

Association between interleukin-36 γ and tumor progression in non-small cell lung cancer

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Abstract. Immunotherapy is effective in improving the survival and prognosis of patients with non-small cell lung cancer (NSCLC), and identifying effective immunomarkers is important for immunotherapy. Interleukin (IL)-36 γ is a novel immunomarker that has an important function in the antitumor immune response. The present study investigated the association between IL-36 γ and NSCLC to provide novel insight into immunotherapy for patients with NSCLC. Tissue microarrays of lung adenocarcinoma and squamous cell carcinoma were purchased for immunohistochemical analysis of IL-36 γ expression levels and clinical parameters. In addition, fresh clinical NSCLC and adjacent normal tissue samples were collected to analyze IL-36 γ mRNA expression levels using quantitative PCR. IL-36 γ protein was primarily located in the cytoplasm, with a small quantity in the nucleus, and IL-36 γ mRNA and protein expression levels in lung cancer tissues were significantly higher compared with those

in adjacent normal tissues. Elevated IL-36 γ protein expression levels were significantly associated with a higher tumor grade of lung adenocarcinoma; however, IL-36 γ mRNA expression levels were inversely associated with the clinical Tumor-Node-Metastasis stage in patients with lung squamous cell carcinoma. In addition, patients with adenocarcinoma with high IL-36 γ protein expression levels tended to longer post-operative survival times. These findings indicate that IL-36 γ may have potential as an immunomarker for prediction of tumor progression and survival in patients with NSCLC.

Introduction

According to the World Health Organization statistics in 2018, lung cancer is the sixth leading cause of cancer-associated mortality worldwide and NSCLC accounts for ~85% of all lung cancer cases (1,2). Although a number of patients with lung cancer may benefit from chemotherapy, radiotherapy or molecular targeted therapy, more effective immunotherapies need to be developed to aid our understanding of the molecular characteristics of lung tumor tissues.

The body is able to recognize and destroy cancer cells through immune surveillance mechanisms (3,4). However, certain characteristics of cancer cells may lead to immune tolerance and can be induced by multiple mechanisms in the tumor microenvironment (TME), including a reduction in the expression of co-stimulatory molecules and cytokines and through the expression of negative immunoregulatory molecules (5,6). Cytokines serve an important role in the antitumor immune response (7,8); therefore, investigation of cytokine expression levels in the TME may provide valuable novel insight into the underlying molecular mechanisms of tumor behavior for cancer immunotherapy.

Interleukin (IL)-36 is a member of the IL-1 family and has several subtypes, including IL-36 α , IL-36 β , IL-36 γ and IL-36 receptor antagonist (9). IL-36 γ interacts with the IL-36 receptor/IL-1RAcP, activating the NF- κ B and mitogen-activated protein kinase signaling pathways. These pathways result in the

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Abbreviations: DCs, dendritic cells; IL-36 γ , interleukin-36 γ ; NSCLC, non-small cell lung cancer; qPCR, quantitative PCR; TME, tumor microenvironment

Key words: non-small cell lung cancer, lung adenocarcinoma, squamous cell carcinoma, IL-36 γ , immunohistochemistry

production of inflammatory mediators, such as cytokines and chemokines, and regulate autoimmune diseases, inflammatory responses and antitumor immune responses. IL-36 γ is primarily expressed in peripheral blood lymphocytes, keratinocytes and bronchial epithelial cells (9). In addition, human macrophages and murine dendritic cells (DCs) express IL-36 γ following stimulation by the toll-like receptor or lipopolysaccharides (10,11). Previous studies have demonstrated that IL-36 γ induces autoimmune diseases such as psoriasis, allergic rhinitis (11) and allergic asthma (12), and is associated with type-1 immune responses (13-15). High IL-36 γ expression levels can stimulate immune differentiation of Th1-type cells, contributing to a positive immune response to infectious diseases (16,17). IL-36 γ -transfected DCs can upregulate the expression levels of T-bet, a T-box transcription factor, transforming the TME and promoting the development of lymphoid organs and inhibiting tumor growth (10,18). IL-36 γ is a novel antitumor cytokine that can promote proliferation of CD4⁺ T lymphocytes, CD8⁺ T lymphocytes, NK cells and $\gamma\delta$ T cells *in vitro* and *in vivo*, promoting tumor eradication in the TME (7).

