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

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Received July 25, 2019; Accepted December 13, 2019

DOI: 10.3892/ol.2020.11319

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)-36γ is a novel immunomarker that has an important function in the antitumor immune response. The present study investigated the association between IL-36γ 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-36γ expression levels and clinical parameters. In addition, fresh clinical NSCLC and adjacent normal tissue samples were collected to analyze IL-36γ mRNA expression levels using quantitative PCR. IL-36γ protein was primarily located in the cytoplasm, with a small quantity in the nucleus, and IL-36γ mRNA and protein expression levels in lung cancer tissues were significantly higher compared with those in adjacent normal tissues. Elevated IL-36γ protein expression levels were significantly associated with a higher tumor grade of lung adenocarcinoma; however, IL-36γ 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-36γ protein expression levels tended to longer post-operative survival times. These findings indicate that IL-36γ may have potential as an immunomarker for prediction of tumor progression and survival in patients with NSCLC.

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Abbreviations: DCs, dendritic cells; IL-36γ, interleukin-36γ; 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-36γ, immunohistochemistry

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-36α, IL-36β, IL-36γ and IL-36 receptor antagonist (9). IL-36γ interacts with the IL-36 receptor/IL-1RAcP, activating the NF-κB and mitogen-activated protein kinase signaling pathways. These pathways result in the

production of inflammatory mediators, such as cytokines and chemokines, and regulate autoimmune diseases, inflammatory responses and antitumor immune responses. IL-36γ is primarily expressed in peripheral blood lymphocytes, keratinocytes and bronchial epithelial cells (9). In addition, human macrophages and murine dendritic cells (DCs) express IL-36γ following stimulation by the toll-like receptor or lipopolysaccharides (10,11). Previous studies have demonstrated that IL-36γ 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-36γ expression levels can stimulate immune differentiation of Th1-type cells, contributing to a positive immune response to infectious diseases (16,17). IL-36γ-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-36γ is a novel antitumor cytokine that can promote proliferation of $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, NK cells and γδT 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-36γ 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-36γ was expressed in lung cancer, especially in lung squamous cell carcinoma. A previous study demonstrated that IL-36γ 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-36γ expression levels in the treatment and diagnosis of patients with NSCLC. Immunohistochemistry and quantitative (q)PCR was used to investigate IL-36γ mRNA and protein expression levels during the progression of NSCLC, and to establish the association between IL-36γ and the clinical and pathological parameters of patients with NSCLC.

Material and methods

Specimens. IL-36γ 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 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.

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

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

IL, interleukin.

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 $2^{-\Delta\Delta Cq}$ 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-36γ 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%;

