Original paper

CMTM6: a potential biomarker in non-small cell lung carcinoma

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Lung cancer is the most common cause of cancer-related deaths worldwide. With the advent of immunotherapy, programmed cell death-1 and programmed death ligand 1 (PD-L1) inhibitors have gained clinical importance, driving research into their regulatory mechanisms. CKLF-like MARVEL transmembrane domain-containing 6 (CMTM6) has been identified as a key regulator of PD-L1. In our study, we aimed to assess the prognostic significance of PD-L1 and CMTM6 expression in patients with non-small cell lung carcinoma (NSCLC).
This study included 100 patients with NSCLC. CMTM6 and PD-L1 expression were evaluated using immunohistochemistry, and H-scores were calculated.
A positive correlation was observed between H-PD-L1 and H-CMTM6 scores. In addition, both immunohistochemical scores were positively correlated with tumor size and grade. Both H-PD-L1 and H-CMTM6 scores of patients with adenocarcinoma were found to be lower than those of squamous carcinoma and other carcinoma types. Non-mucinous adenocarcinomas were grouped according to their predominant pattern (Group 1: lepidic pattern, Group 2: acinar and papillary pattern, Group 3: solid, micropapillary, and complex glandular/cribriform pattern). Group 3 had higher H-PD-L1 and H-CMTM6 scores than the others.
Our study demonstrated a correlation between CMTM6 and PD-L1 in NSCLC and suggested that CMTM6 may serve as a potential adverse prognostic marker.

Key words: PD-L1, prognosis, CMTM6, non-small cell lung carcinoma (NSCLC).

Introduction

According to the Global Cancer Observatory GLOBOCAN 2020 database, lung cancer is the second most commonly diagnosed cancer worldwide, with approximately 2.2 million new cases, and is also the most common cause of cancer-related deaths, with approximately 1.8 million deaths [1]. Lung cancer has been shown to have a 22% rate of regional lymph node metastasis and a 57% rate of distant metastasis at the time of diagnosis [2]. Although chemotherapy and radiotherapy have been used for many years in advanced-stage patients, the elucidation of carcinogenesis and the discovery of genetic alterations in lung cancer have led to developments in targeted therapy. These treatments act by suppressing genetic mutations that are essential for tumor cell survival [3, 4]. Following surgery, radiotherapy, chemotherapy, and targeted therapy, cancer treatment has now entered the era of immunotherapy. Despite this life-extending advancement, only a small subset of patients respond to immunotherapy, so various biomarkers are needed to select the patients most likely to respond to treatment.

In recent years, drugs that block the programmed cell death-1/programmed death ligand 1 (PD-1/PD-L1)

axis have gained prominence and been marketed in tumor immunotherapy. Programmed cell death-1 is an inhibitory receptor found on activated T and B lymphocytes and has two ligands: PD-L1 and programmed cell death ligand 2 (PD-L2) [5]. Programmed cell death-1 and its ligands (PD-L1/PD-L2) are immune checkpoint proteins that primarily function to limit the effector function of T-cells in peripheral tissues during inflammatory responses, limiting autoimmunity [6, 7]. When expressed in the tumor microenvironment, these proteins cause tumor cells to evade the immune system. Anti-PD-L1 drugs restore immune cell activity and promote tumor cell destruction [8]. The success of therapies targeting the PD-1/PD-L1 axis has led to the identification of other pathway members as potential immunotherapy targets, as well as the use of PD-1/PD-L1 expression as a prognostic factor.

CKLF-like MARVEL transmembrane domain containing 6 (CMTM6) was first identified in 2003 and is a type 3 transmembrane protein belonging to the CMTM family. The CMTM family consists of nine members, including CKLF and CMTM1-CMTM8, located in different chromosome regions [9]. CMTM6 is a 183 amino acid protein with a MARVEL domain consisting of 4 transmembrane helices. The MARVEL domain is known to play an important role in regulating the transfer of transmembrane proteins [10]. The CMTM family has significant clinical value in tumors and immune-related diseases [11, 12].

The biological function of CMTM6 was first identified in 2017; it was understood that CMTM6 binds to PD-L1, stabilizes it, and consequently enhances its inhibitory effect on the immune system. It also prevents PD-L1 from being targeted for lysosome-mediated degradation [13, 14]. These findings are consistent with CMTM6 serving as a therapeutic target, either in combination with PD-L1 or independently.

Although previous studies have investigated the prognostic role of CMTM6 in lung cancer, the reported findings remain inconsistent. While some studies have suggested that increased CMTM6 expression is associated with favorable outcomes, others have reported an association with poor prognosis [15–17]. These discrepancies may be attributed to differences in histological subtypes, patient populations, and methodological approaches, underscoring the need for further evaluation in larger and well-characterized cohorts. In our study, we describe our data on the immunohistochemical results of PD-L1 and CMTM6 markers and their relationship with prognostic parameters in 100 non-small cell lung carcinoma (NSCLC) cases diagnosed in 2015–2021.