A previous study demonstrated that a low expression level of IL-33, another member of the IL-1 family, was associated with poor prognosis in patients with lung adenocarcinoma (19). Therefore, the present study aimed to determine if IL-36 γ had a similar association with the prognosis of patients with non-small cell lung carcinoma (NSCLC). By reviewing the The National Center for Biotechnology Information Gene Expression Database (NCBI GEO) database (ncbi.nlm.nih.gov/geo), it was identified that IL-36 γ was expressed in lung cancer, especially in lung squamous cell carcinoma. A previous study demonstrated that IL-36 γ greatly promoted the proliferation and activation of CD8⁺ cells and enhanced the antitumor immune response using animal models (7). Therefore, the present study retrospectively analyzed clinical tissue specimens to investigate the value of IL-36 γ expression levels in the treatment and diagnosis of patients with NSCLC. Immunohistochemistry and quantitative (q)PCR was used to investigate IL-36 γ mRNA and protein expression levels during the progression of NSCLC, and to establish the association between IL-36 γ and the clinical and pathological parameters of patients with NSCLC.

Material and methods

Specimens. IL-36 γ tissue microarrays of lung adenocarcinoma and squamous cell lung cancer were purchased from the Shanghai Xinchao Biological Technology Co., Ltd. Each chip contained 150 tissues, including 75 tumor tissues and 75 corresponding adjacent normal tissues. Among the 75 lung squamous cell carcinoma tissues, one was classified as large cell carcinoma and was excluded from the follow-up analysis. Immunohistochemistry was performed on the tissue chips by Shanghai Xinchao Biological Technology Co., Ltd., in accordance with standard procedures.

Tumor tissues and adjacent normal tissues were also collected from patients with NSCLC (age range, 32-76 years; median age, 61 years; 65 men, 34 women) following surgery at The Third Affiliated Hospital of Soochow University between March and December 2009, and between January 2014 and February 2015. The samples of lung cancer tissue were

Table I. IL-36 γ and GAPDH primer sequences.

Gene	Primer sequence (5'-3')	Fragment length, bp
IL-36 γ		112
Forward	AGGTTGGAGAACAGCCCACATT	
Reverse	GTCCTACCAGTCTTGGCACGG	
GAPDH		189
Forward	GGAAGGTGAAGGTCGGAGTC	
Reverse	CGTTCTCAGCCTTGACGGT	
IL, interleukin.		

confirmed as NSCLC by senior pathologists based on tissue histopathology and morphology, and there were 57 cases of lung adenocarcinoma and 42 cases of lung squamous cell carcinoma. According to the Tumor-Node-Metastasis (TNM) stage criteria for lung cancer by the International Association for the Study of Lung Cancer (20), stages I and IIa were classified as early cases, whereas stages IIb, III and IV were classified as advanced cases (19). The tissues (100 mg) were frozen and stored in nitrogen immediately (-196°C). The present study was approved by The Ethics Committee of Soochow University (Suzhou, China) and all patients provided informed written consent.

Total RNA extraction and qPCR. Total RNA was extracted from patient tumor tissues and adjacent tissues using TRIzol[®] reagent (Ambion; Thermo Fisher Scientific, Inc.). RNA quality was assessed using 1% agarose gel electrophoresis and absorbance was measured at 260/280 nm using a NanoDrop[™] 2000 UV spectrophotometer (Thermo Fisher Scientific, Inc.). RNA was then reverse transcribed into cDNA using a Reverse Transcription kit (Applied Biosystems, Thermo Fisher Scientific, Inc.) on a Bio-Rad T100[™] Thermal Cycler (Bio-Rad Laboratories, Inc.), and the reaction conditions were as follows: 25°C for 10 min, 37°C for 120 min and 85°C for 5 min, followed by maintaining at 4°C. The qPCR assay was performed using a QuantiNova SYBR PCR kit (Qiagen China Co., Ltd.) using a CFX96[™] Real-Time system (Bio-Rad Laboratories, Inc.). The qPCR cycling conditions were as follows: Preheating at 95°C for 2 min, denaturation at 95°C for 5 sec, annealing at 60°C for 10 sec and a final extension at 60°C for 10 sec, for 40 amplification cycles. The results were quantified using the 2^{- $\Delta\Delta C_q$} method (21). The primers were designed and synthesized by Nanjing GenScript Biotech Corp. GAPDH was used as the internal reference and all primer sequences are shown in Table I.

