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Research Article

The Clinical Significance of IL-37-Mediated Regulation of VEGFC Expression in Lung Adenocarcinoma

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Abstract

Background: The aim of our study was to investigate the clinical significance of interleukin-37 (IL-37) and vascular endothelial growth factor C (VEGFC) expression in lung adenocarcinoma as well as its regulatory correlation.

Methods: The TCGA database was used to analyze the correlation between the expression of IL-37 and VEGFC mRNA and its correlation with the prognosis of patients. 60 lung adenocarcinoma tissues and matched clinical data were collected. The protein expression of IL-37 and VEGFC was analyzed by immunohistochemistry, which was used to analyze the correlation between IL-37 and VEGFC protein expression and its clinical significance. Western Blot was used to analyze VEGFC expression with enhanced expression of IL-37 in A549 cells.

Results: The mRNA expression levels of VEGFC and IL-37 were negatively correlated in the TCGA database ($r = -0.36$, $P < 0.0001$). Immunohistochemical staining showed that the expression of IL-37 was negatively correlated with the expression of VEGFC protein in 60 lung adenocarcinoma tissues ($r = -0.38$, $P < 0.001$). The median overall survival time of patients was significantly longer in the IL-37 protein overexpression group than in the lower expression group ($P < 0.05$), while it was significantly shorter in the VEGFC protein high expression group than in the lower expression group ($P < 0.05$). The expression of IL-37 and VEGFC was significantly correlated with the pathological stage ($P < 0.001$), N stage (IL-37, $P=0.037$; VEGFC, $P < 0.001$), and M stage (IL-37, $P=0.037$, VEGFC, $P=0.005$) of lung adenocarcinoma. Increased expression of IL-37 in A549 cells induced a significant downregulation of VEGFC expression.

Conclusion: IL-37 may play an antitumor role in lung adenocarcinoma by downregulating VEGFC and may be useful as a molecular biomarker for survival prediction and has potential as a therapeutic target in lung adenocarcinoma.

Keywords: Interleukin-37; vascular endothelial growth factor c; lung adenocarcinoma; tumor suppressor

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide, mainly due to its high incidence, high degree of malignancy and lack of effective treatments [1,2]. Lung cancer is currently divided into small cell lung cancer and non-small cell lung cancer. Among them, non-small cell lung cancer accounts for 85%-90% of cases. Non-small cell lung cancer is mainly comprised

of squamous cell carcinoma, adenocarcinoma and large cell carcinoma. In recent years, the morbidity of lung adenocarcinoma has increased [3]. Although the current strategies of early detection, early surgery and multidisciplinary treatment are in wide-spread use, the long-term prognosis is still poor because lung cancer is prone to recurrence and metastasis [4]. Therefore, it is necessary to elucidate the molecular mechanism of its recurrence and metastasis to identify biomarkers for predicting patient outcomes and to act as therapeutic targets. Interleukin-37 (IL-37), as a member of the interleukin family, was discovered in 2000 [5-7]. IL-37 inhibits inflammation and the immune response and participates in the pathophysiological process of acute myocardial infarction, sepsis and rheumatoid arthritis [8,9]. Recent studies have shown that IL-37 acts as a tumor suppressor gene in tumors. Zhao et al. [10] found that IL-37 exerts antitumor immunity by recruiting NK cells into the tumor microenvironment. Wang et al. [11] also found that IL-37 inhibits the proliferation of cervical cancer cells by inhibiting the STAT3 signaling pathway, reducing their aggressiveness. Guan et al. [12] showed that IL-37 inhibits the growth of lung cancer, which is partially mediated by blocking tumor angiogenesis. This study analyzed the correlation between IL-37 and VEGF gene expression in lung adenocarcinoma through the TCGA database. Meanwhile, we collected tumor tissue samples from patients with lung adenocarcinoma and analyzed the correlation between IL-37 and VEGFC protein expression and clinical features by immunohistochemistry. We also upregulated the expression of IL-37 in A549 lung adenocarcinoma cells to analyze its effect on the expression of VEGFC and to provide a basis for exploring IL-37 as a target for lung adenocarcinoma treatment.

Materials and Methods

Materials

TCGA Database Information

UCSC Xena (https://xenabrowser.net.datapages/) was used to download The Cancer Genome Atlas (TCGA) (https://cancergenome. nih.gov/) GDC lung adenocarcinoma data. An online heatmap graphic analysis tool and Kaplan–Meier Plotter data were used for online expression analysis and survival analysis.