Material and methods

Study design

In this study, 100 NSCLC cases diagnosed in 2015–2021, who had not received preoperative chemotherapy or radiotherapy, were evaluated.

Hematoxylin-eosin stained preparations were evaluated by a pair of observers for histopathological examination. Clinical information and archived preparations were reviewed, and patient age, gender, tumor size, histological type, grade, lymphovascular invasion, lymph node metastasis, airway spread, distant metastasis, smoking status, pathological tumor-node-metastasis (TNM) stage, clinical stage, and survival time were recorded. Histological typing was performed according to the World Health Organization (WHO) 2021 classification of thoracic tumors. Pattern analysis for non-mucinous adenocarcinomas was given as a percentage, and subtypes were defined according to the predominant histological pattern. Pathological TNM and clinical staging were performed according to the AJCC 8th TNM Staging. Pack-years of smoking were calculated, and patients were categorized into 4 groups: never smoker, light (≤ 10 pack-years) smoker, heavy (> 10 pack-years) smoker, and unknown [18]. Survival time was calculated as the interval between the date of surgery and the date of death for deceased patients, or between the date of surgery and December 8, 2022, for living patients (censored).

Immunohistochemical analysis of PD-L1 and CMTM6 expression

Immunohistochemical staining was performed on the Dako Omnis automated staining platform. A mouse monoclonal antibody against PD-L1 (clone 22C3) and a rabbit polyclonal antibody against CMTM6 were used.

Programmed death ligand 1 expression was analyzed using an H-score-based approach that incorporates both staining intensity and the proportion of positive tumor cells, allowing a more comprehensive assessment of intratumoral heterogeneity [19]. Although the tumor proportion score (TPS) is routinely used in clinical diagnostic practice for treatment decision-making in NSCLC, H-score evaluation was preferred in the present study, as the primary aim was not therapeutic stratification but rather to elucidate the biological and prognostic characteristics of the tumor. Accordingly, the staining intensity of tumor cells was divided into 4 levels according to the staining intensity of alveolar macrophages. 0 represents "No staining," 1+ represents "Membranous staining less intense than alveolar macrophages," 2+ represents "Membranous staining at the same level as alveolar macrophages," and 3+ represents "More membranous