Pathological scoring criteria. All tissue chip staining scores of IL-36 γ protein expression levels were independently assessed by two pathologists under a light microscope at x200 magnification. A positive signal was identified when the cytoplasm or nucleus showed a dark brown color. A total of 10 fields were randomly selected, and the protein positive ratio and color intensity were scored. The staining positive ratio was scored on a 5-point scale based on the percentage of positive staining as follows: 0 points, <5%; 1 point, 6-25%; 2 points, 26-50%;

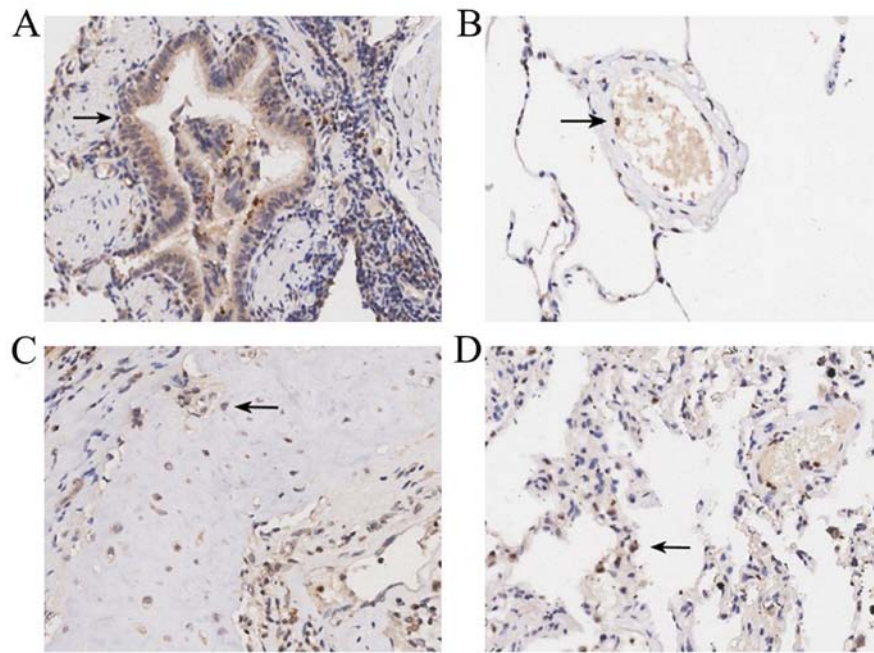


Figure 1. Interleukin-36 γ protein expression levels in normal lung tissues, shown as marked brown deposits (arrows) in (A) bronchial epithelial cells, (B) vascular endothelial cells, (C) chondrocytes and (D) alveolar epithelial cells. Magnification, x200.

3 points, 51-75%; and 4 points, >75%. Color intensity was scored on 4 levels as follows: 0 points, no color; 1 point, light yellow; 2 points, brown; and 3 points, dark brown. The final score was calculated by multiplying the positive ratio and color intensity, with four levels as follows: -, 0 points; +, 1-4 points; ++, 5-8 points; and +++, 9-12 points. Low expression levels were denoted as -/+ and high expression levels were denoted as ++/+++ for statistical analysis (22,23).

Bioinformatics. The GEO (<https://www.ncbi.nlm.nih.gov/>) (dataset no. GDS3966/220322_at/IL-36 γ) and Oncomine databases (<https://www.oncomine.org/>) were used to retrieve IL-36 γ expression data from human tumors.

Statistical analysis. Statistical analyses and graphing were performed using GraphPad Prism 5.0 (GraphPad Software, Inc.). The data are presented as the mean \pm standard deviation. IL-36 γ mRNA results were obtained using the Mann-Whitney U test. Fisher's exact test was used to analyze protein expression levels of IL-36 γ or the association between IL-36 γ protein expression levels and clinical parameters. A χ^2 test was used to analyze the association between IL-36 γ protein expression levels and tumor pathological grade. Patient survival was analyzed using the Kaplan-Meier survival analysis and log-rank test, and the Cox hazard ratio model. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

IL-36 γ protein is expressed in normal tissues. Immunohistochemical analysis of the tissue microarrays was used to explore the expression patterns of IL-36 γ protein in tumor-adjacent normal tissues compared with NSCLC tissues. Positive expression signals of IL-36 γ were primarily located in the cytoplasm, with weaker staining identified in the nucleus,

shown as brown particles. In tumor-adjacent normal tissues, IL-36 γ was expressed in various cell types, including bronchial epithelial cells (Fig. 1A), vascular endothelial cells (Fig. 1B), chondrocytes (Fig. 1C) and alveolar epithelial cells (Fig. 1D).

IL-36 γ protein is expressed in tumor cells. Based on the results of the immunohistochemical staining, it was revealed that IL-36 γ was expressed in lung cancer cells, including lung adenocarcinoma (Fig. 2A) and lung squamous cell carcinoma (Fig. 2B). Positive expression signals (brown particles) of IL-36 γ were also primarily located in the cytoplasm, with weaker staining identified in the nucleus.

