Original Article

Preliminary exploration of poor prognostic factor IL-33 and its involvement in perioperative immunotherapy in stage II–III lung squamous cell carcinoma: a retrospective cohort study

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Background: Current knowledge about the prognostic role of interleukin-33 (IL-33) in lung squamous cell carcinoma (LUSC) remains limited, particularly in stage II–III patients. This study aimed to verify the correlation between IL-33 expression and poor prognosis in stage II–III LUSC patients at both gene and protein levels and to investigate the potential role of IL-33 blockade in combination with immune checkpoint inhibitors (ICIs) in perioperative immunotherapy.

Methods: A retrospective analysis was conducted of 103 patients with stage II–III LUSC who underwent surgical resection at Tianjin Medical University Cancer Institute & Hospital from November 1, 2004, to November 30, 2006. Of these, 83 patients were included based on complete follow-up data, and were divided into a gene expression group (38 patients) and a protein expression group (45 patients). IL-33 expression was analyzed using real-time quantitative polymerase chain reaction (RT-qPCR) and immunohistochemistry (IHC). The correlation between IL-33 expression and overall survival (OS) was assessed using Kaplan-Meier survival analysis. Additionally, IHC results from 20 patients were used to explore the correlation between IL-33, programmed death ligand 1 (PD-L1), and Ki-67 expression levels. The total follow-up time exceeded 60 months, and the study endpoint was OS.

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Results: Patients with high IL-33 expression had significantly shorter OS compared to those with low IL-33 expression, both at the gene (P=0.006) and protein expression (P=0.01). Logistic regression analysis confirmed IL-33 as an independent prognostic factor for poor survival in stage II–III LUSC (P_{gene}=0.04, $P_{protein}$=0.009). Additionally, a significant positive correlation was observed between the protein expression of IL-33 (P=0.03), PD-L1 (P<0.001), and Ki-67 (P=0.01), indicating that high expression of these markers is associated with worse prognosis.

Conclusions: High IL-33 expression in cancer tissues is associated with poor prognosis in stage II–III LUSC. IL-33 blockade combined with ICIs may provide new treatment regimens and ideas for perioperative immunotherapy in stage II–III LUSC patients.

Keywords: Stage II–III lung squamous cell carcinoma (stage II–III LUSC); interleukin-33 (IL-33); programmed death ligand 1 (PD-L1); perioperative immunotherapy

Submitted Jul 15, 2024. Accepted for publication Sep 03, 2024. Published online Sep 18, 2024.
doi: 10.21037/jtd-24-1122
View this article at: https://dx.doi.org/10.21037/jtd-24-1122

Introduction

Lung cancer remains the leading cause of cancer-related morbidity and mortality worldwide, as highlighted by the 2022 Report on the Burden of Malignant Tumors in China released by the National Cancer Center, which indicates that lung cancer has the highest incidence and mortality rates among all cancers (1). Globally, the 5-year overall survival (OS) rate for lung cancer ranges from 10% to 20%, with a specific rate of 19.7% reported in China. Studies indicated that early diagnosis and treatment are crucial for improving the survival outcomes of lung cancer patients (2,3). Lung squamous cell carcinoma (LUSC), a common type of non-small cell lung cancer (NSCLC), accounts for approximately 25% of all NSCLC cases. The prognosis of LUSC can be particularly poor due to the high likelihood of metastasis, as illustrated in recent case reports highlighting the gene profiling of metastatic sites post-surgery (4,5). Given the aggressive nature of LUSC and the lack of targetable genetic alterations, there is an urgent need to identify molecular biomarkers that can predict prognosis and guide treatment strategies.

In recent years, several prognostic biomarkers have been identified in NSCLC, such as epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) rearrangements (6,7). These biomarkers have been instrumental in advancing targeted therapies and improving patient outcomes. However, these biomarkers are primarily associated with lung adenocarcinoma (LUAD) rather than LUSC, highlighting a significant gap in the identification of reliable prognostic markers for LUSC (8). Previous study has also been limited by small sample sizes and a lack of comprehensive analysis across different NSCLC subtypes, resulting in inconclusive findings, especially in the context of LUSC (9). Furthermore, recent bioinformatics analyses have revealed distinct molecular profiles in LUSC compared to LUAD, further underscoring the need for specific research focused on LUSC biomarkers (10).

Interleukin-33 (IL-33) is a member of the IL-1 cytokine family and has been shown to play a complex role in tumor biology, influencing tumor transformation, growth, and metastasis in various malignant tumors, including

Highlight box

Key findings

- Interleukin-33 (IL-33) was identified as an independent prognostic factor for patients with stage II and III lung squamous cell carcinoma (LUSC).

