



Expression of CMTM4 shows clinical significance in lung cancer

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Background: Human chemokine-like factor (CKLF)-like MARVEL transmembrane domain-containing family (CMTM) is implicated in the pathogenesis of immune, reproductive systems and various cancers. However, the expression and clinical significance of CMTM4 in lung cancer have not been identified.

Methods: We performed immunohistochemistry to detect the expression of CMTM4 in 75 paired lung adenocarcinoma and non-tumor lung tissues. The correlation between CMTM4 expression and clinical significance of lung cancer patients was analyzed by Chi-square test. Kaplan-Meier method and Log-Rank test were used to calculate the survival time of lung cancer patients.

Results: We found that CMTM4 was positively expressed in 34/75 (45.3%) cases of lung adenocarcinoma tissues, while positively expressed in 59/75 (78.6%) cases of non-tumor lung tissues, suggesting a lower expression of CMTM4 in lung adenocarcinoma tissues than non-tumor lung tissues ($P < 0.05$). In addition, the negative expression of CMTM4 was associated with gender, smoking, and metastasis in lung cancer patients. Moreover, lung cancer patients with negative expression of CMTM4 had a shorter survival time than the patients with positive expression of CMTM4. COX regression analysis showed that CMTM4 was an independent prognostic factor for the overall survival of lung cancer patients.

Conclusions: Our study supports that CMTM4 can be used as a new marker for the treatment and prognosis of lung cancer.

Keywords: CMTM4; lung cancer; metastasis; prognosis

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Introduction

Lung cancer is a malignant tumor that has the highest incidence and mortality around the world, which has become a serious public health problem (1). In China, lung cancer is also one of the most threatening malignant tumors to the health of the population (2). Lung cancer is divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) according to the clinical and

histopathological characteristics of patients, and NSCLC accounts for about 80%. NSCLC is further classified into squamous cell carcinoma, adenocarcinoma and large cell carcinoma (3). The most common histologic subtype of lung cancer in most countries is lung adenocarcinoma (4). The etiology of lung cancer is still not completely clear, a large number of data show that long-term heavy smoking has a very close relationship with the occurrence of lung cancer (5). Studies have shown that long-term smokers

are 10 to 20 times more likely to develop lung cancer than nonsmokers (6,7). Early diagnosis and treatment of lung cancer is the key to improve the survival rate and prognosis of lung cancer patients.

In recent years, there are many studies focusing on cancer biomarkers, and emerging biomarkers have been shown to play a role in cancer. As a novel gene family, the human chemokine-like factor (CKLF)-like MARVEL transmembrane domain-containing family (CMTM) is implicated in reproduction, immunity, and carcinogenesis. High expression of CMTM2 and CMTM3 was found in the male testes and important in spermatogenesis (8,9). Furthermore, CMTM4 cooperates with its family member CMTM6, can participate in immune escape through synergistic protection of PD-L1 (10). CMTM1 was found to be associated with chemotherapy resistance and prognosis in patients with NSCLC (11). Our previous studies have identified that CMTM2, CMTM4, CMTM6, and CMTM7 were down-regulated in hepatocellular carcinoma (HCC) tissues (12-15). These findings suggest that CMTM family members are potential important cancer biomarkers for cancer diagnosis and therapy.

CMTM4 is the most conserved chemokine containing the MARVEL domain on chromosome 16q22.1 (16). CMTM4 exhibits tumor suppressive roles in renal clear cell carcinoma (CCRCC) and can be an effective target for CCRCC therapy (17), as well as a risk factor of poor prognosis for HCC (13). It was found that CMTM4 could cause cell cycle arrest at G2/M phase and inhibit HeLa cell proliferation without inducing apoptosis (18). Moreover, there was an interaction between CMTM4 and PD-L1 in cancer tissues. CMTM4 could protect PD-L1 as a target for lysosomal degradation and prevent the clearance of tumor cells from body immune cells, showing an important role of CMTM4 in cancer immunotherapy (10). However, the expression and clinical significance of CMTM4 in lung cancer have not been elucidated.

To explore the expression and clinical significance of CMTM4 in lung cancer, we conducted immunohistochemistry (IHC) analysis in 75 paired lung adenocarcinoma and non-tumor lung tissues. We found that CMTM4 was down-regulated in lung cancer tissues and had a relationship with metastasis and prognosis in lung cancer patients.

We present the following article in accordance with the REMARK reporting checklist (available at: <http://dx.doi.org/10.21037/tcr-20-1254>).