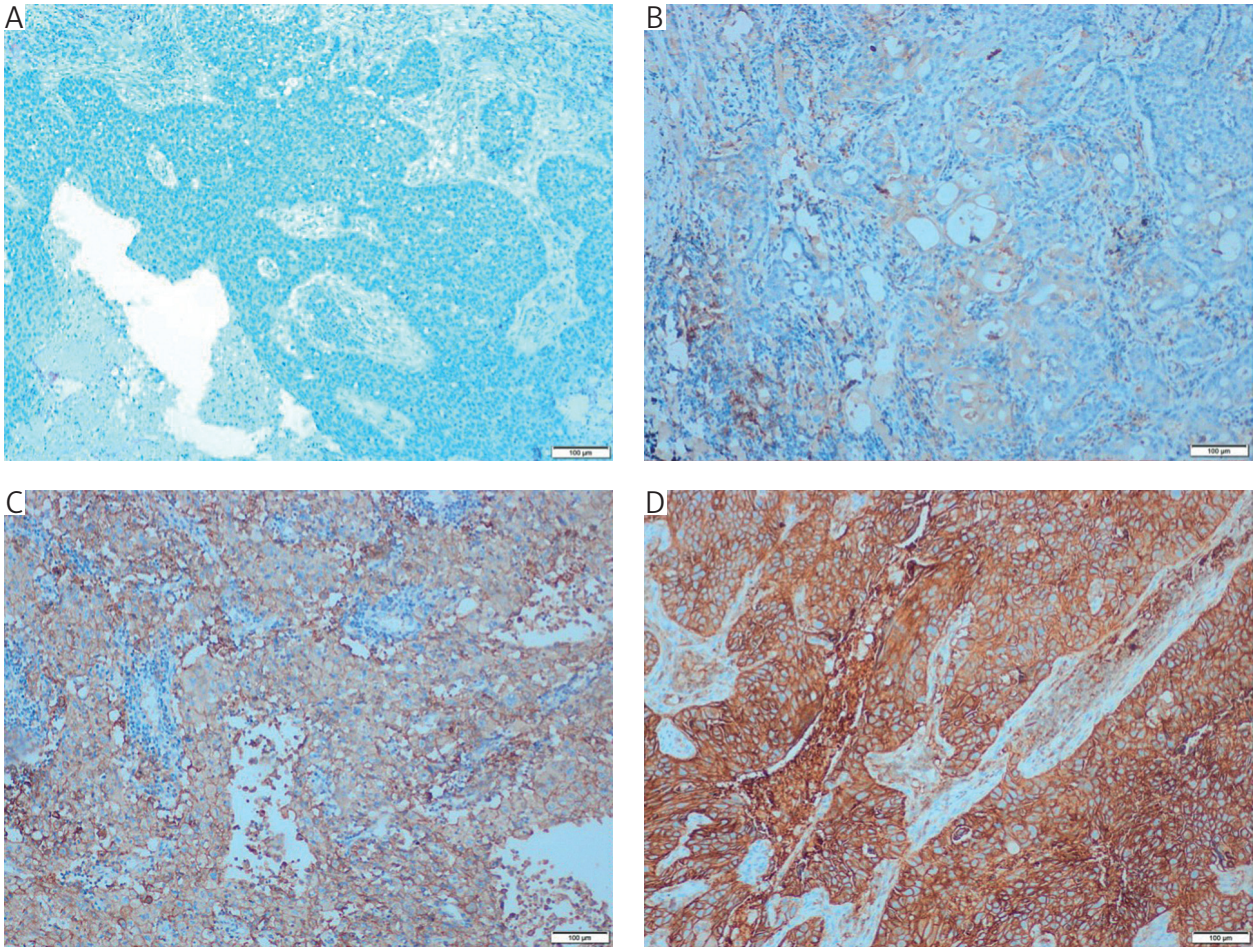

Figure 1. Representative immunohistochemistry images for programmed death ligand 1
Immunohistochemical staining intensities range from low to high (0–3); scale bar = 100 μm.

staining than alveolar macrophages." Programmed death ligand 1 staining percentage was obtained by scanning 3 randomly selected fields at 200× magnification. Following these evaluations, the H-score was calculated with the formula (0 × percentage of areas with a staining intensity of 0) + (1 × percentage of areas with a staining intensity of 1+) + (2 × percentage of areas with a staining intensity of 2+) + (3 × percentage of areas with a staining intensity of 3+). The H-PD-L1 score is a value of 0–300, and an H-score was calculated for each case. For statistical evaluation, H-PD-L1 score was divided into 2 groups as < 75 and ≥ 75 [20]. For clinical relevance, PD-L1 TPS values were also calculated.

H-scoring was performed to assess CMTM6 immunoreactivity, with cytoplasmic or membranous staining considered positive. Staining intensities were classified into four categories [20]. Staining intensity was scored as follows: 0, no staining; 1, weak/pale yellow staining; 2, moderate/yellow-brown staining; and 3, strong/brown staining. The H-CMTM6 score was calculated with the formula (0 × percentage of areas with staining intensity 0) + (1 × percentage of areas with staining intensity 1+) + (2 ×percentage of areas with staining intensity 2+) + (3 × percentage of areas with staining intensity 3+). The H-CMTM6 score is a value of 0–300, and for statistical evaluation, the H-CMTM6 score was divided into two groups as < 70 and ≥ 70 [20]. Staining intensities for PD-L1 and CMTM6 are given in Figure 1, 2.

Statistical analysis

The obtained data were analyzed using the SPSS 27.0 package program. In the descriptive analysis, frequency data were expressed using number (*n*) and percentage (%), and numerical data were expressed using mean ± standard deviation and median (1–3 quartile). The conformity of numerical data to normal distribution was examined using the Kolmogorov-Smirnov test. The distribution of normally distributed numerical data in two independent groups was assessed with the independent samples *t*-test, and the distribution of non-normally distributed numerical data was assessed with the Mann-Whitney *U* test. Numerical data not normally distributed in more than two groups were assessed with the Kruskal-Wallis test. *Post hoc* analysis for variables found to be