What is known, and what is new?

- This study verified the correlation between IL-33 and a poor prognosis in stage II–III LUSC at the gene and protein expression levels.
- This study explored the potential role of IL-33 blockade combined with immune checkpoint inhibitors (ICIs) in perioperative immunotherapy for stage II–III LUSC patients.

What is the implication, and what should change now?

- This study suggested that IL-33 blockade combined with ICIs in perioperative immunotherapy may improve the effect of anti-tumor immunotherapy. However, further confirmation is required through relevant animal experiments and clinical trials.

NSCLC (11). IL-33 can exert its effects either by directly acting on tumor cells or indirectly affecting immune cells and tumor microenvironment. As is reported, the tumor microenvironment plays a critical role in the progression and prognosis of LUSC, with recent studies correlating various microenvironment-related markers to clinical outcomes (12). A study has shown that serum IL-33 levels are significantly higher in NSCLC patients than both healthy volunteers and individuals with benign lung diseases, and IL-33 serves as an independent prognostic factor for NSCLC (13). However, Yang *et al.* (14) found that lower IL-33 expression is associated with a poorer prognosis in LUAD. To date, there are no clear reports on relevant studies of IL-33 in LUSC, indicating a gap that this study aims to address.

Currently, multidisciplinary treatment combined with individualized treatment is recommended for the treatment of lung cancer (15). In recent years, clinical trials focusing on perioperative immunotherapy, or perioperative immunotherapy combined with chemotherapy for resectable stage II–III NSCLC have yielded promising preliminary results, offering hope for surgical intervention and the prognosis of locally advanced patients (16,17). This study retrospectively analyzed the prognostic role of IL-33 in stage II–III LUSC at both the gene transcription and protein expression levels, using clinical data from patients who underwent surgical resection. The study further explored the correlation between IL-33 and programmed death ligand 1 (PD-L1) expression, aiming to identify potential new targets for perioperative immunotherapy in stage II–III LUSC patients. We present this article in accordance with the REMARK reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1122/rc).

Methods

Clinical data and experimental methods

Clinical data

This retrospective cohort study included data from 103 patients who underwent surgical resection of LUSC at Tianjin Medical University Cancer Institute & Hospital from November 1, 2004, to November 30, 2006. These patients had not received any anti-tumor treatments, such as chemotherapy, radiotherapy, targeted therapy, or immunotherapy before surgery. All patients were pathologically confirmed to have stage II–III LUSC postoperatively, with a total follow-up time exceeding 60 months. The primary endpoint of the study was OS.

The 103 patients were divided into two main groups for analysis. In the first group, the relationship between IL-33 expression and prognosis was evaluated in 83 patients. These patients were further randomly assigned to either a gene expression group (group A, 38 patients) or a protein expression group (group B, 45 patients). The remaining 20 patients (group C) were used for correlation analysis and Kaplan-Meier survival analysis of IL-33, PD-L1, and Ki-67 expression based on IHC results.

This research was approved by the Ethics Committee of Tianjin Medical University (No. Ek2024055) and was conducted in accordance with the principles outlined in the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all patients. Tissue samples were rapidly frozen and stored at −80 ℃ for subsequent analysis.

Sample size estimation and follow-up procedures

Sample size estimation was conducted based on prior studies suggesting that IL-33 might be a significant prognostic factor in NSCLC. We calculated that a minimum sample size of 85 patients would be necessary to detect a meaningful difference in survival outcomes with a power of 0.80 and an alpha level (α) of 0.05. Follow-up procedures included regular clinical evaluations, imaging studies, and laboratory tests at 3-month intervals during the first 2 years post-surgery, followed by 6-month intervals until 5 years post-surgery.

Assessment of baseline clinical factors and measurement of prognosis outcome

Baseline clinical factors such as sex, age, smoking history, and tumor stage were assessed at the time of diagnosis. Prognostic outcome was measured in terms of OS, defined as the time from the date of surgery to the date of death from any cause.