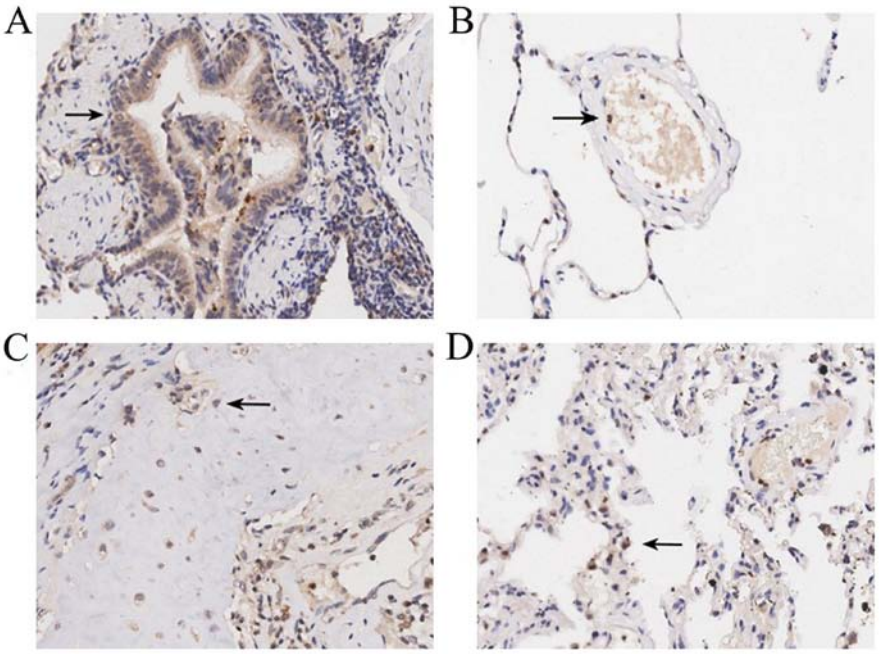

Figure 1. Interleukin-36γ protein expression levels in normal lung tissues, shown as marked brown deposits (arrows) in (A) bronchial epithelial cells, (B) vascular endothelial cells, (C) chondrocytes and (D) alveolar epithelial cells. Magnification, x200.

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-36γ) and Oncomine databases (https://www.oncomine.org/) were used to retrieve IL-36γ 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 ± standard deviation. IL-36γ mRNA results were obtained using the Mann-Whitney U test. Fisher's exact test was used to analyze protein expression levels of IL-36γ or the association between IL-36γ protein expression levels and clinical parameters. A χ^2 test was used to analyze the association between IL-36γ 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-36γ protein is expressed in normal tissues. Immunohistochemical analysis of the tissue microarrays was used to explore the expression patterns of IL-36γ protein in tumor-adjacent normal tissues compared with NSCLC tissues. Positive expression signals of IL-36γ were primarily located in the cytoplasm, with weaker staining identified in the nucleus, shown as brown particles. In tumor-adjacent normal tissues, IL-36γ 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-36γ protein is expressed in tumor cells. Based on the results of the immunohistochemical staining, it was revealed that IL-36γ 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-36γ were also primarily located in the cytoplasm, with weaker staining identified in the nucleus.

IL-36γ 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-36γ protein expression levels were evaluated in 75 lung adenocarcinoma tumor tissues, 74 squamous cell carcinoma tissues and the corresponding adjacent normal tissues. IL-36γ 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-36γ expression levels in tumor tissues, whereas only 2 (3%) exhibited higher IL-36γ 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-36γ expression levels in tumor tissues, whereas only 1 (1%) of the adjacent tissue samples exhibited higher IL-36γ expression levels ($P<0.0001$; Table III).

IL-36γ 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-36γ 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-36γ 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

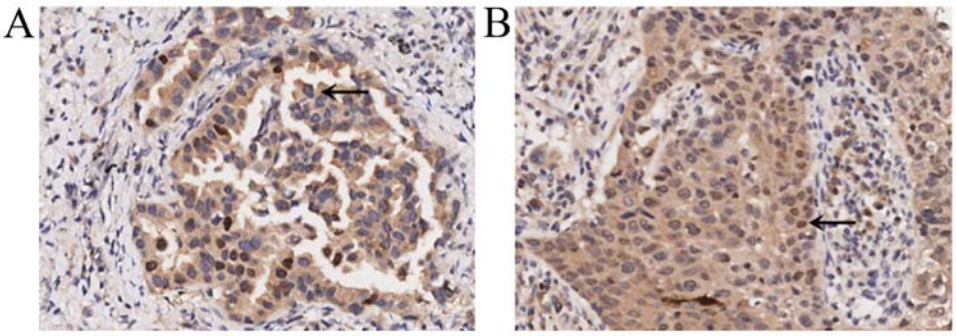

Figure 2. Interleukin-36γ protein expression levels in lung tumor tissues, shown as marked brown deposits (arrows) in (A) lung adenocarcinoma and (B) lung squamous cell carcinoma. Magnification, x200.