IL-36 γ protein and mRNA expression levels in tumor tissues of lung adenocarcinoma and squamous cell carcinoma are significantly higher compared with those in adjacent normal tissues. Based on the pathological scoring criteria, IL-36 γ protein expression levels were evaluated in 75 lung adenocarcinoma tumor tissues, 74 squamous cell carcinoma tissues and the corresponding adjacent normal tissues. IL-36 γ protein expression levels were higher in the cancer tissues compared with those in the corresponding adjacent normal tissues (Fig. 3). Among the 75 patients with lung adenocarcinoma, 39 (52%) exhibited higher IL-36 γ expression levels in tumor tissues, whereas only 2 (3%) exhibited higher IL-36 γ expression levels in adjacent normal tissues ($P < 0.0001$; Table II). Among the 74 patients with squamous cell carcinoma, 42 (57%) exhibited significantly higher IL-36 γ expression levels in tumor tissues, whereas only 1 (1%) of the adjacent tissue samples exhibited higher IL-36 γ expression levels ($P < 0.0001$; Table III).

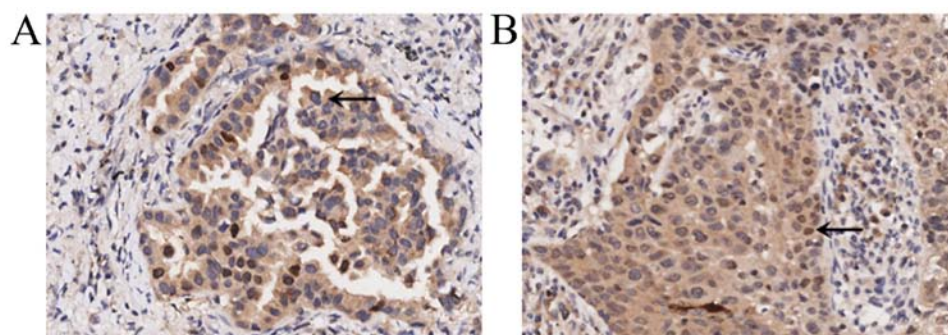
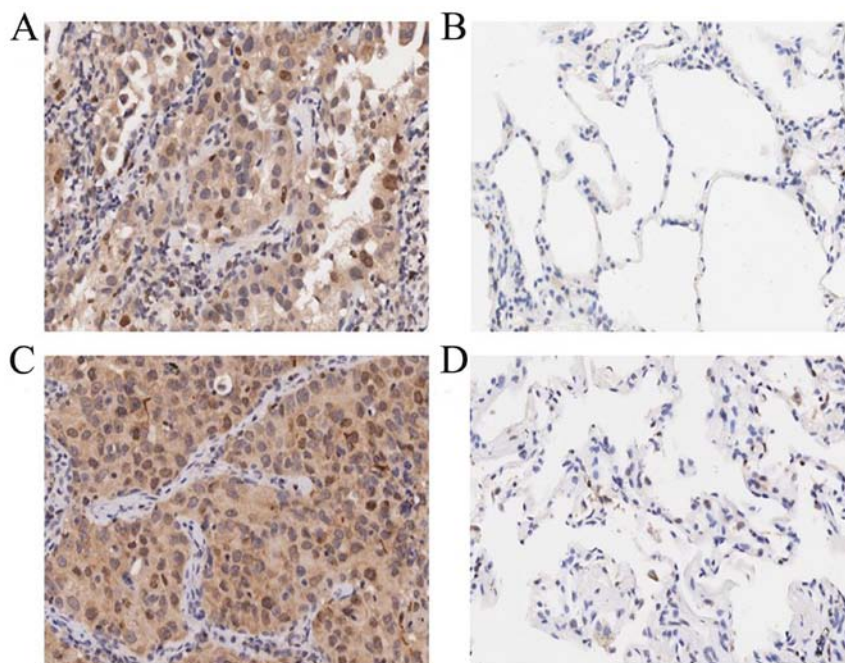
IL-36 γ mRNA expression levels were also analyzed in patients recruited from the Third Affiliated Hospital of Soochow University, including 57 cases of lung adenocarcinoma (29 cases in stage I/IIa and 28 cases in stage IIb/III/IV) and 42 cases of squamous cell carcinoma (22 cases in stage I/IIa and

Table II. Interleukin-36 γ expression levels in lung adenocarcinoma and adjacent normal tissues (n=75).

Group	Low expression, n (%)	High expression, n (%)	P-value
Adenocarcinoma	36 (48.0)	39 (52.0)	<0.0001
Adjacent tissues	73 (97.3)	2 (2.7)	<0.0001

Table III. Interleukin-36 γ expression levels in lung squamous cell carcinoma and adjacent normal tissues (n=74).

