, Pan JF, Yan J, Hu JH, Wang Z, Dai Z, Fan J, Zhou J. IL-17 induces AKT-dependent IL-6/JAK2/STAT3 activation and tumor progression in hepatocellular carcinoma. Mol Cancer 2011; 10: 150.

[28] Huang Q, Du J, Fan J, Lv Z, Qian X, Zhang X, Han J, Chen C, Wu F, Jin Y. The effect of proinflammatory cytokines on IL-17RA expression in NSCLC. Med Oncol 2014; 31: 144.

[29] Gu K, Li MM, Shen J, Liu F, Cao JY, Jin S, Yu Y. Interleukin-17-induced EMT promotes lung cancer cell migration and invasion via NF-kappaB/ZEB1 signal pathway. Am J Cancer Res 2015; 5: 1169-79.

[30] Chang SH, Dong C. Signaling of interleukin-17 family cytokines in immunity and inflammation. Cell Signal 2011; 2: 1069-75.

[31] Liu RY, Zeng Y, Lei Z, Wang L, Yang H, Liu Z, Zhao J, Zhang HT. JAK/STAT3 signaling is required for TGF-beta-induced epithelial-mesenchymal transition in lung cancer cells. Int J Oncol 2014; 44: 1643-51.

[32] Li L, Han R, Xiao H, Lin C, Wang Y, Liu H, Li K, Chen H, Sun F, Yang Z, Jiang J, He Y. Metformin sensitizes EGFR-TKI-resistant human lung cancer cells in vitro and in vivo through inhibition of IL-6 signaling and EMT reversal. Clin Cancer Res 2014; 20: 2714-26.

[33] Tania M, Khan MA, Fu J. Epithelial to mesenchymal transition inducing transcription factors and metastatic cancer. Tumour Biol 2014; 35: 7335-42.

[34] Shih JY, Yang PC. The EMT regulator slug and lung carcinogenesis. Carcinogenesis 2011; 32: 1299-304.

[35] Sato M, Shames DS, Hasegawa Y. Hasegawa, Emerging evidence of epithelial-to-mesenchymal transition in lung carcinogenesis. Respirology 2012; 17: 1048-59.

[36] Zhao Y, Yao J, Wu XP, Zhao L, Zhou YX, Zhang Y, You QD, Guo QL, Lu N. Wogonin suppresses human alveolar adenocarcinoma cell A549 migration in inflammatory microenvironment by modulating the IL-6/STAT3 signaling pathway. Mol Carcinog 2015; 54 Suppl 1: E81-93.

[37] Chen W, Gao Q, Han S, Pan F, Fan W. The CCL2/CCR2 axis enhances IL-6-induced epithelial-mesenchymal transition by cooperatively activating STAT3-Twist signaling. Tumour Biol 2015; 36: 973-81.

[38] Zhao Z, Cheng X, Wang Y, Han R, Li L, Xiang T, He L, Long H, Zhu B, He Y. Metformin inhibits the IL-6-induced epithelial-mesenchymal transition and lung adenocarcinoma growth and metastasis. PLoS One 2014; 9: e95884.

[39] Zhang L, Tao L, Shi T, Zhang F, Sheng X, Cao Y, Zheng S, Wang A, Qian W, Jiang L, Lu Y. Paeonol inhibits B16F10 melanoma metastasis In vitro and In Vivo via disrupting proinflammatory cytokines-mediated NF-kappaB and STAT3 pathways. IUBMB Life 2015; 67: 778-88.

[40] Sekine Y, Hata A, Koh E, Hiroshima K. Lung carcinogenesis from chronic obstructive pulmonary disease: characteristics of lung cancer from COPD and contribution of signal transducers and lung stem cells in the inflammatory microenvironment. Gen Thorac Cardiovasc Surg 2014; 62: 415-21.