Real-time quantitative polymerase chain reaction (RT-qPCR) analysis for gene expression

Total RNA was extracted using the TRIzol kit (Thermo, Grand Island, NY, USA) according to the manufacturer's instructions. The concentration and purity of the RNA was detected by UV-1800 ultraviolet spectrophotometer (Shimadzu, Kyoto, Japan), and the total RNA contents were calculated. A Molony murine leukemia virus (M-MLV) reverse transcription kit (Promega, Madison, WI, USA) was

Table 1 Primer design for RT-qPCR

Gene names	Primer sequence (5'-3')	Segment length (bp)
IL-33	Forward primer 5'-GTGACGGTGTTGATGGTAAGAT-3'	94
	Reverse primer 5'-AGCTCCACAGAGTGTTCCTTG-3'	
β-actin	Forward primer 5'-GAGCACAGAGCCTCGCCTTT-3'	129
	Reverse primer 5'-GGTGAGCTGCGAGAATAGCC-3'	

RT-qPCR, real-time quantitative polymerase chain reaction; IL-33, interleukin-33.

used to reverse transcribe the RNA into complementary DNA (cDNA) according to the instructions. An equal amount of RNA (1 μg) was reverse transcribed into cDNA for detection in the SYBR Premium Ex Taq™ 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The primers were synthesized by Santa Cruz (Dallas, TX, USA), and their sequences are listed in *Table 1*. The reverse transcription reaction mixture was 20 μL in total, comprising 10 μL of 2× SYBR Green premix, 0.4 μL each of 10 μmol/L forward and reverse primers, 2 μL of cDNA, with the remainder made up of double-distilled water. The reaction conditions were as follows: pre-denaturation at 95 ℃ for 2 minutes, followed by denaturation at 95 ℃ for 45 seconds, annealing at 60 ℃ for 20 seconds for 40 cycles, and final extension at 72 ℃ for 5 minutes to terminate the reaction. All the experiments were conducted in triplicate. ΔCT and ΔΔCT were calculated as follows: $\Delta CT = CT_{target\ gene} - CT_{reference\ gene}$; $\Delta\Delta CT = \Delta CT_{sample} - \Delta CT_{sample\ average}$. The relative expression quantity of the messenger RNA was calculated by $2^{-\Delta\Delta CT}$.

Immunohistochemistry (IHC) for protein expression

The streptavidin peroxidase method for IHC was used to detect the protein expression of IL-33 in the LUSC tissues. The paraffin-embedded tissue sections were sliced at a thickness of 4 μm. After baking at 65 ℃ for 20 minutes and deparaffinization in xylene, all the samples were rehydrated in graded alcohol and then blocked with 30 mL/L H_2O_2. Antigens were retrieved with heated citrate buffer (pH 6.0) for more than 20 minutes. The samples were blocked with rabbit anti-human serum. Subsequently, the sections were incubated overnight at 4 ℃ with appropriately diluted primary antibodies: IL-33 (1:200; bs-2633R; Bioss, Woburn, MA, USA); Ki-67 (1:200; bs-23103R; Bioss); PD-L1 (1:200; bs-22022R; Bioss). Following this, sections were incubated at room temperature for 30 minutes with secondary mouse anti-rabbit antibody labeled with streptavidin-horseradish peroxidase (HRP) (1:1,000). Finally, 3,3'-diaminobenzidine (DAB) staining kit (Polymer, Mentor, OH, USA) was used for visualization.

Under a light microscope, five representative high-power fields (400× magnification) were selected for each section and observed, and yellow to brownish-yellow or dark brown indicated a positive reaction.

Method for determining IL-33 IHC results

The positive rate referred to the percentage of positively stained cells in the cancer tissues, and was calculated as follows: 0–10%, a score of 0; 11–25%, a score of 1; 26–50%, a score of 2; 51–75%, a score of 3; and >75%, a score of 4.

In relation to the staining intensity score, the positively stained cells in the LUSC samples were categorized into the following four ranks based on their staining intensity: negative (which was scored as 0) for unstained cells; weakly positive (which was scored as 1) for cells showing a light yellow stain; moderately positive (which was scored as 2) for cells exhibiting a brownish-yellow stain; and strongly positive (which was scored as 3) for cells displaying a dark brown stain.

In relation to the results, the following formula was used: total score of each post-stain section = score of intensity of staining for that section × the number of staining positive cells for that section. A final score of ≤5 was defined as low expression and a final score >5 was defined as high expression (18).

Method for determining the Ki-67 IHC results

Five high-power fields were randomly selected for each slide, and the cell positive rate was calculated as follows: 0–5%: a score of 1; 6–50%: a score of 2; 51–75%: a score of

3; and 76–100%: a score of 4. A final score of ≤2 was defined as low expression, and a final score of >3 was defined as high expression (19).

Method for determining PD-L1 IHC results

Scoring was based on the proportion of positive cells. The positive cell proportion score involved randomly selecting five high-power fields (400×) under a light microscope, calculating the percentage of tumor cells showing positive staining in each field relative to all the tumor cells present, and using the average percentage across fields as the positive cell percentage. Based on the proportion of positive cells, the cells were scored as follows: no tumor cells stained: a score of 0; 1–49% stained, a score of 1; and >50% stained, a score of 2. A final score of 0–1 was defined as low expression, and a final score of 2 was defined as high expression (20).