Group	Low expression, n (%)	High expression, n (%)	P-value
Squamous cell carcinoma	32 (43.2)	42 (56.8)	<0.0001
Adjacent tissues	73 (98.6)	1 (1.4)	<0.0001

Figure 2. Interleukin-36 γ protein expression levels in lung tumor tissues, shown as marked brown deposits (arrows) in (A) lung adenocarcinoma and (B) lung squamous cell carcinoma. Magnification, x200.Figure 3. IL-36 γ protein expression levels are upregulated in non-small cell lung cancer, shown as brown deposits. (A) High IL-36 γ expression levels in lung adenocarcinoma tissues. (B) Low IL-36 γ expression levels in adenocarcinoma-adjacent tissues. (C) High IL-36 γ expression levels in squamous cell carcinoma tissues. (D) Low IL-36 γ expression levels in squamous cell carcinoma-adjacent tissues. Magnification, x200. IL, interleukin.

20 cases in stage IIb/III/IV). IL-36 γ mRNA expression levels were significantly increased in both lung adenocarcinoma and

squamous cell carcinoma tumor tissues compared with those in normal tissues ($P<0.01$ and $P<0.05$, respectively; Fig. 4).

Table IV. Association between IL-36 γ protein expression levels and clinicopathological features of patients with adenocarcinoma.

Clinicopathological feature	n (%)	IL-36 γ expression levels		P-value
		-/+	++/+++	
Sex				0.6466
Men	40/75 (53.3)	18	22	
Women	35/75 (46.7)	18	17	
Age, years				0.8147
<60	31/73 (42.5)	16	15	
≥ 60	42/73 (57.5)	20	22	
Pathological grade				0.0302 ^a
I	13/75 (17.3)	8	5	
II	49/75 (65.3)	26	23	
III	13/75 (17.3)	2	11	
Tumor size, cm				0.2781
<5.5	58/75 (77.3)	30	28	
≥ 5.5	17/75 (22.7)	6	11	
Lymph node metastasis				0.3668
N0-N1	43/57 (75.4)	18	25	
N2-N3	14/57 (24.6)	8	6	
Clinical stage				>0.9999
I/IIa	36/58 (62.1)	17	19	
IIb/III/IV	22/58 (37.9)	11	11	

Due to incomplete patient information in the case data, the group sizes for each feature is not the same. ^aP<0.05. IL, interleukin.

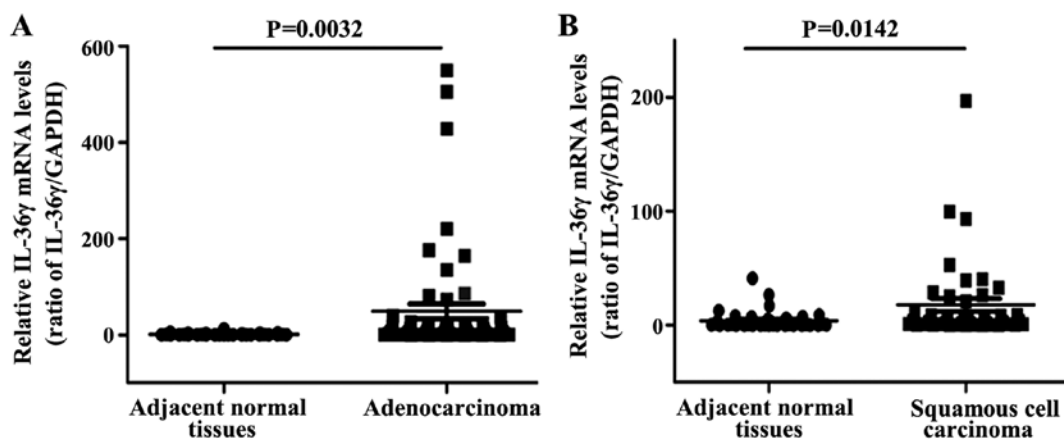


Figure 4. IL-36 γ mRNA expression levels are upregulated in non-small cell lung cancer. IL-36 γ mRNA expression levels in (A) adenocarcinoma tissues (n=57) and (B) squamous cell carcinoma (n=42) compared with respective adjacent normal tissues (P<0.05). IL, interleukin.

High IL-36 γ protein and mRNA expression levels are associated with tumor pathological grade in lung adenocarcinoma and clinical TNM stage in squamous cell carcinoma. The association between IL-36 γ protein expression levels and the clinical pathological parameters of NSCLC were investigated. Higher IL-36 γ protein expression levels were significantly associated with a higher tumor pathological grade of lung adenocarcinoma (P<0.05; Table IV). Meanwhile, there was no association between IL-36 γ protein expression level and all other assessed clinical pathological parameters in the

74 cases of lung squamous cell carcinoma (Table V). However, IL-36 γ mRNA expression level was inversely associated with the clinical TNM stage of the patients with squamous cell carcinoma, which was lower in the late stages (stage IIb/III/IV) than in the early stages (stage I/IIa) (P<0.05; Fig. 5B).