Methods

Tissue samples

Seventy-five paired lung adenocarcinoma and non-tumor lung tissues were collected from the Affiliated Guilin People's Hospital of Guilin Medical University from 2014 to 2016. These cases were diagnosed as lung cancer by pathological method and had no treatment before operation. All patients had detailed clinical data and signed the informed consent. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of Guilin Medical University (GLMC2014003).

The clinic-pathological features of lung cancer patients were shown in *Table 1*. Some variables in *Table 1* were defined as follows. Patients had a history of smoking, alcohol intake, allergy, and diseases diagnosed were defined as "Yes" of smoking, alcohol intake, allergy history, and past history. According to the latest WHO classification (19), these cases were all invasive adenocarcinomas and classified into 5 subtypes, acinar predominant, solid predominant, papillary predominant, mucinous adenocarcinoma, and fetal adenocarcinoma. The TNM classification was conducted according to 8th Edition of the TNM Classification for Lung Cancer (20).

IHC

The tissues were made on a tissue microarray from Fanpu (Guilin, China). And then the IHC assay was performed to detect the expression of CMTM4 by an IHC kit (Maixin biotechnologies, Fuzhou, China) according to the previous method (14). After incubated with primary CMTM4 antibody (NBP1-84457, Novus, CO, USA) and secondary anti-rabbit antibody, the tissue microarray was color-developed by 3,3'-aminobenzidine tetrahydrochloride (DAB), and observed under a microscope for 5–10 min until the appropriate color appeared. And then it was counterstained with hematoxylin, dehydrated, transparent, and fixed.

The IHC results were read by two professional pathologists according to the intensity of staining and the percentage of positive cells. For the inconsistency of the results, a third pathologist was invited to reread the tissue microarray. Five visual fields ($\times 400$) were observed in each section. The score for percentage of positive cells was defined as: 0 for $\leq 10\%$, 1 for 11–25%, 2 for 26–50%,

Table 1 Association between the expression of CMTM4 and clinicopathological features of lung cancer patients

Variables	Total	CMTM4 staining		P
		Negative	Positive	
Gender				0.035*
Male	43	19	24	
Female	32	22	10	
Age, year				0.324
<50	12	5	7	
≥50	63	36	27	
Smoking				0.010*
No	43	29	14	
Yes	32	12	20	
Alcohol intake				1.000
No	67	37	30	
Yes	8	4	4	
Allergy history				0.498
No	73	39	34	
Yes	2	2	0	
Past history				0.648
No	57	32	25	
Yes	18	9	9	
Family history of lung cancer				0.453
No	74	41	33	
Yes	1	0	1	
CEA (ng/mL)				0.837
<5	56	31	25	
≥5	19	10	9	
CA125 (U/mL)				1.000
<35	73	40	33	
≥35	2	1	1	
CA199 (U/mL)				1.000
≤37	74	40	34	
>37	1	1	0	

Table 1 (continued)**Table 1** (continued)

Variables	Total	CMTM4 staining		P
		Negative	Positive	
Pathological classification				0.251
Acinar predominant	31	15	16	
Solid predominant	13	7	6	
Papillary predominant	22	11	11	
Mucinous adenocarcinoma	5	5	0	
Fetal adenocarcinoma	4	3	1	
Tumor diameter (cm)				0.311
<5	55	32	23	
≥5	20	9	11	
TNM stage				1.000
I+II	69	38	31	
III+IV	6	3	3	
Lymphatic metastasis				0.030*
No	36	15	21	
Yes	39	26	13	

*, indicate significance.

3 for 51–75%, and 4 for >75%. The score for intensity of staining was defined as: 0 for no color, 1 for light color, 2 for middle, and 3 for dark brown color. H-score (percentage × intensity) is for CMTM4 expression. H-score >4 was defined as positive expression of CMTM4 and ≤4 was defined as negative expression of CMTM4.

Statistical analysis

All data were statistically analyzed by SPSS 19.0 software. Chi-square test was used to analyze the expression difference of CMTM4 between lung adenocarcinoma and non-tumor lung tissues. Kaplan-Meier method and Log-Rank test were used to compare the survival curves between different groups. $P < 0.05$ was defined as statistically significant.