Statistical analysis

Data were analyzed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as median with interquartile ranges, and categorical variables were presented as frequencies and percentages. The hierarchical data were tested using a rank-sum test. The survival curve was drawn using the Kaplan-Meier method, and the survival analysis was performed using the log-rank test. Pearson correlation analysis was used to evaluate the relationships among the groups. The independent prognostic role of IL-33 (both gene and protein expression) in OS was assessed using multivariate logistic regression analysis. The regression model included IL-33 expression, tumor-node-metastasis (TNM) stage, T stage, and N stage as independent variables. Statistical significance was set at P<0.05, and all tests were two-sided.

Results

Relationship between IL-33 expression and clinicopathological characteristics in patients with stage II–III LUSC

IL-33 gene and protein expression in the LUSC tissues were analyzed to determine their correlation with the clinicopathological characteristics of the patients. The study included 103 patients, with 38 patients in the *IL-33* gene expression group and 45 patients in the IL-33 protein expression group, all of whom had complete medical records. The analysis revealed no significant correlation between IL-33 expression (both gene and protein levels) and the gender, age, and smoking status of the patients (P>0.05 for all comparisons). However, a statistically significant correlation was observed between IL-33 expression and TNM stage, T stage, and N stage (P<0.05) (*Table 2*).

Survival analysis of IL-33 in patients with stage II–III LUSC

Survival analysis was conducted to evaluate the relationship between *IL-33* gene expression and OS in patients with stage II–III LUSC. In the *IL-33* gene expression group (n=38), the median survival time for patients with high *IL-33* gene expression group (n=22) was 28 months, while the low *IL-33* gene expression group (n=16) did not reach the median survival time, indicating a significantly higher survival rate in the low expression group (P=0.006) (*Figure 1A*).

Similarly, in the IL-33 protein expression group (n=45), the high IL-33 protein expression group (n=28) had a median survival time of 26.3 months, whereas the low IL-33 protein expression group (n=17) did not reach the median survival time, demonstrating better survival outcomes in the low expression group (P=0.01) (*Figure 1B*).

Logistic regression analysis predicting prognostic factors in patients with LUSC

A multiple regression analysis was conducted to identify predictive variables using IL-33, TNM stage, T stage, and N stage as the independent variables, and OS as the dependent variable. The regression analysis revealed that both *IL-33* gene expression and IL-33 protein expression were significant independent prognostic factors for OS. Specifically, the $IL\text{-}33_{(\text{gene expression})}$ group had an *F* score of 7.761 (P<0.05), and the $\text{IL-33}_{(\text{protein expression})}$ group had an *F* score of 4.414 (P<0.05). The overall significance test of the multiple linear regression indicated that the regression model was significant, which suggested that at least one independent variable would have an effect on the dependent variable. The stepwise regression analysis revealed that the independent risk factor affecting OS in stage II–III LUSC was IL-33 (P<0.05). Thus, in this study, the higher level of IL-33 in the cancer tissue of the stage II–III LUSC patients, the shorter the OS, which in turn indicated a poorer prognosis (*Tables 3*,*4*).

Table 2 Relationship between *IL-33* gene (protein) expression and the clinical parameters of patients with LUSC

Clinicopathological characteristics	*IL-33* gene expression			IL-33 protein expression		
	Low expression (n=16)	High expression (n=22)	P	Low expression (n=17)	High expression (n=28)	P
Gender, n (%)			0.18			0.72
Male	15 (93.75)	17 (77.27)		13 (76.47)	20 (71.43)	
Female	1 (6.25)	5 (22.73)		4 (23.53)	8 (28.57)	
Age (years), n (%)			0.52			0.27
<60	12 (75.00)	10 (45.45)		7 (41.18)	7 (25.00)	
≥60	4 (25.00)	12 (54.55)		10 (58.82)	21 (75.00)	
Smoking, n (%)			0.17			0.26
No	3 (18.75)	1 (4.55)		4 (23.53)	3 (10.71)	
Yes	13 (81.25)	21 (95.45)		13 (76.47)	25 (89.29)	
TNM staging, n (%)			0.03			0.02
IIA–IIIA	15 (93.75)	14 (63.64)		16 (94.12)	18 (64.29)	
IIIB	1 (6.25)	8 (36.36)		1 (5.88)	10 (35.71)	
T staging, n (%)			0.02			0.04
T1–T3	16 (100.00)	16 (72.73)		16 (94.12)	19 (67.86)	
T4	0 (0.00)	6 (27.27)		1 (5.88)	9 (32.14)	
N staging, n (%)			0.02			0.049
N0–N1	12 (75.00)	8 (36.36)		13 (76.47)	13 (46.43)	
N2	4 (25.00)	14 (63.64)		4 (23.53)	15 (53.57)	

IL-33, interleukin-33; LUSC, lung squamous cell carcinoma; TNM, tumor-node-metastasis.