Association between IL-36 γ protein expression levels and prognosis in patients with NSCLC. After excluding patients with no clinical stage data and a lack of follow-up data, 38 patients with lung adenocarcinoma were followed for

Table V. Association between IL-36 γ protein expression levels and clinicopathological features of patients with squamous cell carcinoma.

Clinicopathological feature	n (%)	IL-36 γ expression levels		P-value
		-/+	++/+++	
Sex				
Men	68/74 (91.9)	31	37	0.2258
Women	6/74 (8.1)	1	5	
Age, years				
<60	26/73 (35.6)	13	13	0.4682
\geq 60	47/73 (64.4)	19	28	
Pathological grade				
I	5/74 (6.7)	3	2	0.1475
II	61/74 (82.4)	28	33	
III	8/74 (10.8)	1	7	
Tumor size, cm				
<5.5	47/74 (63.5)	18	29	0.3312
\geq 5.5	27/74 (36.5)	14	13	
Lymph node metastasis				
N0-N1	56/62 (90.3)	26	30	>0.9999
N2-N3	6/62 (9.7)	3	3	
Clinical stage				
I/IIa	40/62 (64.5)	18	22	0.7929
IIb/III/IV	22/62 (35.5)	11	11	

Due to incomplete patient information in the case data, the group sizes for each feature is not the same. IL, interleukin.

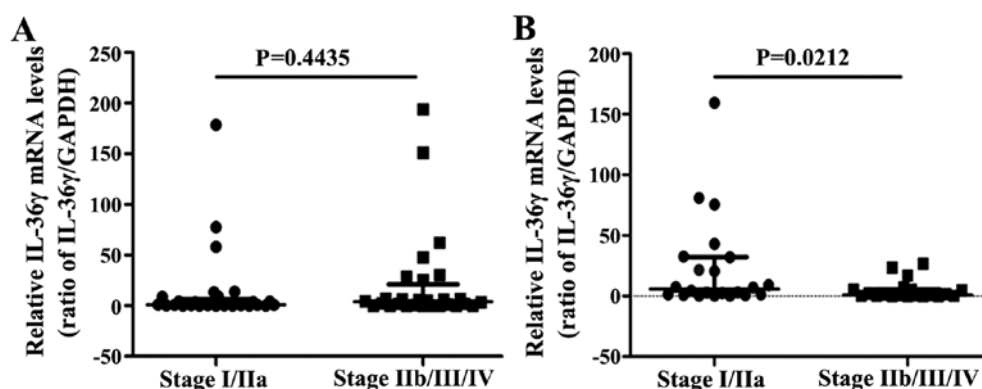


Figure 5. Association between IL-36 γ mRNA expression levels and clinical TNM stage. IL-36 γ mRNA expression and clinical TNM stage in (A) adenocarcinoma tissues (n=57) and (B) squamous cell carcinoma (n=42). IL, interleukin; TNM, Tumor-Node-Metastasis.

5 years. The overall 5-year survival rate of patients with NSCLC was 39% (15/38). In addition, 74 patients with squamous cell carcinoma were followed up for 3 years and these patients had a 3-year overall survival rate of 78% (58/74). Kaplan-Meier survival analysis and a log-rank test demonstrated that patients with lung adenocarcinoma and high IL-36 γ protein expression levels experienced a longer survival time; however, this difference was not statistically significant ($P=0.1343$; Fig. 6A). In addition, IL-36 γ protein expression levels were not associated with survival in patients with squamous cell carcinoma ($P>0.05$; Fig. 6B).

Association between clinical parameters and survival of patients with NSCLC. A Cox hazard ratio model was also built. Univariate and multivariate survival analyses were performed on IL-36 γ expression level, sex, age, pathological grade, tumor size and T stage in patients with lung adenocarcinoma and squamous cell carcinoma (Tables VI and VII). No correlation was discovered between lung adenocarcinoma survival and any of the above variables (Table VI). There was a significant association between tumor size and survival in patients with lung squamous cell carcinoma in the univariate and multivariate analyses (Table VII). With $HR<1$ for patients

Table VI. Survival analysis using Cox's regression model in patients with lung adenocarcinoma (n=75).

Clinicopathological feature	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
IL36 γ expression levels (low:high)	1.276 (0.815-1.999)	0.2860	1.402 (0.880-2.234)	0.1547
Sex (men:women)	0.610 (0.344-1.083)	0.0912	0.563 (0.286-1.108)	0.0965
Age (<60: \geq 60 years)	1.163 (0.663-2.041)	0.5980	0.917 (0.499-1.684)	0.7797
Pathological grade (I:II:III)	0.729 (0.446-1.192)	0.2080	0.737 (0.380-1.429)	0.3667
Tumor size (<5.5: \geq 5.5 cm)	0.414 (0.147-1.164)	0.0944	0.385 (0.080-1.849)	0.2329
T stage (T1:T2:T3)	0.659 (0.319-1.362)	0.2600	1.011 (0.346-2.953)	0.9844

IL, interleukin; HR, hazard ratio; CI, confidence interval.