Lung Adenocarcinoma Tissue Specimen Collection

Sixty pathologically diagnosed lung adenocarcinoma tissue specimens were collected from the First Affiliated Hospital of Jinan University. Meanwhile, we collected the clinical data of the patients, including the patient's age, sex, tumor TNM analysis, and degree of differentiation. The patients were followed up for 5 years. The pathological staging and TNM staging of patients with lung adenocarcinoma refer to the seventh edition of the International Association for the Study of Lung Cancer (IALSC) staging standards [13]. All patients signed an informed consent form. Their specific clinicopathological information is shown in Table 1.

Table 1: Clinicopathologic characteristics of 60 cases of patients with lung adenocarcinoma.

Characteristics	No. of case (%)	Characteristics	No. of case (%)
Age (y)		T4	10(20.0)
≤65	29(48.3)	**N classification**	
> 65	31(51.7)	N0	26(52.0)
Gender		N1	10(20.0)
Male	35(58.3)	N2	16(26.7)
Female	25(41.70)	N3	5 (8.3)
Differentiation		**M classification**	
Poor	25(41.7)	M0	53(88.3)
Moderate	27(45.0)	M1	7(11.7)
Well	8(13.3)	**Smoking**	
Pathological stage		Yes	32(63.9)
Ⅰ A	10(16.7)	No	28(36.1)
Ⅰ B	10(16.7)	**IL-37 expression**	
IIA	6 (10.0)	Negative/Low	31 (51.7)
IIB	8 (13.3)	High	29(48.3)
IIIA	11 (18.3)	**VEGFC expression**	
IIIB	8 (13.3)	Low expression	26(43.3)
Ⅳ	7(11.7)	High expression	34(56.7)

T classification		Vital status	
T1	18(30.0)	Death	31 (51.7)
T2	21(35.0)	Alive	29(48.3)
T3	7 (14.0)		

Lung Adenocarcinoma Cell Lines

The A549, H322, and PC-9 human lung adenocarcinoma cell lines and the normal control NHBE human bronchial epithelial cell line were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences.

Methods

Immunohistochemical Staining Analysis

The protein expression of IL-37 and VEGFC in paraffin-embedded lung adenocarcinoma tissue of patients with lung adenocarcinoma was analyzed by immunohistochemical staining with a pika universal streptavidin-HRP kit (CoWin Biosciences, Beijing, China), rabbit anti-IL-37 polyclonal antibody purchased from Sigma, and rabbit anti-VEGFC polyclonal antibody purchased from Gene Tex. All were applied at a working concentration of 1:200 antibody, and all steps were performed according to the instructions provided by the DAB chromogenic kit (CoWin Biosciences, Beijing, China). The pathological sections were stained and analyzed by two pathologists. The expression intensity of IL-37 and VEGFC in the lung adenocarcinoma sections was scored as previously described [14,15]. In brief, the staining intensity scores were as follows: 0 points (no staining), 1 point (slight staining: light yellow), 2 points (moderate staining: yellowish brown), and 3 points (heavy staining: brown). The number of stained positive cells was scored as follows: 0 points (no stained positive cells), 1 point (<10% positive cells), 2 points (10-50% positive cells), and 3 points (>50% positive cells). The expression intensity of the IL-37 and VEGFC protein staining was evaluated by the staining index (staining index = number of positive cells × staining intensity), which was recorded as 0, 1, 2, 3, 4, 6, and 9. The best cutoff value based on the log-rank test was used to define the protein expression levels of IL-37 and VEGFC. Among them, an IL-37 protein staining index >4 indicated high expression, and a VEGFC protein staining index >6 indicated high expression.

Cell Culture and Western Blot Analysis

The A549, H322, PC-9 and NHBE cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, CA) containing 10% fetal bovine serum (Gibco BRL, Carlsbad, CA), 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were grown in a 37 °C incubator containing 5% CO2. Total protein was extracted from the lung adenocarcinoma cells using a KEYGENE protein extraction kit. The proteins were separated by 10% SDS–PAGE and then transferred to PVDF membranes (Millipore), blocked with 5% skim milk, and incubated with rabbit anti-IL-37 antibodies (1:200, 24 kD, Sigma), rabbit anti-VEGFC antibodies (1:200, 47 kD, Gene Tex) and internal reference antibody rabbit anti-GAPDH (1:2500, BIOSS ANTIBODIES, Beijing, China) overnight at 4 °C. After rewarming at room temperature, 0.1% Tween-20 in PBS (PBST) was used to wash the membrane 3 to 5 times for 5 minutes each time. Following incubation with the HRP cross-linked mouse anti-rabbit II antibody (1:3000, CoWin Biosciences, Beijing, China), the cells were incubated for 1 hour at room temperature, washed with PBST 3~5 times, and then developed with ECL luminescent solution (CoWin Biosciences, Beijing, China). The blots were analyzed by the gel imager after recording images.