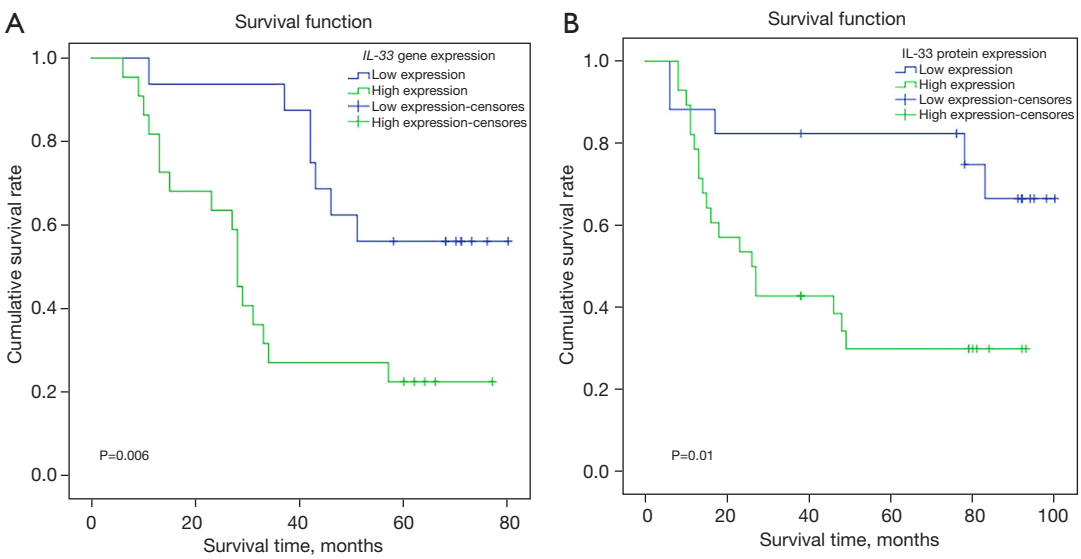

Figure 1 Survival curve of IL-33 gene (A) and protein (B) expression in stage II–III LUSC. IL-33, interleukin-33; LUSC, lung squamous cell carcinoma.

Table 3 Logistic multivariate regression analysis of prognosis of patients with stage II–III LUSC (*IL-33* gene expression group)

Prognostic factors	*t*	P
IL-33 gene expression	–2.138	0.04
TNM	–1.802	0.08
T	0.260	0.80
N	–1.907	0.07

LUSC, lung squamous cell carcinoma; IL-33, interleukin-33; TNM, tumor-node-metastasis.

Table 4 Logistic multivariate regression analysis of prognosis of patients with stage II–III LUSC (IL-33 protein expression group)

Prognostic factors	*t*	P
IL-33 protein expression	–2.755	0.009
TNM	–0.041	0.49
T	–0.238	0.81
N	–0.697	0.49

LUSC, lung squamous cell carcinoma; IL-33, interleukin-33; TNM, tumor-node-metastasis.

Survival analysis and correlation analysis for IHC of Ki-67, PD-L1, and IL-33

IHC staining results were shown in *Figure 2*. The relationship between protein expression of Ki-67, PD-L1, and IL-33 and survival time in IHC samples from 20 patients with stage II–III LUSC as demonstrated in *Figure 3*. The low expression groups for Ki-67, PD-L1, and IL-33 did not reach the median survival time. In contrast, the high expression groups had significantly shorter median survival times: 10 months for the Ki-67 high expression group (P=0.01), 9 months for the PD-L1 high expression group (P<0.001), and 16 months for the IL-33 high expression group (P=0.03).

In the correlation analysis between IL-33 and the protein expressions of Ki-67 and PD-L1, the results demonstrated a positive correlation between IL-33 and Ki-67 protein expression (r=0.503, P=0.02), indicating statistical significance. Additionally, IL-33 and PD-L1 protein expression also exhibited a significant positive correlation (r=0.612, P=0.004). These results suggested that the expression levels of IL-33 may be correlated with those of Ki-67 and PD-L1 (*Table 5*).