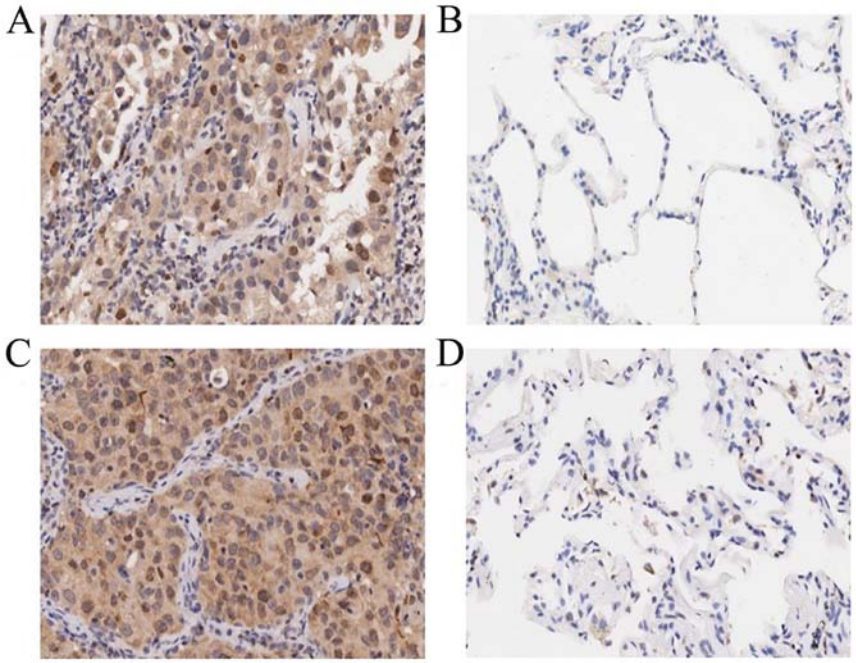

Figure 3. IL-36γ protein expression levels are upregulated in non-small cell lung cancer, shown as brown deposits. (A) High IL-36γ expression levels in lung adenocarcinoma tissues. (B) Low IL-36γ expression levels in adenocarcinoma-adjacent tissues. (C) High IL-36γ expression levels in squamous cell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

20 cases in stage IIb/III/IV). IL-36γ 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-36γ protein expression levels and clinicopathological features of patients with adenocarcinoma.

Clinicopathological feature	n (%)	IL-36γ expression levels -/+	IL-36γ 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. [a]P<0.05. IL, interleukin.

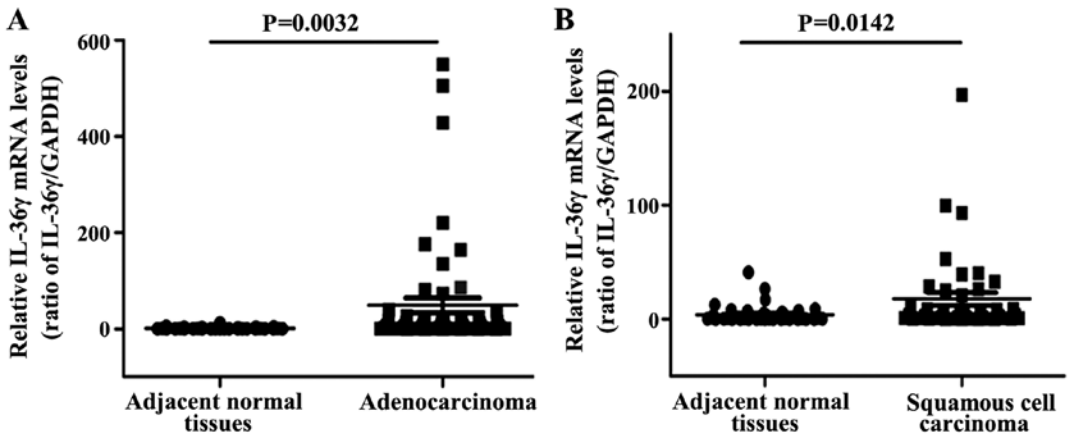

Figure 4. IL-36γ mRNA expression levels are upregulated in non-small cell lung cancer. IL-36γ mRNA expression levels in (A) adenocarcinoma tissues (n=57) and (B) squamous cell carcinoma (n=42) compared with respective adjacent normal tissues (P<0.05). IL, interleukin.