Plasmid Construction, Transfection and Western Blot Analysis

The recombinant IL-37 (NM_014439.3) cDNA plasmid pcDNA3.1(+)-IL-37 and the control empty plasmid pcDNA3.1(+) were purchased from Gene Copoeia. A549 cells were cultured to logarithmic growth phase and transfected with Lipo2000 transfection reagent. The total cell protein was extracted 72 hours after transfection, and IL-37 protein expression was detected along with any change in the VEGFC protein expression. The A549 parent cell line and the A549 cell line transfected with empty plasmid pcDNA3.1(+) or overexpression plasmid pcDNA3.1(+)-IL-37 were compared.

Statistical Analysis

Statistical analysis of the data was performed with SPSS 16.0 software (SPSS Inc., Chicago, IL). The correlations of the IL-37 and VEGFC mRNA and protein expression with the prognosis of the patients were analyzed by Kaplan–Meier and log-rank tests. Bivariable correlation analysis was performed by Pearson correlation test (bivariate normal distribution) and Sperman correlation test (abnormal distribution). The mean density of IL-37 and VEGFC staining in the lung adenocarcinoma tissue was analyzed by IPP graphical analysis software to express its staining intensity. And the Mann–Whitney U test was used to analyze the relationship between IL-37 and VEGFC protein expression and the clinical characteristics of the patients. P value less than 0.05 was considered statistically significant.

Results

Relationship between IL-37 and VEGFC Expression and its Relationship with the Patient Prognosis Shown by The TCGA Database

There was a total of 572 lung adenocarcinoma patients in the TCGA database. The heatmap shows the relationship between IL-37 and VEGFA, VEGFB, and VEGFC expression at the mRNA level. Among them, IL-37 and VEGFC expression were significantly negatively correlated, r=-0.36 and P <0.05, indicating a significant regulatory relationship between IL-37 and VEGFC expression (Figures 1A & 1B). Among the 572 patients with lung adenocarcinoma, 283 patients had high IL-37 mRNA expression, accounting for 49.5%, and 289 patients had low IL-37 mRNA expression, accounting for 50.5%. The 10-year overall survival time of patients with high IL-37 mRNA expression was significantly longer than that of patients with low IL-37 expression (P=0.038), suggesting that IL-17 may be a tumor suppressor gene in lung adenocarcinoma. There were 285 patients with high VEGFC expression, accounting for 49.8%, and 287 patients with low VEGFC expression, accounting for 50.2%. The 10-year overall survival time of patients with high VEGFC mRNA expression was significantly shorter than that of patients with low VEGFC expression, suggesting that VEGFC acts as an oncogene in lung adenocarcinoma. Based on the correlation analysis of its expression, IL-37 may have an antitumor effect by downregulating VEGFC.