Results

CMTM4 is down-regulated in lung cancer tissues

We first analyzed the expression of CMTM4 in lung adenocarcinoma tissues from TCGA database according

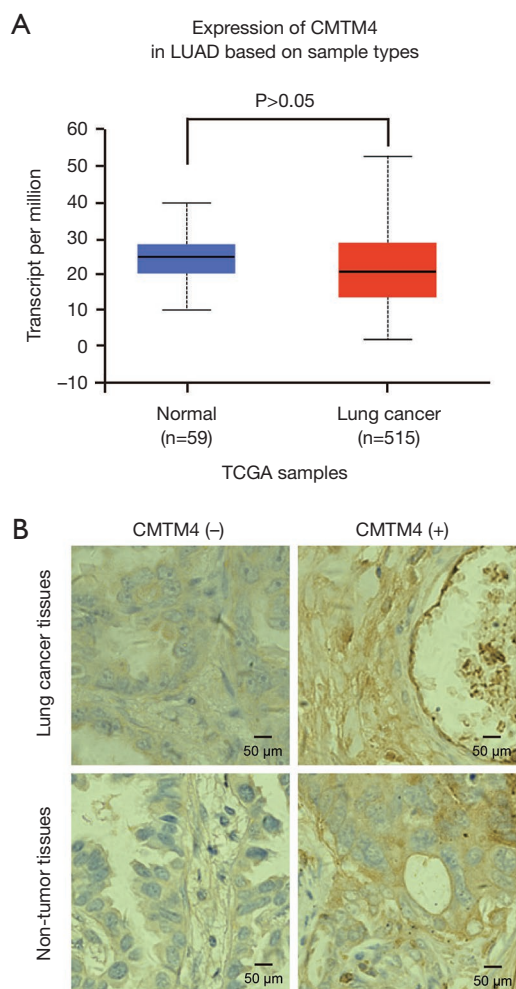


Figure 1 CMTM4 expression in lung cancer tissues. (A) Relative mRNA expression of CMTM4 in normal lung and lung cancer tissues in TCGA database ($P>0.05$); (B) Representative CMTM4 protein expression was detected in lung cancer tissues and non-tumor tissues by immunohistochemical analysis at 400 \times magnification.

to the previous method (21). As shown in *Figure 1A*, there was no difference of CMTM4 expression between lung adenocarcinoma tissues and normal lung tissues in TCGA database ($P>0.05$). Then the expression of CMTM4 in lung adenocarcinoma and corresponding non-tumor lung tissues was identified by IHC staining. As shown in *Figure 1B*, CMTM4 was mainly located in the cytoplasm and cell membrane. Moreover, CMTM4 was positively expressed in 34/75 (45.3%) cases of lung adenocarcinoma tissues, while positively expressed in 59/75 (78.6%) cases

Table 2 CMTM4 expression in paired lung cancer tissues and adjacent non-tumor tissues

Lung cancer tissues	Adjacent non-tumor tissues		Total
	Positive	Negative	
Positive	30	4	34
Negative	29	12	41
Total	59	16	75

$P<0.05$, P value is based on the McNemar χ^2 test.

of non-tumor lung tissues (*Table 2*). After the McNemar χ^2 test, the positive expression of CMTM4 in 75 cases of lung adenocarcinoma tissues was significantly lower than that in the paired non-tumor lung tissues ($P<0.05$). The IHC results suggest that CMTM4 is down-regulated in lung cancer tissues.

CMTM4 expression is related with the clinic-pathological features of lung cancer patients

According to the IHC results of CMTM4 expression, we divided the 75 cases of lung cancer patients into CMTM4 positive group and CMTM4 negative group. The clinic-pathological features of these patients were collected to analyze the relationship with CMTM4 expression, such as gender, age, smoking, alcohol intake, allergy history, past history, family history, CEA, CA125, CA199, pathological classification, tumor diameter, TNM stage, and metastasis. After chi-square test, we found that CMTM4 expression had a significant correlation with gender, smoking, and metastasis ($P<0.05$, *Table 2*), but was not related with other clinic-pathological features of lung cancer patients ($P>0.05$). In addition, through multivariate logistic regression analysis, we confirmed that CMTM4 expression was only significantly positive correlated to smoking in these lung cancer patients (OR=3.452, $P<0.05$, *Table 3*).

CMTM4 expression is correlated with the prognosis of lung cancer patients

Furthermore, we performed a survival analysis to explore the relationship between CMTM4 expression and the prognosis of these lung cancer patients. As shown in *Figure 2*, the expression of CMTM4 was significantly associated with the survival of these lung cancer patients ($P=0.012$). Moreover, the survival rate of these patients in the CMTM4 negative group was significantly lower than that

Table 3 Multivariable logistic regression analysis of CMTM4 expression in lung cancer patients

Variables	B	Wald	P value	OR	95% CI for OR	
					Lower	Upper
Smoking	1.239	6.417	0.011*	3.452	1.324	9.005

*, indicate significance.