High IL-36γ 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-36γ protein expression levels and the clinical pathological parameters of NSCLC were investigated. Higher IL-36γ 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-36γ protein expression level and all other assessed clinical pathological parameters in the 74 cases of lung squamous cell carcinoma (Table V). However, IL-36γ 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-36γ 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-36γ protein expression levels and clinicopathological features of patients with squamous cell carcinoma.

Clinicopathological feature	n (%)	IL-36γ 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
≥60	47/73 (64.4)	19	28	
Pathological grade				0.1475
I	5/74 (6.7)	3	2	
II	61/74 (82.4)	28	33	
III	8/74 (10.8)	1	7	
Tumor size, cm				0.3312
<5.5	47/74 (63.5)	18	29	
≥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.

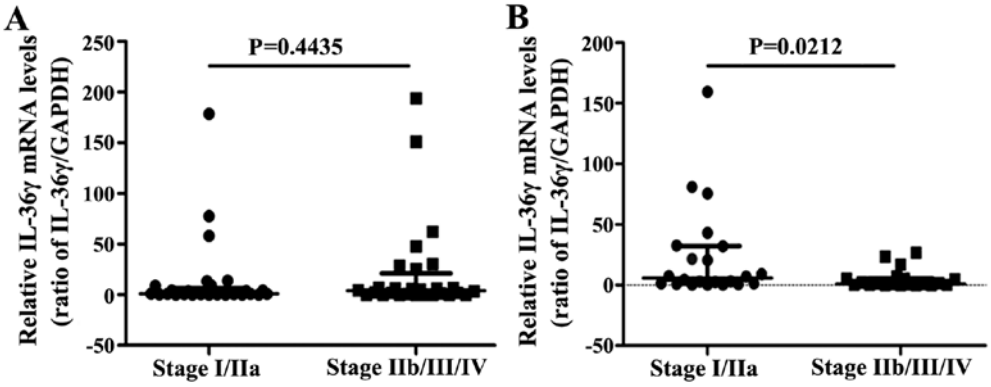

Figure 5. Association between IL-36γ mRNA expression levels and clinical TNM stage. IL-36γ mRNA expression and clinical TNM stage in (A) adenocarcinoma tissues (n=57) and (B) squamous cell carcinoma (n=42). IL, interleukin; TNM, Tumor-Node-Metastasis.

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-36γ protein expression levels experienced a longer survival time; however, this difference was not statistically significant (P=0.1343; Fig. 6A). In addition, IL-36γ 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-36γ 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).

	Univariate analysis		Multivariate analysis	
Clinicopathological feature	HR (95% CI)	P-value	HR (95% CI)	P-value
IL36γ 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:≥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:≥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).

	Univariate analysis		Multivariate analysis	
Clinicopathological feature	HR (95% CI)	P-value	HR (95% CI)	P-value
IL36γ 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:≥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:≥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

[a]P<0.01. IL, interleukin; HR, hazard ratio; CI, confidence interval.