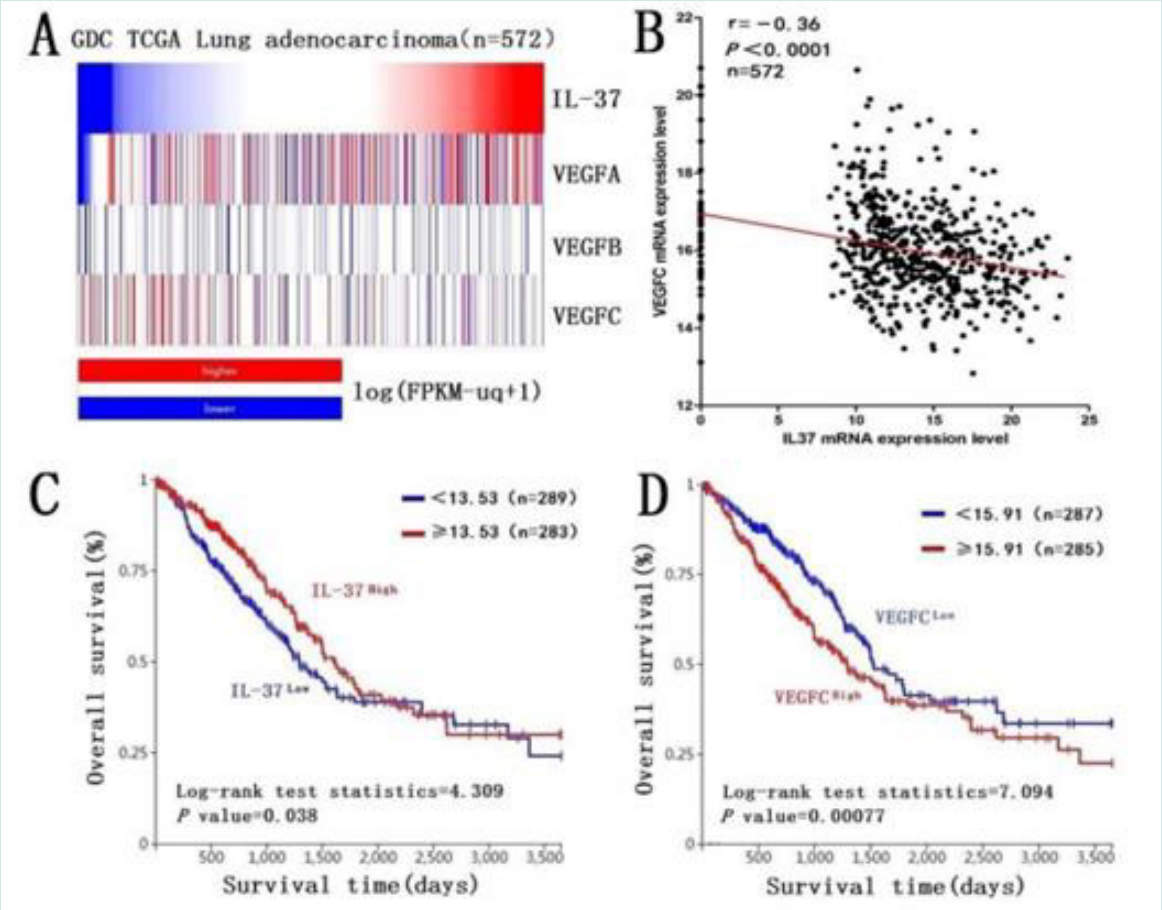

Figure 1(A): The correlation among IL-37 and VEGFA, VEGFB, and VEGFC mRNA levels in lung adenocarcinoma tissues from the TCGA database.

Figure 1(B): Correlation between IL-37 and VEGFC mRNA expression in lung adenocarcinoma with a correlation coefficient of -0.36, p < 0.0001.

Figure 1(C): Survival analysis shows that the overall survival time is better in lung adenocarcinoma patients with high IL-37 mRNA expression than in those with low IL-37 mRNA expression (P=0.038).

Figure 1(D): Survival analysis shows that lung adenocarcinoma patients with low VEGFC mRNA expression have better overall survival times than those with high VEGFC mRNA expression (P=0.00077).

Figure 1: The correlation between IL-37 and VEGFC expression and their relationship with the prognosis of patients with lung adenocarcinoma from the TCGA database.

Expression of IL-37 and VEGFC Protein in Tumor Tissues and Tumor Cells of Patients with Lung Adenocarcinoma

To further confirm that at the level of protein expression, IL-37 produces antitumor effects by regulating VEGFC, we performed immunohistochemical staining analysis of IL-37 and VEGFC protein expression in tissue specimens of 60 patients with lung adenocarcinoma. The results showed that among 60 patients with lung adenocarcinoma, there were 29 cases with high expression of IL-37 protein, accounting for 48.3%. A total of 31 patients had low expression of IL-37, accounting for 51.7%; 34 patients had high expression of VEGFC, accounting for 56.7%; and 26 patients had low expression of VEGFC, accounting for 43.3%, as shown in Figure 2A. In A549, H322, and PC-9 lung adenocarcinoma cells, IL-37 protein expression was significantly lower than its expression in NHBE normal bronchial mucosal epithelial cells, while VEGFC protein was the opposite, and the expression was significantly higher in lung adenocarcinoma cells. For its expression in normal bronchial epithelial cells, see Figure 2C. These data suggest that IL-37 may be a tumor suppressor gene, while VEGFC is an oncogene. The expression intensity of IL-37 and VEGFC in continuous sections of tumor tissues of 60 patients with lung adenocarcinoma was analyzed by the average optical density. The results showed that the protein expression of IL-37 and VEGFC were significantly negatively correlated r=-0.38 and $P < 0.05$, which is consistent with the results at the mRNA expression level, as shown in Figure 2B.