Table VII. Survival analysis using Cox's regression model in patients with squamous cell carcinoma (n=74).

Clinicopathological feature	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
IL36 γ protein expression levels (low:high)	0.920 (0.535-1.583)	0.7630	1.439 (0.698-2.967)	0.3237
Sex (men:women)	0.882 (0.372-2.088)	0.7750	1.072 (0.393-2.925)	0.8922
Age (<60: \geq 60 years)	0.659 (0.343-1.265)	0.2100	0.526 (0.231-1.195)	0.1248
Pathological grade (I:II:III)	1.014 (0.570-1.804)	0.9610	0.807 (0.399-1.631)	0.5504
Tumor size (<5.5: \geq 5.5 cm)	0.355 (0.172-0.735)	0.0053 ^a	0.258 (0.104-0.642)	0.0036 ^a
T stage (T1:T2:T3)	0.902 (0.490-1.663)	0.7420	1.552 (0.778-3.093)	0.2122

^aP<0.01. IL, interleukin; HR, hazard ratio; CI, confidence interval.

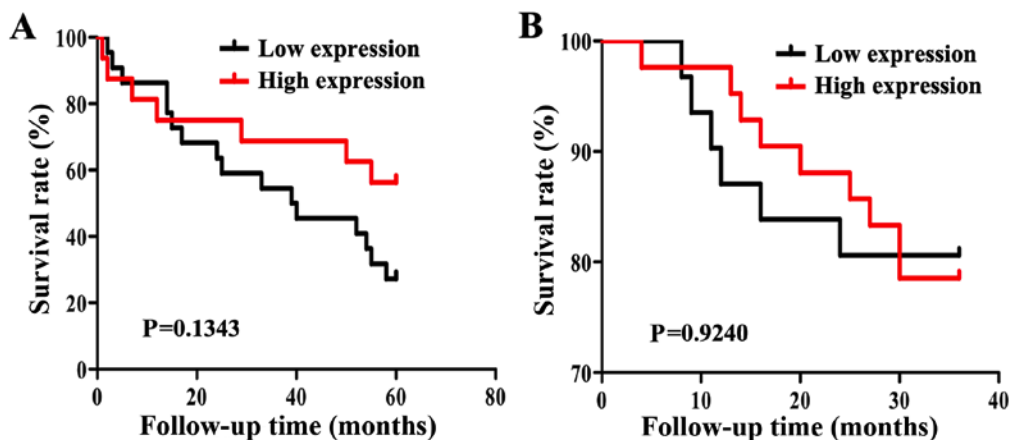


Figure 6. Association between IL-36 γ expression levels and the survival of patients with non-small cell lung cancer. (A) The 5-year survival follow-up data for patients with lung adenocarcinoma with low and high IL-36 γ expression levels (n=38). (B) The 3-year survival follow-up data for patients with squamous cell carcinoma with low and high IL-36 γ expression levels (n=74). IL, interleukin.

with squamous cell carcinoma, this suggests that the smaller the tumor the longer the survival time (P<0.01; Table VII).

Discussion

In the present study, IL-36 γ mRNA and protein expression levels were upregulated in NSCLC. Elevated IL-36 γ protein expression levels were significantly associated with a higher tumor grade of

lung adenocarcinoma, and IL-36 γ mRNA expression levels were inversely associated with clinical TNM stage in patients with squamous cell carcinoma. In addition, higher IL-36 γ expression in patients with adenocarcinoma tended to prolong survival, although this was not statistically significant. These data suggest that IL-36 γ may have an antitumor role in NSCLC.

IL-33 and IL-36, both members of the cytokine IL-1 family, primarily function as an 'alarmins', which are released

following tissue injury (9) or during infection (11,24) and are associated with the antitumor immune response (7,25,26). These cytokines can enhance the function of immune cells such as CD8⁺ T lymphocytes and NK cells by promoting the secretion and expression of effector cytokines, thereby functioning in the antitumor immune response (7,25). In our previous study, IL-33 had an antitumor effect in NSCLC (19). IL-33 expression levels were downregulated in tumor tissues and upregulated IL-33 expression levels were associated with longer survival times in patients with lung adenocarcinoma. Therefore, the present study aimed to determine whether IL-36 had a similar effect.