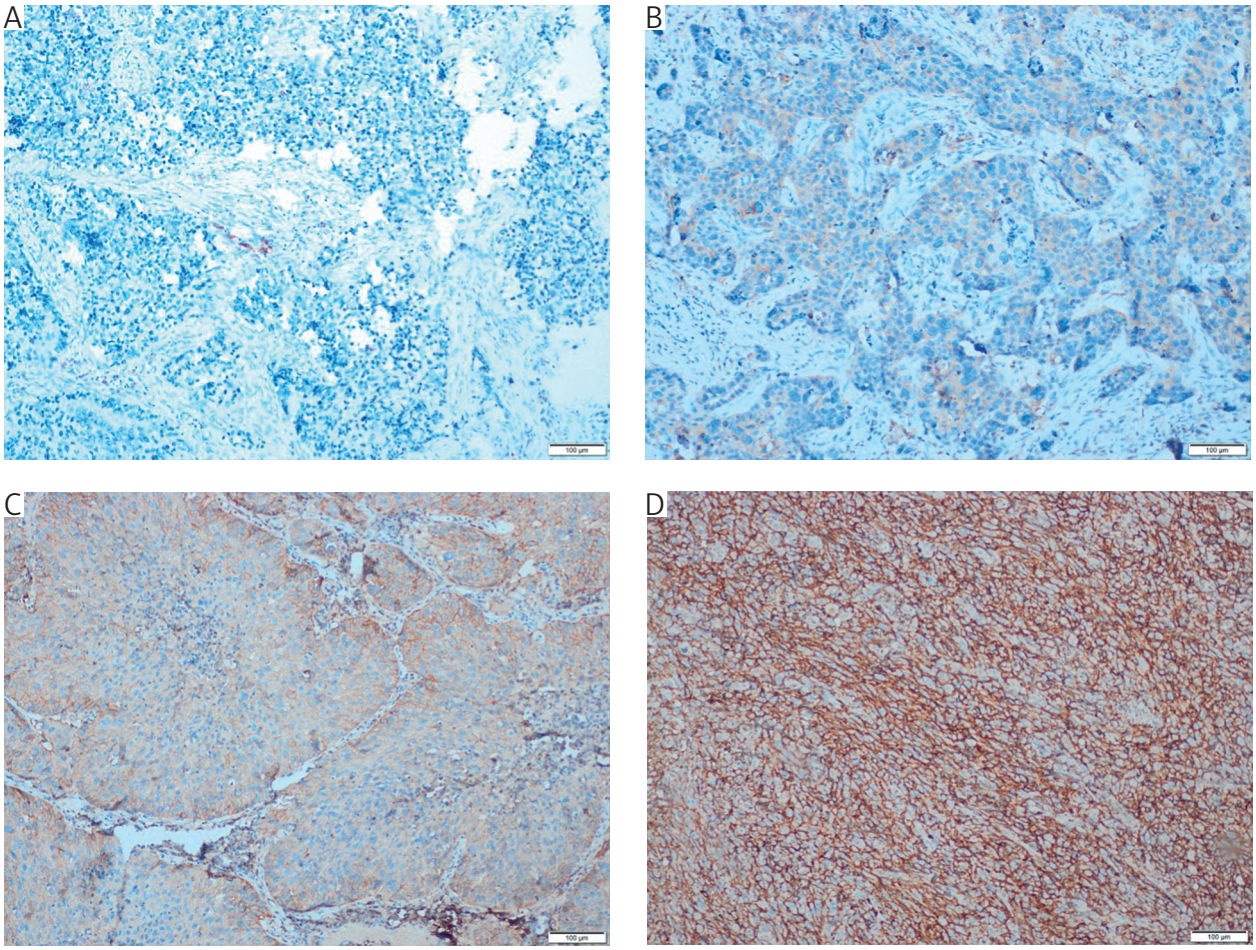

Figure 2. Representative immunohistochemistry images for CMTM6
Immunohistochemical staining intensities range from low to high (0–3); scale bar = 100 μm.

significant in the Kruskal-Wallis test was performed using the Mann-Whitney *U* test with Bonferroni correction. The relationship between non-normally distributed numerical data and ordinal data was assessed using Spearman correlation analysis, and the relationship between categorical and numerical data was assessed using point-biserial correlation analysis. Univariate analysis of survival was performed using the log-rank test. In multivariate analysis, independent predictors of survival were examined using Cox regression analysis with backward selection using potential factors identified in previous analyses. Survival rates were calculated using Kaplan-Meier survival analysis. Results were evaluated at a 95% confidence interval and significance was assessed at a $p < 0.05$ level.

Results

Demographic data and clinicopathological features

This study included 100 patients diagnosed with NSCLC. Of these, 83.0% ($n = 83$) were male and 17.0% ($n = 17$) were female. The mean age of the patients was 61.65 ±8.55 (min: 40, max: 81). The median smoking duration of the patients was 30.00 (min: 0.00, max: 150.00) pack-years, and smoking information was not available for 10 patients. The mean tumor diameter was 3.70 ±2.07 cm. Of the 100 diagnosed cases, 62 (62.0%) were adenocarcinoma, 32 (32.0%) were squamous cell carcinoma, 2 (2.0%) were adenosquamous carcinoma, 3 (3.0%) were pleomorphic carcinoma, and 1 (1.0%) was adenoid cystic carcinoma. Of the adenocarcinomas, 56 (90.3%) were non-mucinous adenocarcinoma, 5 (8.1%) were mucinous adenocarcinoma, and 1 (1.6%) was mixed mucinous and non-mucinous adenocarcinoma.

Evaluation of immunohistochemical results and clinicopathological parameters

The relationship between H-PD-L1 score and TPS was evaluated. A very strong positive correlation was observed ($r = 0.99$, $p < 0.001$), indicating excellent agreement between the two scoring methods. The raw H-scores and TPS values are provided in the Supplementary material.

The median H-PD-L1 score of all patients included in the study was determined to be 10.00 (1st quartile: 0.00 – 3rd quartile: 70.00), and the median H-CMTM6 score was determined to be 40.00 (1st quartile: 10.00 – 3rd quartile: 100.00). H-PD-L1 and H-CMTM6 scores of patients with tumor diameter ≥ 5 cm were statistically significantly higher than those with tumor diameter < 5 cm ($p = 0.014$, $p = 0.013$, respectively). Both H-PD-L1 and H-CMTM6 scores of patients with adenocarcinoma were found to be statistically significantly lower than those with squamous carcinoma and other carcinoma types ($p = 0.004$, $p = 0.001$, respectively). The H-PD-L1 and H-CMTM6 scores of histological grade 1 patients were found to be statistically significantly lower than those of grade 2 and grade 3 patients ($p = 0.011$, $p = 0.043$, respectively). The H-PD-L1 score of clinical stage 1 patients was found to be statistically significantly lower than that of patients in other stages (stages 2, 3, and 4) ($p = 0.020$). A comparison of patient immunohistochemistry scores with clinicopathological parameters is shown in Table I.