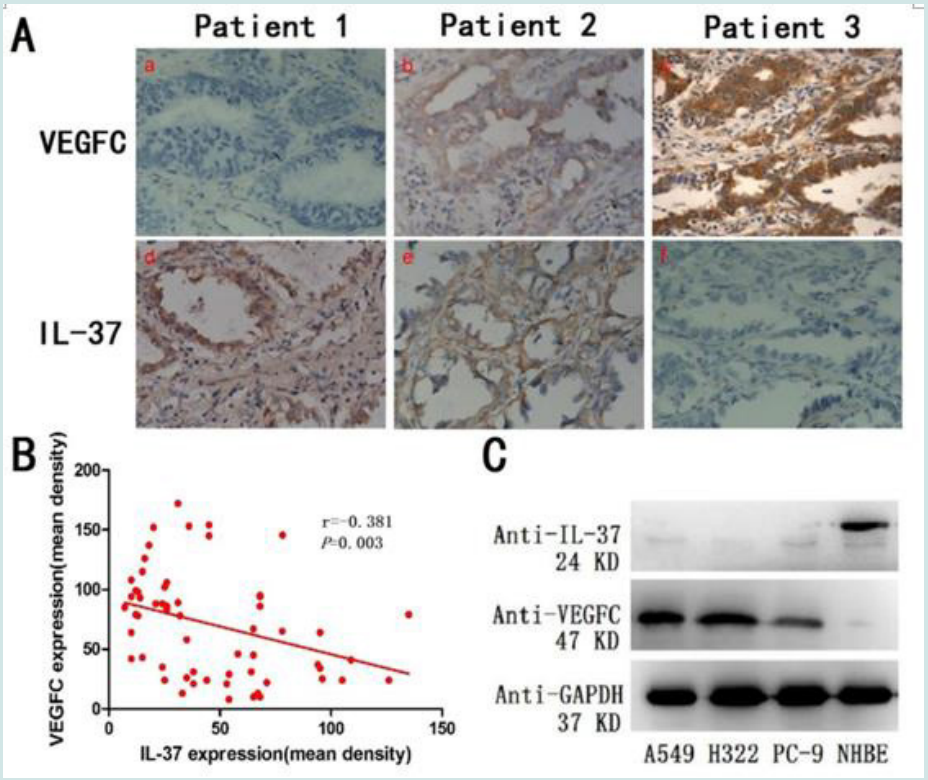

Figure 2(A): Representative image from 60 cases of lung adenocarcinoma with low expression of VEGFC and high expression of IL-37 by using IHC analysis (200). Scale bar: 20 μm.
Figure 2(B): The correlation between IL-37 and VEGFC protein expression from IHC analysis, r= −0.381, $P < 0.05$. C, IL-37 and VEGFC protein expression in A549, H322, PC-9 and NHBE cell lines.
Figure 2: The expression correlation between IL-37 and VEGFC in lung adenocarcinoma tissues and cells.

Relationship between the Protein Expression Level of IL-37 and VEGFC and the Overall Survival Time of the Patients

The tumor tissues of 60 patients with lung adenocarcinoma were analyzed by immunohistochemical staining. The log-rank test showed that the 5-year cumulative survival rate of the IL-37 protein high expression group was significantly better than that of the IL-37 low expression group (P=0.015). The 5-year cumulative survival rate of the VEGFC protein high expression group was significantly lower than that of the VEGFC protein high expression group (P=0.004). Kaplan–Meier analysis showed that for lung adenocarcinoma patients with high expression of IL-37 protein,

the 5-year median overall survival time was 54 months, while with low expression of IL-37 protein, the 5-year median overall survival time was 36 months. The difference was significant, suggesting a protective effect of IL-37 protein in patients with lung adenocarcinoma.

Meanwhile, the expression of VEGFC protein in tumor tissues of 60 patients with lung adenocarcinoma was analyzed. Among them, 34 cases had high expression, accounting for 56.7%. The 5-year median overall survival time of lung adenocarcinoma patients with high VEGFC expression was 31 months, while the 5-year median overall survival time of lung adenocarcinoma patients with low VEGFC protein expression was 57 months ($P < 0.05$), indicating that VEGFC in lung adenocarcinoma tissue plays a role in promoting cancer. See Figures 3A & 3B.

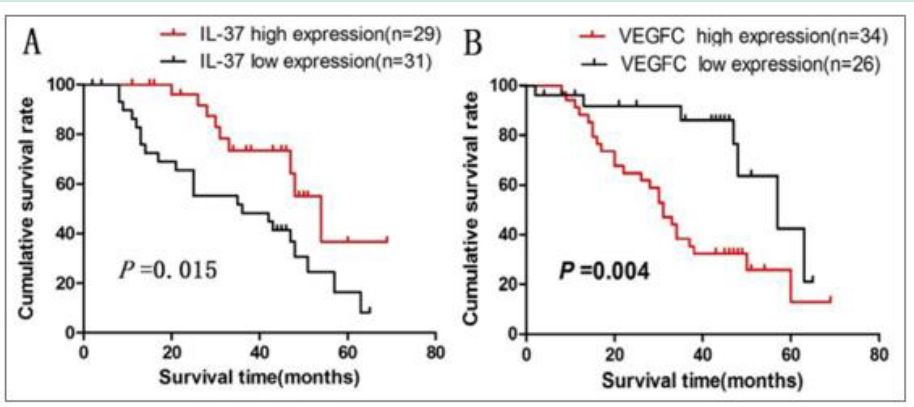

Figure 3(A): The overall survival time of patients with lung adenocarcinoma was significantly different between the high (red line)- and low (black line)-IL-37 expression groups (P=0.015).
Figure 3(B): The overall survival time of patients with lung adenocarcinoma was significantly different between the high (black line)- and low (red line)-VEGFC expression groups (P=0.004).
Figure 3: The correlation between IL-37 or VEGFC protein expression and the overall survival rate of patients with lung adenocarcinoma.