Discussion

In the context of LUSC, the identification of reliable biomarkers is crucial for improving diagnostic and prognostic accuracy. Recent studies, such as a research from Zhao *et al.* (21), have explored a range of potential biomarkers for LUSC, providing valuable insights into the molecular underpinnings of the disease. These efforts highlight the ongoing need to validate biomarkers that can be applied in clinical settings to guide treatment decisions. As is reported, IL-33 expression was identified as a poor prognostic factor in stage II and III LUSC (22). In this retrospective study, we used the RT-qPCR and IHC methods to validate its expression at both the gene transcription and protein translation levels, respectively. The high expression of both the *IL-33* gene and protein significantly shortened the survival time of patients. Further, the high expression of IL-33 at both the gene and protein levels was significantly associated with advanced tumor T stage, N stage, and TNM stage, suggesting a poor prognosis. The multiple stepwise regression suggested that IL-33 was probably an independent risk factor for a poor prognosis of LUSC, which is consistent with relevant literature reports.

IHC research has shown that IL-33 is more highly expressed in LUAD and LUSC tissues than normal tissues, suggesting that IL-33 may be involved in tumor progression (23). As a cancer promoter, IL-33 increases tumor invasiveness in head and neck squamous cell carcinoma (24) and oral squamous cell carcinoma (25). The high expression of IL-33 is associated with poorer prognosis and shorter survival time, which suggests that IL-33 promotes the progression of squamous cell carcinoma. Additionally, studies on other malignant tumors, such as colorectal cancer, gastric cancer (26), and breast cancer (27), have also suggested that the high IL-33 expression promotes tumor invasion and metastasis, which is positively correlated with tumorigenesis and tumor progression. In our study, through IHC analysis of tumor tissues from LUSC patients, we also found that patients with high expression of IL-33 had significantly shorter survival periods compared to those with low expression.

Ki-67, also known as MKi67, is used to assess cell proliferation activity and differentiation potential. Previous studies have shown its association with the occurrence, progression, metastasis, and prognosis of various cancers (28,29). PD-L1, on the other hand, is involved in the