Non-mucinous adenocarcinomas were categorized according to their predominant histological pattern. Among 56 cases, the acinar pattern was predominant in 20 patients, the solid pattern in 15, the lepidic pattern in 14, the papillary pattern in 4, the complex glandular/cribriform pattern in 2, and the micropapillary pattern in 1 patient. According to the WHO 2021 classification and the IASLC non-mucinous adenocarcinoma grading system, three distinct groups were established. Group 1 consists of the lepidic pattern, Group 2 consists of the acinar and papillary pattern, and Group 3 consists of the solid, micropapillary and complex glandular/cribriform pattern [21]. It was determined that the H-PD-L1 and H-CMTM6 scores of Group 3, which contained high-grade patterns, were statistically higher than the other 2 groups ($p = 0.001$, $p = 0.001$, respectively) (Table II).

The relationships between H-PD-L1 and H-CMTM6 staining scores and clinicopathological parameters, including age, smoking status (pack-years), tumor diameter, grade, and stage, were analyzed. A good positive correlation was found between the H-PD-L1 and H-CMTM6 scores ($r = 0.636$, $p < 0.001$) (Figure 3), and the relationships between the other parameters are shown in Table III.

Patient survival times were compared according to clinicopathological features and immunohistochemical scores. Survival time was significantly shorter in patients with visceral pleural invasion ($p < 0.001$), distant metastases ($p = 0.003$), and stage IV disease ($p = 0.014$). No significant differences in survival were observed between patients with low (< 75) and high (≥ 75) H-PD-L1 scores ($p = 0.688$), or between those with low (< 70) and high (≥ 70) H-CMTM6 scores ($p = 0.362$). Overall survival according to clinicopathological features and immunohistochemical score groups is compared in Table IV.

Discussion

CMTM6 is a type 3 transmembrane protein belonging to the CMTM family, identified as a PD-L1 regulator in 2017 [13, 14]. CMTM6 increases the PD-L1 protein pool without affecting PD-L1's transcriptional level [14]. By binding to PD-L1 on the cell surface, it prevents PD-L1 from being targeted for lysosome- and proteasome-mediated degradation, thereby prolonging the half-life of PD-L1 [22]. These data indicate that the cooperative interaction between CMTM6 and PD-L1 may have a potential role in NSCLC progression. In addition to its role in stabilizing PD-L1, CMTM6 has increasingly been recognized as a contributor to the shaping of the tumor immune microenvironment in NSCLC. In lung cancer, the tumor microenvironment represents a dynamic and dual-function system that can either restrain or promote tumor progression [23]. Within this microenvironment, tumor-infiltrating lymphocytes (TIL), along with other immune cells, endothelial cells, and fibroblasts, play a critical role in determining the effectiveness of immunotherapy and overall disease prognosis [24, 25].

Evidence regarding the relationship between CMTM6 expression, immune cell infiltration, and clinical outcomes remains heterogeneous in the literature, likely reflecting differences in histological subtypes and tumor-specific microenvironmental contexts. Dai *et al*. [26] demonstrated an association between CMTM6 expression and increased TIL abundance in NSCLC; this finding also indicates that CMTM6 may play a role in modulating antitumor immune responses. Notably, despite increased immune cell infiltration, insufficient T-cell activation may still impair effective antitumor immunity. Importantly, CMTM6 may also modulate the function of dendritic cells and suppress the pro-inflammatory activity of eosinophils within the tumor microenvironment, potentially limiting effective T-cell activation. These effects are suggested by mechanistic studies and preliminary evidence and could contribute to immune evasion, promoting T-cell exhaustion and reducing overall antitumor immune responses in NSCLC.

Consistent with this concept, other studies in NSCLC have reported that CMTM6 expression is associated with $CD8^+$ and $CD4^+$ T-cell infiltration, although these correlations are generally weak to moderate in strength. In contrast, no significant association has been observed between CMTM6 expression and B-cell infiltration [25]. These findings indicate that CMTM6 may influence the functional state of tumor-infiltrating T-cells rather than directly

Table I. Comparison of clinicopathological parameters and immunohistochemistry scores (H-CMTM6 and H-PD-L1) in patients