Relationship between IL-37 and VEGFC Protein Expression and the Clinical Characteristics of the Patients.

Low expression of IL-37 protein and high expression of VEGFC protein are related to a poor prognosis, but it is still unclear which functions of adenocarcinoma cells are mainly affected by IL-37 and VEGFC protein. We used the Mann–Whitney U test to analyze the correlation between the age, sex, degree of differentiation, pathological stage and TNM stage of the patients with the IL-37 and VEGFC protein expression levels. The results showed that the protein expression levels of IL-37 and VEGFC were correlated with those in lung adenocarcinoma patients. The pathological stage, N stage, and M stage were significantly related (Table 2), suggesting that IL-37 and VEGFC may affect the invasion and metastasis ability of lung adenocarcinoma cells. Combined with the negative correlation between the expression of IL-17 and VEGFC in lung adenocarcinoma tissue, these results suggest that IL-37 affects the invasion and metastasis of lung adenocarcinoma cells by downregulating VEGFC.

Table 2: The correlation between IL-37 and VEGFC protein expression levels and Clinicopathologic characteristics in 60 cases of patients with lung adenocarcinoma.

Clinicopathologic characteristics	IL-37 expression		Mann-Whitney U	VEGFC expression		Mann-Whitney U
	None/Low (n)	High (n)	(P value)	None/Low (n)	High (n)	(P value)
Age(y)						
≤65	15	11	0.09	11	18	0.83
>65	11	20		15	16	
Gender						
Male	20	15	0.69	19	16	0.32
Female	13	12		7	18	

Smoking						
No	11	17	0.075	10	18	0.27
Yes	20	12		16	16	
Differentiation						
Poor	11	14	0.32	12	15	0.73
Moderate /well	20	15		14	21	
Pathological stage						
Ⅰ	5	15	< 0.001	14	6	< 0.001
II	6	8		5	9	
III	14	5		6	13	
Ⅳ	6	1		1	6	
T stage						
T1	10	8	0.48	8	10	0.63
T2	10	11		8	13	
T3	2	5		2	5	
T4	5	5		4	6	
N stage						
N0	18	8	0.018	18	8	< 0.0001
N1-3	13	21		8	26	
M stage						
M0	30	23	0.037	26	27	0.005
M1	1	6		0	7	

Effect of Upregulating the Expression of IL-37 Protein in A549 Cells on the Expression of VEGFC

To further confirm whether IL-37 plays an antitumor effect in lung adenocarcinoma by downregulating VEGFC protein, we upregulated the expression of IL-37 protein in the lung adenocarcinoma cell line A549 to analyze its effect on VEGFC protein expression. The A549 cell line was transfected with the empty plasmid and the IL-37 full-length cDNA plasmid overexpressing IL-37. Total cell protein was extracted 72 hours after transfection, and Western Blot analysis was performed with the total protein of the parent A549 cells acting as a control. The results showed that in the A549 cell line transfected with the full-length IL-37 cDNA plasmid, IL-37 protein expression was significantly higher than that in the parental cell line and the A549 cell line transfected with the empty plasmid, indicating successful transfection. Meanwhile, Western Blot analysis showed that when upregulating the expression of IL-37 in A549 cells, the protein expression level of VEGFC was significantly lower than that in the A549 cell line transfected with the empty vector and the parental cell line. The difference was significant, suggesting that IL-37 can downregulate the expression of VEGFC protein in A549 cells. The results are shown in Figures 4A & 4B.