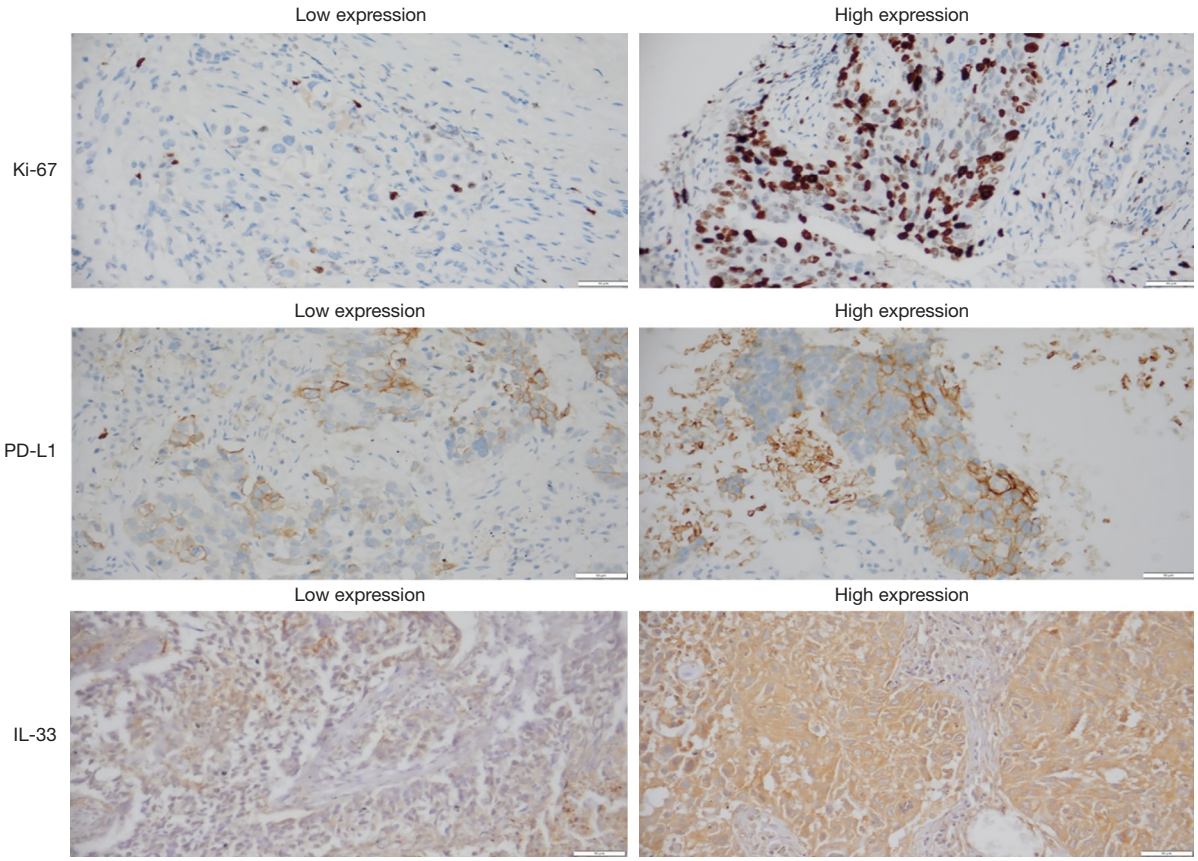

Figure 2 IHC staining (×400; 50 μm) of Ki-67, PD-L1, and IL-33. IHC, immunohistochemistry; PD-L1, programmed death ligand 1; IL-33, interleukin-33.

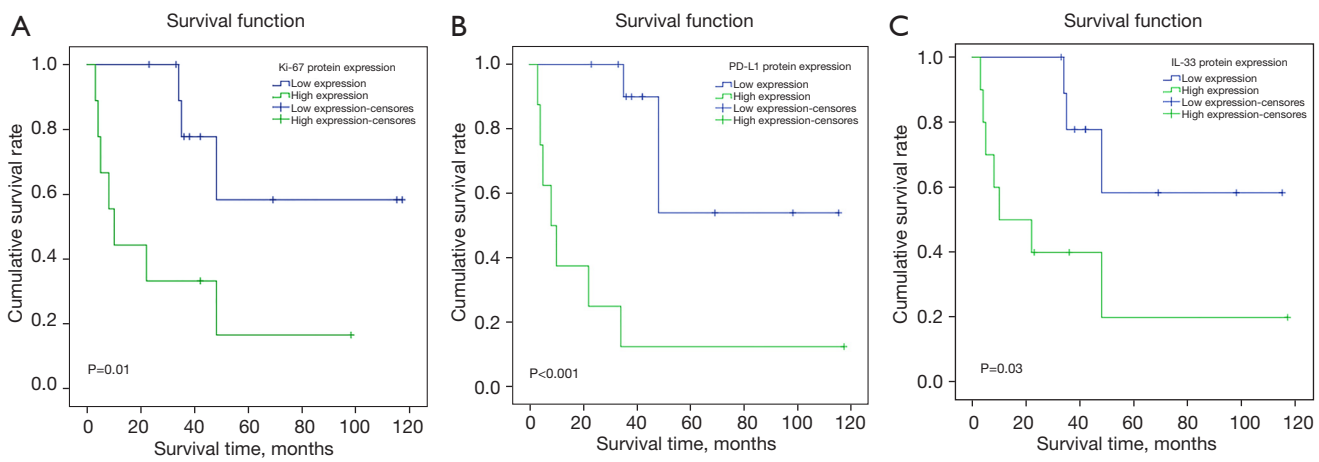

Figure 3 Survival curve of Ki-67 (A), PD-L1 (B), and IL-33 (C) protein expression in stage II–III LUSC. PD-L1, programmed death ligand 1; IL-33, interleukin-33; LUSC, lung squamous cell carcinoma.

Table 5 Correlation analysis between IL-33, Ki-67, and PD-L1 protein expression in stage II–III LUSC

Protein	IL-33	
	Pearson correlation coefficient	P value
Ki-67	0.503	0.02
PD-L1	0.612	0.004

IL-33, interleukin-33; PD-L1, programmed death ligand 1; LUSC, lung squamous cell carcinoma.

immune evasion mechanism of NSCLC, particularly focusing on the alterations in PD-L1 expression from precancerous lesions to invasive cancer stages and its implications for immunotherapy. It has been observed that heightened PD-L1 expression facilitates tumor cell immune evasion (30). In our survival analysis, patients with high expression of Ki-67, and PD-L1 had significantly shorter survival periods compared to those with low expression. Furthermore, we observed a positive correlation between IL-33 expression and Ki-67 protein expression, as well as between IL-33 expression and PD-L1 expression.