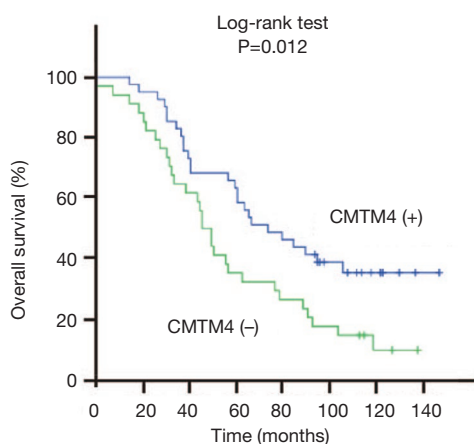


Figure 2 The relationship between CMTM4 expression and the prognosis of lung cancer patients from IHC result.

in the CMTM4 positive group. In addition, we did COX regression analysis on the overall survival of lung cancer patients after surgery and found that CMTM4 expression was an independent factor affecting the prognosis of these lung cancer patients (OR=2.003, 95% CI: 1.152–3.483), $P < 0.05$, Table 4).

Discussion

As a leading cause of death in both men and women worldwide, lung cancer has become a global problem and attracted a lot of attention. In this study, we found that the expression of CMTM4 was lower in lung adenocarcinoma tissues than that in the corresponding non-tumor tissues. Moreover, down-regulated CMTM4 was associated with gender, smoking, and metastasis in lung cancer patients. In addition, CMTM4 expression was an independent prognostic factor for the overall survival of lung cancer patients. Our study suggests a suppressor role of CMTM4 in lung cancer.

CMTM family members are widely expressed and play an important role in various cellular processes,

especially in carcinogenesis. Increased expression of CMTM1_v17 in breast cancer cells can promote cell proliferation and resistance to tumor necrosis factor- α (TNF- α)-induced apoptosis (22). Apart from affecting sperm and testosterone production, CMTM2 is also found down-regulated in HCC tissues and associated with the prognosis of HCC patients (12). CMTM3 is an independent prognostic marker for gastric cancer (23), while CMTM5 is an independent indicator of prognosis for oral squamous cell carcinoma (24), and CMTM7 is an independent prognostic factor in NSCLC patients (25). CMTM8 regulates cell proliferation and apoptosis by influencing EGFR and related signaling pathways through the MARVEL region (26).

In consistent with its family members, CMTM4 also shows anti-tumorigenic activity in several cancers, such as CCRCC, HCC, and colorectal cancer (13,17,27). Our study detected down-regulated CMTM4 expression in lung adenocarcinoma tissues, further supporting its suppressor role in lung cancer. The reason is mainly in the location of the *CMTM4* gene on chromosome 16q22.1, a locus that harbors a number of tumor-suppressor genes (16). Furthermore, in accord with our previous study of CMTM4 in HCC (13), this study also found that the expression of CMTM4 was associated with some clinic-pathological features of lung cancer patients, gender, smoking, and metastasis. As all we know, smoking is the main risk factor for lung cancer. Anti-smoking strategies are the most effective and efficient way to reduce the high incidence and mortality of lung cancer (28). Through the relationship between CMTM4 expression and smoking status of lung cancer patients, we can conclude some etiology of lung cancer caused by smoking. The exact mechanism needs to be further explored in other models. Of particular concern, the identification of CMTM4 as a critical PD-L1 regulator provides that CMTM4 can be used as an immunotherapeutic target for cancer (10). Our study also showed that the negative expression of CMTM4 was correlated with shorter survival time of lung cancer patients than the positive expression of CMTM4, implying

Table 4 COX regression analysis for overall survival of lung cancer patients after surgery

Variables	B	Wald	P value	OR ^a	95% CI for OR	
					Lower	Upper
CMTM4	0.695	6.067	0.014*	2.003	1.152	3.483
Smoking	-0.112	0.159	0.690	0.894	0.515	1.551

*, indicate significance. ^a, adjusted for age and gender.

a potential prognosis marker of CMTM4 in lung cancer.

In addition, there are some limits in our study. First, we detected CMTM4 expression only in lung adenocarcinoma tissues and the sample size was small. Second, we had small proportion of patients in high stage and didn't exclude the other possible therapy that patients had received after surgery. Third, we didn't detect PD-L1 expression to see its correlation with CMTM4. In future, we need to increase the sample size and select more subtype of lung cancer to solve these shortcomings.

Conclusions

In conclusion, we first clarified that CMTM4 was down-regulated in lung adenocarcinoma tissues and correlated to gender, smoking, and metastasis in lung cancer patients. Furthermore, down-regulated CMTM4 expression in lung cancer tissues had a significant relationship with the prognosis of patients. Our findings suggest CMTM4 as a potential biomarker that can be used in lung cancer diagnosis and prognosis.

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Footnote

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uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr-20-1254>). The 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. All patients had detailed clinical data and signed the informed consent. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the medical ethics committee of Guilin Medical University (GLMC2014003).

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