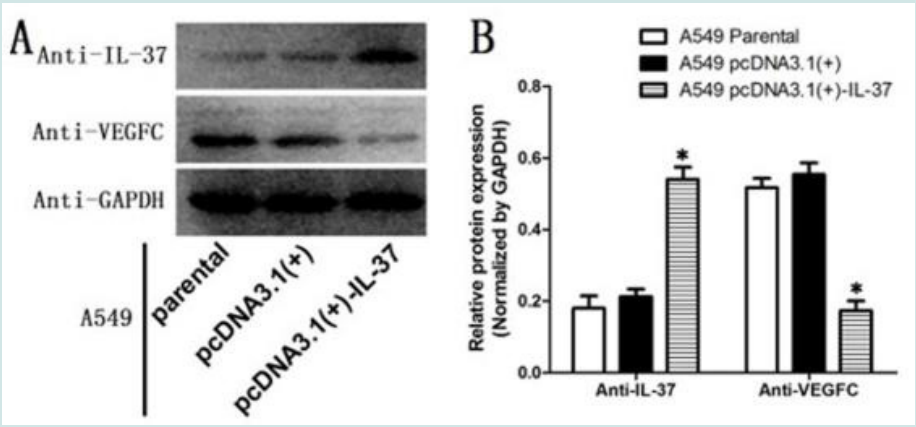

Figure 4(A): Western Blot analysis shows that enhanced expression of IL-37 inhibits VEGFC expression.
Figure 4(B): The graph shows that with overexpression of IL-37, the VEGF protein was decreased significantly compared with that in the control groups. *p < 0.05.
Figure 4: Overexpression of IL-37 inhibited VEGFC protein expression in A549 cells.

Discussion

As an inhibitor of human innate immunity and the inflammatory response, IL-37 is highly expressed in inflammatory tissues, thereby inhibiting the damage caused by excessive immunity to the body [16]. In recent years, studies have found that IL-37 plays an antitumor effect in liver cancer, cervical cancer, and non-small cell lung cancer [10,11]. In non-small cell lung cancer, IL-37 inhibits tumor growth and invasion by inhibiting tumor angiogenesis [12]. However, the mechanism and clinical significance of IL-37 in non-small cell lung cancer, especially in lung adenocarcinoma, has not been further explored.Our analysis found that in the TCGA lung adenocarcinoma database, IL-37 and VEGFC were significantly negatively correlated at the mRNA level. The 10-year cumulative survival time of patients with high IL-37 or low VEGFC expression was significantly longer than that of patients with low IL-37 or high VEGFC expression. At the protein level, we performed immunohistochemical staining analysis on tissue specimens of 60 patients with lung adenocarcinoma. It is found that patients with high IL-37 expression had a 5-year median overall survival time of 54 months, while those patients who had low IL-37 protein expression, the 5-year median overall survival time was 36 months. The difference between them was significant, suggesting a protective effect of IL-37 protein in patients with lung adenocarcinoma. Meanwhile, the expression of VEGFC protein in tumor tissues of 60 patients with lung adenocarcinoma was analyzed. Among them, 34 cases had high expression, accounting for 56.7%. The 5-year median overall survival time of lung adenocarcinoma patients with high expression of VEGFC was 31 months, and the 5-year median overall survival time of lung adenocarcinoma patients with low expression of VEGFC protein was 57 months, P<0.05, suggesting that VEGFC plays a role in promoting cancer, leading to a poor prognosis of patients with lung adenocarcinoma. Zhao et al. [10] showed that in liver cancer tissues, the expression of IL-37 is negatively correlated with tumor size, suggesting that IL-37 inhibits tumor growth. Guan et al. [12] also showed that IL-37 is expressed in tissues adjacent to lung adenocarcinoma, with its expression in lung adenocarcinoma tissues is significantly downregulated, and the low expression of IL-37 is associated with a poor prognosis. Downregulation of IL-37 is significantly related to advanced TNM staging, suggesting that IL-37 inhibits the progression of non-small cell lung cancer. We found through correlation analysis that the level of IL-37 expression is mainly related to the frontal N stage and M stage of lung adenocarcinoma patients, suggesting that IL-37 may be related to tumor invasion and metastasis. IL-37 can inhibit the angiogenesis of lung adenocarcinoma, possibly by inhibiting the expression of CD34 in the tumor microenvironment, thereby inhibiting tumor growth [12]. Tumor angiogenesis is critical to tumor formation, progression, invasion and metastasis, and the main promoters of tumor angiogenesis are the VEGF family [17], which includes VEGFA, VEGFB, VEGFC and VEGFD. Through the analysis of the TCGA lung adenocarcinoma database data, we found that the expression of IL-37 was mainly negatively correlated with the expression of VEGFC, r=-0.36 (P < 0.0001). There was no significant correlation with VEGFA and VEGFB, suggesting that IL-37 may have a specific negative regulatory relationship with VEGFC. In lung adenocarcinoma cells, Western Blot analysis showed that the expression of IL-37 was significantly downregulated in A549, H322, and PC-9 cells, while the expression was significantly increased in normal bronchial epithelial cells. In contrast, VEGFC is highly expressed in lung adenocarcinoma cells and downregulated in normal tissues. The immunohistochemical staining analysis of IL-37 and VEGFC in 60 lung adenocarcinoma tissues showed that

there was a significant negative correlation, indicating that IL-37 may have an antitumor angiogenesis effect by downregulating VEGFC. To further confirm the regulation of VEGFC by IL-37, we upregulated the protein expression of IL-37 in A549 cells. Compared with the control group, the expression of VEGFC was significantly downregulated, indicating that IL-37 can downregulate the expression of VEGFC. Based on our findings, IL-37's inhibitory effect on tumor angiogenesis in lung adenocarcinoma may occur through downregulation of VEGFC. However, how IL-37 affects the expression of VEGFC needs to be studied further at the molecular mechanism level.