Clinicopathological parameters	H-PD-L1 score (median, IQR)	*p*-value	H-CMTM6 score (median, IQR)	*p*-value
Age				
< 62 (n = 51)	20.0 (0–90)		50.0 (10–100)	
≥ 62 (n = 49)	0.0 (0–50)	0.097	30.0 (10–90)	0.360
Sex				
Female (n = 17)	10.0 (0–70)		60.0 (20–100)	
Male (n = 83)	10.0 (0–80)	0.965	40.0 (10–100)	0.850
Tumor size				
< 5 cm (n = 73)	0.0 (0–60)		40.0 (10–90)	
≥ 5 cm (n = 27)	40.0 (0–260)	0.014*	90.0 (20–190)	0.013*
Histological type				
Adenocarcinoma (n = 62)	0.0 (0–40)		30.0 (10–80)	
Squamous carcinoma (n = 32)	30.0 (0–100)		90.0 (40–142.5)	
Other* (n = 6)	210.0 (55–237)	0.004*	210.0 (57.4–220)	0.001*
Histological grade				
Grade 1 (n = 30)	0.0 (0–12.5)		20.0 (7.5–80)	
Grade 2 (n = 37)	20.0 (0–95)		40.0 (10–120)	
Grade 3 (n = 33)	20.0 (0–105)	0.011*	80.0 (20–105)	0.043*
Lymphovascular invasion				
Absent (n = 50)	0.0 (0–45)		30.0 (10–90)	
Present (n = 50)	15.0 (0–90)	0.178	60.0 (20–112.5)	0.094
Lymph node metastasis				
Absent (n = 64)	0.0 (0–55)		40.0 (10–90)	
Present (n = 36)	20.0 (0–97.5)	0.105	80.0 (20–120)	0.068
Visceral pleural invasion				
Absent (n = 84)	10.0 (0–70)		40.0 (10–100)	
Present (n = 16)	10.0 (0–90)	0.414	40.0 (10–110)	0.817
Airway spread				
Absent (n = 15)	0.0 (0–40)		40.0 (10–90)	
Present (n = 85)	10.0 (0–80)	0.171	40.0 (10–100)	0.522
Distant metastasis				
Absent (n = 75)	10.0 (0–80)		40.0 (10–100)	
Present (n = 24)	5.0 (0–62.5)	0.631	40.0 (0–97.5)	0.522
Stage				
Stage I (n = 39)	0.0 (0–40)		30.0 (10–80)	
Stage II–IV (n = 60)	10.0 (0–90)	0.020*	55.0 (12.5–107.5)	0.185
Smoking status				
Never (n = 21)	10.0 (0–70)		70.0 (20–100)	
Light (n = 10)	0.0 (0–10)		30.0 (7.5–40)	
Heavy (n = 59)	20.0 (0–100)	0.064	40.0 (10–130)	0.088

H-CMTM6 score – histological CKLF-like MARVEL transmembrane domain-containing 6 score, H-PD-L1 score – histological programmed death ligand 1 score
** Median value is given.*
*** Mann-Whitney U test/Kruskal Wallis test was used.*
*Other histological type * Adenosquamous carcinoma + pleomorphic carcinoma + adenoid cystic carcinoma*

Table II. Comparison of H-PD-L1 and H-CMTM6 scores among three histologic pattern groups in non-mucinous adenocarcinomas

HISTOLOGIC PATTERN GROUP	H-PD-L1 (MEDIAN, IQR)	*P*-VALUE	H-CMTM6 (MEDIAN, IQR)	*P*-VALUE
Group 1 (n = 14)	0.00 (0.00–22.50)		10.00 (0.00–55.00)	
Group 2 (n = 24)	0.00 (0.00–7.50)	0.001*	20.00 (0.00–80.00)	0.001*
Group 3 (n = 18)	40.00 (7.50–17.50)*		90.00 (47.50–115.00)*	

H-CMTM6 score – histological CKLF-like MARVEL transmembrane domain-containing 6 score, H-PD-L1 score – histological programmed death ligand 1 score, IQR – interquartile range
** Median value (IQR) is given.*
*** P-values were calculated using the Kruskal-Wallis test.*
Group 1 – lepidic pattern, Group 2 – acinar + papillary pattern, Group 3 – solid + micropapillary + complex glandular/cribriform pattern

determining the magnitude of lymphocyte infiltration. By enhancing PD-L1 stability and prolonging PD-1/PD-L1 signaling, CMTM6 may contribute to T-cell exhaustion and suppression of cytotoxic T-cell activity, thereby enabling immune evasion despite increased immune cell presence. This mechanism may help explain why elevated immune infiltration does not always translate into effective antitumor immune responses in NSCLC.

The tumor microenvironment, particularly TIL and other immune cells, is known to play a crucial role in immunotherapy response and prognosis. However, as the primary aim of this study was to evaluate the prognostic significance of CMTM6 expression, its association with PD-L1, and its relationships with clinicopathological features, tumor microenvironment-related cellular components were not analyzed due to the retrospective study design and methodological limitations related to the standardized and quantitative assessment of tumor-associated immune cells. This should be considered a limitation of the study. Future studies integrating CMTM6 expression with the tumor immune microenvironment may provide more comprehensive insights into the immunobiology of NSCLC and its clinical implications for immunotherapy.