Currently, surgery remains the primary treatment for early-stage NSCLC, but the indications for surgery in stage T1–3N2M0 (i.e., stage IIIA–IIIB) are still greatly disputed. However, the KEYNOTE-671 study revealed that pembrolizumab combined with chemotherapy significantly improved progression-free survival and pathological response outcomes in early resectable NSCLC patients compared to chemotherapy alone (31,32). In the meanwhile, feasibility and safety evaluations in neoadjuvant/perioperative immunotherapy clinical trials are ongoing. Wang *et al.* (33) have shown that IL-33 is a cancer-promoting factor in NSCLC cell growth and metastasis. Blocking IL-33 effectively inhibited the growth of NSCLC xeno-transplanted tumors in immunodeficient mice; thus, blocking IL-33 may be a promising method for treating NSCLC. Yue *et al.* (34) found that the simultaneous administration of IL-33 and programmed cell death protein 1 (PD-1) blockers significantly enhanced the efficacy of anti-PD-1 therapy in LUAD-bearing mice, showing better tumor control compared to anti-PD-1 alone. In the melanoma mouse model, PD-1 blockade combined with the cytokine IL-33 significantly inhibited tumor progression and improved their survival rates without immune-related adverse events, demonstrating the efficacy and safety of combination therapy, and lays the foundation for preclinical research (35). In mouse models of 4T1 breast cancer and CT26 colon cancer, the use of the co-blockade of the IL-33/ST2 and PD-L1/PD-1 axes was more effective in preventing tumor progression than a single blockade of either axis, which could provide a potential new approach to tumor immunotherapy (36). These findings highlight the potential of IL-33 in clinical treatment.

Although this study has produced significant findings, it is also important to acknowledge its various limitations. Firstly, the samples used in this study were relatively old, lacking modern positron emission tomography (PET) computed tomography (CT) scanning equipment in the early stages, which may have led to underestimation of disease staging in some patients. Furthermore, prolonged sample storage time may have caused protein degradation and changes in gene expression, potentially introducing bias into the experimental data. Secondly, the sample size was relatively limited, which may affect the generalizability and statistical significance of the findings, further validation of our results in larger cohorts is needed. Thirdly, we did not differentiate between survival outcomes among patients with stage II, IIIA, and IIIB, which may have limited a comprehensive understanding of the role of IL-33 across different disease stages. Finally, although we observed an association between IL-33 and the prognosis of LUCS patients, the specific biological mechanisms underlying this relationship require further investigation.

Conclusions

In summary, RT-qPCR and IHC techniques were used to validate molecular markers at the transcriptional and translational levels. The statistical analysis identified IL-33 as an independent prognostic factor for patients with stage II and III LUSC. It is highly likely that IL-33 will become a novel target for immunotherapy in LUSC. This study introduced the known prognostic adverse factors of Ki-67 and PD-L1 to further confirm the involvement of IL-33 in the malignant proliferation of tumor cells, and also suggested that IL-33 blockade combined with immune checkpoint inhibitors (ICIs) in perioperative immunotherapy could improve the effect of anti-tumor immunotherapy. However, further confirmation is required through relevant animal experiments and clinical trials.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1122/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1122/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1122/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1122/coif). N.S. obtained research grants from Eli Lilly, Chugai Pharmaceutical, Taiho Pharmaceutical, Pfizer, Ono Pharmaceutical, Nippon Kayaku, Takeda Pharmaceutical, Eisai, Shionogi, Daiichi Sankyo, and Boehringer Ingelheim and received speaking honoraria from Eli Lilly, AstraZeneca, MSD, Chugai Pharmaceutical, Taiho Pharmaceutical, Pfizer, Ono Pharmaceutical, Nippon Kayaku, Takeda Pharmaceutical, Daiichi Sankyo, Boehringer Ingelheim, Novartis, Kyowa Kirin, and Bristol Myers Squibb. W.M.B. received honoraria for lectures or educational events from AstraZeneca, Boehringer, Novartis, MSD, BMS, Lilly, Pfizer and Roche, received support for attending meetings and/or travel from Boehringer, Roche Pharma, and AstraZeneca, served on advisory board of Astra Zeneca, Boehringer, Novartis, MSD, Lilly Pharma, BMS, and Roche, and received equipment, material, drugs, medical writing, gifts or other services from Boehringer for medical writing. W.M.B.'s patent application EP21183549.1 (method for predicting a clinical response towards an immune checkpoint inhibitor based on pretreatment therewith) was filed with regard to the results of this study. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This work was performed according to the Helsinki Declaration (as revised in 2013) and was approved by the Institutional Ethics Committee of Tianjin Medical University (No. Ek2024055). Written informed consent was obtained from all patients.

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(English Language Editor: L. Huleatt)

Cite this article as: Liu Y, Liu P, Zhang R, Seki N, Forest F, Brueckl WM, Zhao C, Zhang C, Yu J. Preliminary exploration of poor prognostic factor IL-33 and its involvement in perioperative immunotherapy in stage II–III lung squamous cell carcinoma: a retrospective cohort study. J Thorac Dis 2024;16(9):6204-6215. doi: 10.21037/jtd-24-1122