Conclusion

Upregulation of the expression of IL-37 in lung adenocarcinoma cells significantly downregulated the expression of VEGFC protein, suggesting that IL-37 may play a role as a tumor suppressor gene in lung adenocarcinoma by downregulating the expression of VEGFC. IL-37 may be a useful molecular marker for survival prediction and has potential as a therapeutic target in lung adenocarcinoma.

Ethics Statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of Jinan University, and the patients' written informed consent was waived by the Ethics Committee.

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Authors' Contributions

Chuang Hu designed this study; Wenjia Lai collected the clinical data; Xingdong Cai performed statistical analyses; Renfa Lai and Chuang Hu gave critical comments and suggestions; Wenjia Lai and Chuang Hu drafted the manuscript.

References

1. Aberle DR, Adams AM, Berg CD (2011) National Lung Screening Trial Research Team: Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 365: 395-409.
2. Wu H, Qiao N, Wang Y (2013) Association between the telomerase reverse transcriptase (TERT) rs2736098 polymorphism and cancer risk: Evidence from a case-control study of non-small-cell lung cancer and a meta-analysis. PLoS One 8(11): e76372.
3. Pao W, Girard N (2011) New driver mutations in non-small-cell lung cancer. Lancet Oncol 12(2): 175-180.
4. Torre LA, Bray F, Siegel RL (2015) Global cancerstatistics, 2012. CA Cancer J Clin 65(2): 87-108.
5. Smith DE, Renshaw BR, Ketchem RR (2000) Four new members expand the interleukin-1 superfamily. J Biol Chem 275(2): 1169-1175.
6. Kumar S, McDonnell PC, Lehr R (2000) Identification and initial characterization of four novel members of the interleukin-1 family. J Biol Chem 275(14): 10308-10314.
7. Busfield SJ, Comrack CA, Yu G (2000) Identification and gene organization of three novel members of the IL-1 family on human chromosome 2. Genomics 66(2): 213-216.
8. Xu D, Wang A, Jiang F (2015) Effects of interleukin-37 on cardiac function after myocardial infarction in mice. Int J Clin Exp Pathol 8(5): 5247-5251.
9. Xia L, Shen H, Lu J (2015) Elevated serum and synovial fluid levels of interleukin-37 in patients with rheumatoid arthritis: Attenuated the production of inflammatory cytokines. Cytokine 76(2): 553-557.
10. Zhao JJ, Pan QZ, Pan K (2014) Interleukin-37 mediates the antitumor activity in hepatocellular carcinoma: role for CD57+ NK cells. Sci Rep 4(7503): 5177.
11. Wang S, An W, Yao Y (2015) Interleukin 37 Expression Inhibits STAT3 to Suppress the Proliferation and Invasion of Human Cervical Cancer Cells. J Cancer 6(10): 962-969.
12. Guanqun Ge, Aiqin wang, Jingyue Yang (2016) Interleukin-37 suppresses tumor growth through inhibition of angiogenesis in non-small cell lung cancer. Journal of Experimental &Clinical Cancer Research 35(1): 1-10.
13. Postmus PE, Brambilla E, Chansky K (2007) The IASLC Lung Cancer Staging Project: proposals for revision of the M descriptors in the forthcoming (seventh) edition of the TNM classification of lung cancer. J Thorac Oncol 2: 686-693.
14. Cai XD, Zhou YB, Huang LX (2012) Reduced expression of Krüppel-like factor 17 is related to tumor growth and poor prognosis in lung adenocarcinoma. Biochem Biophys Res Commun 418(1): 67-73.
15. Lu JK, Wang YQ, Sun YH (2008) Investigation of the relationship between COX-2 expression and multidrug resistance in non-small cell lung cancer. Chinese Journal of Pathophysiology 24(3): 605-616.
16. Nold MF, Nold-Petry CA, Zepp (JA 2010) IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol 11: 1014-1022.
17. Kachroo P, Lee MH, Zhang L (2013) IL-27 inhibits epithelial-mesenchymal transition and angiogenic factor production in a STAT1-dominant pathway in human non-small cell lung cancer. J ExpClin Cancer Res 32(1): 97.

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