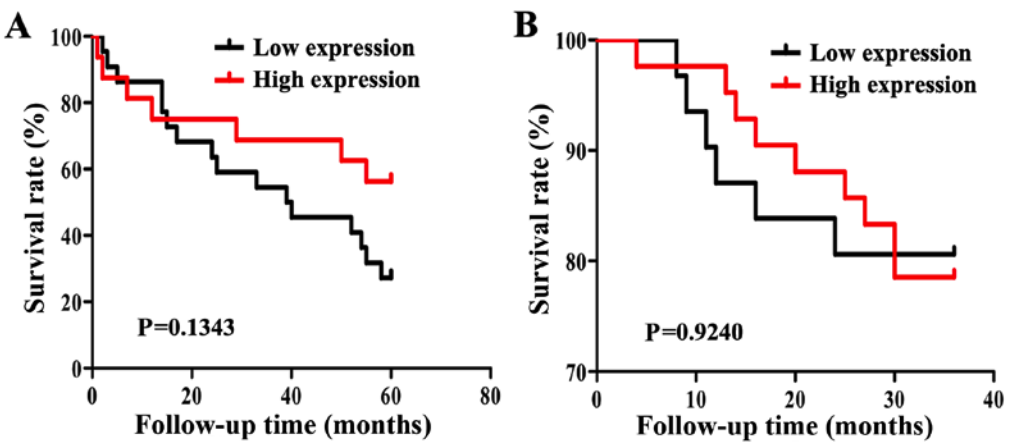

Figure 6. Association between IL-36γ expression levels and the survival of patients with non-small cell lung cancer. (A) The 5-year survival follow-up data for patients with lung adenocarcinoma with low and high IL-36γ expression levels (n=38). (B) The 3-year survival follow-up data for patients with squamous cell carcinoma with low and high IL-36γ 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-36γ mRNA and protein expression levels were upregulated in NSCLC. Elevated IL-36γ protein expression levels were significantly associated with a higher tumor grade of lung adenocarcinoma, and IL-36γ mRNA expression levels were inversely associated with clinical TNM stage in patients with squamous cell carcinoma. In addition, higher IL-36γ expression in patients with adenocarcinoma tended to prolong survival, although this was not statistically significant. These data suggest that IL-36γ 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-36γ 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-36γ in the differentiation of Th cells and type-1 immune responses (7,16,17). Our previous study showed that IL-36γ 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-36γ, melanoma tumor cells and breast cancer cells have previously been transfected to overexpress full-length IL-36γ in our laboratory (7). A mouse model demonstrated that IL-36γ 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-36γ 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-36γ 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-36γ, as an inflammatory cytokine, may serve an important role in inflammatory diseases and antitumor immunotherapy.

The IL-36γ protein is expressed in keratinocytes, bronchial epithelial cells and brain tissue, and IL-36γ expression levels in macrophages and neutrophils are significantly increased during infection (9,31,33). IL-36γ 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-36γ 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-36γ 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-36γ in a diffuse pattern.

IL-36γ mRNA and protein expression levels were significantly increased in NSCLC tissues compared with those in adjacent normal tissues in the present study. Higher IL-36γ 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-36γ 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-36γ expression levels were higher in the early stage compared with those in the advanced stage (7). It suggestes a potential antitumor effect of IL-36γ in squamous cell carcinoma.

Furthermore, in the present study, survival analysis showed that patients with adenocarcinoma with high IL-36γ 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-36γ and tumors (7,32). Although the underlying mechanism of IL-36γ in the antitumor immune response has been studied in animal models, the role of IL-36γ in human tumors is unclear (7). In the present study, IL-36γ expression patterns in human tumor tissues were investigated, aiming to determine the association between IL-36γ 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-36γ in NSCLC. The present study may also provide novel insight into the value of IL-36γ 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-36γ mRNA and protein expression levels and clinical parameters, and the mechanism underlying IL-36γ function to better determine its value for clinical application. Immunofluorescence co-localization of cellular markers (such as CD4, CD8 and CD56) and IL-36γ in tumor tissues may aid the identification of cell types that secret IL-36γ, facilitating further investigation of the underlying mechanisms of IL-36γ function.

Acknowledgements

Not applicable.

Funding

The present study was funded by The National Natural Science Foundation of China (grant nos. 81273208 and 31607916), The Major Basic Research Project of the Natural Science Foundation of the Jiangsu Higher Education Institutions (grant no. 18KJA180011) and The Suzhou Applied Basic Research-Medical Guidance Program (grant no. SYSD2016106).

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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