In this study, we investigated the prognostic significance of PD-L1 and CMTM6 expression in NSCLC, as well as their associations with clinicopathological features. For both markers, H-scores were calculated,

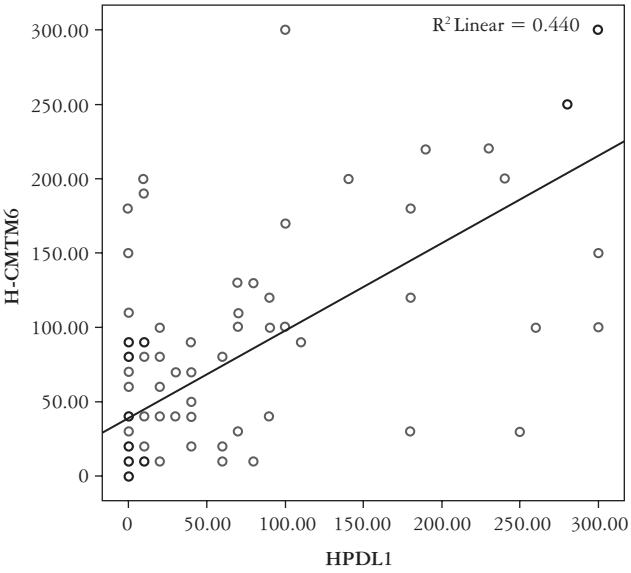

Figure 3. Scatter plot showing the correlation between programmed death ligand 1 and CMTM6 immunohistochemistry scores (H-CMTM6-H-PD-L1)

Linear regression line is shown. Coefficient of determination (R^2) is indicated. Spearman correlation coefficient (r) and p-value are reported in the text.

and in addition, PD-L1 TPS were also determined. Our analyses demonstrated that the H-PD-L1 score shows strong concordance with TPS-based classification. This finding supports the validity of the H-score-based approach while also indicating that it provides additional information regarding staining intensity and intratumoral heterogeneity.

Table III. Correlation between clinicopathological features and immunohistochemistry scores in patients

CLINICOPATHOLOGICAL PARAMETERS	H-PD-L1 SCORE		H-CMTM6 SCORE	
	R	*P*-VALUE	*R*	*P*-VALUE
Age	–0.178	0.076	–0.14	0.166
Smoking status (pack/years)	0.19	0.073	0.082	0.444
Tumor size	0.254	0.011*	0.241	0.016*
Histological grade	0.241	0.016*	0.218	0.029*
Stage	0.121	0.509	0.112	0.271

H-CMTM6 score – histological CKLF-like MARVEL transmembrane domain-containing 6 score, H-PD-L1 score – histological programmed death ligand 1 score
** Spearman rank correlation coefficient (r) is presented.*
*** p-values < 0.05 were considered statistically significant.*

Table IV. Comparison of clinicopathological features and immunohistochemical scores with overall survival

Clinicopathological parameters	*n* (%)	Median survival (months)	*p*-value
Sex			
Female	17 (17.0)	57.40	0.140
Male	83 (83.0)	51.86	
Age			
< 62	51 (51.0)	58.00	0.279
≥ 62	49 (49.0)	48.59	
Histological type			
Adenocarcinoma	64 (64.0)	52.85	0.444
Squamous carcinoma	32 (32.0)	53.00	
Other	4 (4.0)	–	
Tumor size			
< 5 cm	73 (73.0)	52.60	0.758
≥ 5 cm	27 (27.0)	52.90	
Histological grade			
Grade 1	30 (30.0)	56.14	0.239
Grade 2	37 (37.0)	49.84	
Grade 3	33 (33.0)	48.51	
Lymphovascular invasion			
Absent	50 (50.0)	58.00	0.181
Present	50 (50.0)	47.95	
Lymph node metastasis			
Absent	64 (64.0)	54.00	0.864
Present	36 (36.0)	51.70	
Visceral pleural invasion			
Absent	84 (84.0)	58.15	< 0.001*
Present	16 (16.0)	29.77	
Airway spread			
Absent	15 (15.0)	58.12	0.195
Present	85 (85.0)	51.77	
Distant metastasis			
Absent	76 (76.0)	60.11	0.003*
Present	24 (24.0)	39.29	
Stage			
Stage 1	39 (39.0)	59.05	0.014*
Stage 2	24 (24.0)	61.01	
Stage 3	11 (11.0)	50.50	
Stage 4	25 (25.0)	38.77*	
H-PD-L1 score			
Low (< 75)	76 (76.0)	54.19	0.688
High (≥ 75)	24 (24.0)	50.99	
H-CMTM6 score			
Low < 70	57 (57.0)	51.59	0.362
High ≥ 70	43 (43.0)	53.75	

H-CMTM6 score – histological CKLF-like MARVEL transmembrane domain-containing 6 score, H-PD-L1 score – histological programmed death ligand 1 score
** Data are presented as median survival (months). P-values were determined using the log-rank test.*
Other histological type: adenosquamous carcinoma + pleomorphic carcinoma + adenoid cystic carcinoma

The analysis revealed that patients with tumor diameters ≥ 5 cm had significantly higher H-PD-L1 and H-CMTM6 scores compared to those with tumor diameters < 5 cm. Similarly, Dai *et al*. [26] reported higher CMTM6 expression in NSCLC patients with tumor diameters ≥ 5 cm, supporting a role for CMTM6 in promoting tumor progression. Another study evaluating CMTM6 expression in lung adenocarcinomas reported significant associations with lymph node metastasis and T stage; these findings are consistent with CMTM6 serving as a poor prognostic factor [15].