As a subtype of IL-36, IL-36 γ functions in a variety of skin inflammatory reactions and immunopathological processes, such as psoriasis, inflammatory megacolon and infectious diseases (16,27-31). Previous studies have demonstrated the involvement of IL-36 γ in the differentiation of Th cells and type-1 immune responses (7,16,17). Our previous study showed that IL-36 γ promoted cell activation and expressed IFN- γ , granzyme-B and other type 1 effectors *in vitro* by stimulating cultured human peripheral blood CD4⁺ T lymphocytes and CD8⁺ T lymphocytes (7). Therefore, due to these characteristics of IL-36 γ , melanoma tumor cells and breast cancer cells have previously been transfected to overexpress full-length IL-36 γ in our laboratory (7). A mouse model demonstrated that IL-36 γ overexpression inhibited tumorigenesis and metastasis by promoting the proliferation of CD8⁺ T and NK cells, and the production of the effector cytokines IFN- γ and TNF- α . Thus, the survival time of tumor-bearing mice was prolonged. In addition, IL-36 γ is also used as an adjuvant for tumor vaccines to induce antigen-specific immune responses (7). A recent breast cancer lung metastasis model study indicated that IL-36 γ has an important effect in improving the antitumor immune response by enhancing the type-1 immune response, inhibiting lung metastasis (32). Overall, these studies suggest that IL-36 γ , as an inflammatory cytokine, may serve an important role in inflammatory diseases and antitumor immunotherapy.

The IL-36 γ protein is expressed in keratinocytes, bronchial epithelial cells and brain tissue, and IL-36 γ expression levels in macrophages and neutrophils are significantly increased during infection (9,31,33). IL-36 γ may affect a variety of cells, including stromal cells, DCs, macrophages and lymphocytes by inducing a series of related inflammatory responses, including the promotion of synthesis and activity of IL-12, IL-8 and IL-6, and the chemokines CXCL1 and CCL20 (31,33-35). In the present study, immunohistochemistry of tissue microarrays showed that IL-36 γ is expressed in various cell types, including bronchial epithelial cells, vascular endothelial cells, chondrocytes and alveolar epithelial cells. Positive signals were primarily located in the cytoplasm, with weak staining in the nucleus. According to previous reports and the NCBI GEO and Oncomine databases, IL-36 γ is expressed in several other tumor tissues, including melanoma, colorectal cancer, head and neck cancer and lung cancer. The results of the present study also suggest that NSCLC cells express high levels of IL-36 γ in a diffuse pattern.

IL-36 γ mRNA and protein expression levels were significantly increased in NSCLC tissues compared with those in adjacent normal tissues in the present study. Higher IL-36 γ protein expression levels in adenocarcinoma tissues were significantly associated with higher tumor pathological grades, but

there was no association observed in squamous cell carcinoma. IL-36 γ mRNA expression levels in squamous cell carcinoma were inversely associated with the clinical TNM stage, which is consistent with a previous report investigating melanoma and lung cancer progression that demonstrated that IL-36 γ expression levels were higher in the early stage compared with those in the advanced stage (7). It suggests a potential antitumor effect of IL-36 γ in squamous cell carcinoma.

Furthermore, in the present study, survival analysis showed that patients with adenocarcinoma with high IL-36 γ protein expression levels had longer survival times ($P > 0.05$); however, the lack of information on patient treatment is a limitation when evaluating the survival time of patients. In addition, the Cox risk model indicated that the survival of patients with squamous cell carcinoma was associated with tumor size.

Prior to the present study, there have been a few studies on the association between IL-36 γ and tumors (7,32). Although the underlying mechanism of IL-36 γ in the antitumor immune response has been studied in animal models, the role of IL-36 γ in human tumors is unclear (7). In the present study, IL-36 γ expression patterns in human tumor tissues were investigated, aiming to determine the association between IL-36 γ mRNA and protein expression levels and clinical and pathological parameters in NSCLC. The findings of the present study have provided valuable information that may inform later studies of potential mechanisms underlying the function of IL-36 γ in NSCLC. The present study may also provide novel insight into the value of IL-36 γ as an immunotherapy target for NSCLC treatment. Therefore, further specimens should be collected and the sample size expanded to further study the associations between IL-36 γ mRNA and protein expression levels and clinical parameters, and the mechanism underlying IL-36 γ function to better determine its value for clinical application. Immunofluorescence co-localization of cellular markers (such as CD4, CD8 and CD56) and IL-36 γ in tumor tissues may aid the identification of cell types that secrete IL-36 γ , facilitating further investigation of the underlying mechanisms of IL-36 γ function.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JW and YZ designed and directed the study. LL and HH conducted the experiments and wrote the original manuscript.

DX, HZ and LS analyzed the data. YF and YG collected clinical specimens and acquired the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Committee of Soochow University (Suzhou, China) and all patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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