In our study, a positive correlation was observed between CMTM6 and PD-L1 expression score in NSCLC cases. Similarly, Koh *et al*. [20] reported a positive correlation between CMTM6 and PD-L1 at both immunohistochemical and mRNA levels in NSCLC. Wang *et al*. [17] also demonstrated that in lung adenocarcinomas, CMTM6 expression positively correlated with PD-L1 at both mRNA and protein levels. Gao *et al*. [27] study further confirmed this correlation in lung cancer and reported that no CMTM6-negative, PD-L1-positive cases were observed. This observation indicates that CMTM6 may be required for PD-L1 expression. Consistently, in our study, no cases with CMTM6-negative but PD-L1-positive expression were observed.

In other cancer types, similar correlations between CMTM6 and PD-L1 have been reported. Yugawa *et al*. [28] evaluated hepatocellular carcinoma and observed co-expression of PD-L1 and CMTM6; cases with high CMTM6 expression exhibited more microscopic intrahepatic metastases, higher rates of intrahepatic recurrence, and higher-grade tumor cells. Similarly, Pang *et al*. [29] reported that in oral squamous cell carcinomas, high CMTM6 expression was associated with higher pathological stage and elevated PD-L1 expression. Taken together, these data indicate that the cooperative interaction between CMTM6 and PD-L1 may represent a general mechanism across different cancer types.

Our findings showed that, among NSCLC cases, H-CMTM6 and H-PD-L1 scores were found to be statistically significantly lower in adenocarcinoma cases compared to squamous carcinoma and other carcinoma types. Zugazagoitia *et al*. [25] reported higher CMTM6 expression in the squamous cell carcinoma subgroup of NSCLC cases. Similarly, Dai *et al*. [26] study on NSCLC also found higher CMTM6 levels in squamous cell carcinoma.

In our study, non-mucinous adenocarcinomas were classified according to their predominant histological pattern. In our statistical grouping (Group 1: lepidic pattern; Group 2: acinar + papillary pattern; Group 3: solid + micropapillary + complex glandular/cribriform pattern), Group 3, representing higher-grade histological patterns, was associated with higher PD-L1 and CMTM6 expression levels.

Gagné *et al*. [30], in their study evaluating PD-L1 heterogeneity in lung adenocarcinomas, reported higher PD-L1 expression in solid and micropapillary adenocarcinomas. Similarly, Reiniger *et al*. [31] associated a lepidic growth pattern with lower PD-L1 expression. To our knowledge, this is the first study to comparatively evaluate CMTM6 expression across different non-mucinous adenocarcinoma growth patterns.

In our study, H-CMTM6 and H-PD-L1 scores in grade 1 patients were found to be significantly lower compared to those in grade 2 and grade 3 patient groups. In a study by Pawełczyk *et al*. [32] investigating the role of PD-L1 expression in NSCLC cases, higher PD-L1 expression was observed with a higher grade of malignancy, and lower PD-L1 expression was found in grade 1 adenocarcinomas compared to grades 2 and 3. Similarly, in a study by Jiang *et al*. [33], a high PD-L1 score was associated with higher histological grade. However, in Dai *et al*. [26] NSCLC study, no significant association was observed between CMTM6 expression and histological grade.

The 5-year survival rate for NSCLC is 23%, making it the leading cause of cancer-related death [1, 34]. In our study, no significant association was observed between immunohistochemical scores of PD-L1 and CMTM6 (H-PD-L1, H-CMTM6) and patient survival. Dai *et al*. [26] study in NSCLC demonstrated that patients with high CMTM6 expression had significantly shorter overall survival compared to those with low CMTM6 expression, and multivariate analyses indicated that CMTM6 is an independent predictor of survival. In contrast, a study including 109 NSCLC cases reported that high CMTM6 expression was associated with better prognosis based on Kaplan-Meier survival analysis [16]. Likewise, in a study of 80 lung adenocarcinoma cases, patients with high CMTM6 expression tended to have better overall survival [17]. Overall, these findings indicate that CMTM6 may serve as a potential prognostic marker in NSCLC patients. However, given the inconsistencies reported in the literature, its prognostic role should be interpreted with caution and further validated in larger, well-characterized cohorts, taking into account tumor biology and the immune context.

Conclusions

In summary, we report that CMTM6 is expressed in NSCLC and that its expression is positively correlated with PD-L1. CMTM6 expression is associated with tumor size and grade, suggesting a role in promoting tumor progression. These findings highlight CMTM6 as a potential prognostic marker and a promising target for immunotherapy in NSCLC. Further studies are warranted to evaluate its utility in predicting response to immune checkpoint inhibitors and to explore the underlying mechanisms through

which CMTM6 influences the tumor immune microenvironment.

Disclosures

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4. Conflicts of interest: None.

Supplementary material

The supplementary Excel file provides the raw immunohistochemical data for programmed death ligand 1 (tumor proportion score and H-score) and CMTM6 (H-score). For each case, the percentages of tumor cells exhibiting different staining intensities (0–3+) are reported.

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