Clinical and Experimental Immunology, 2022, **207**, 65–71
https://doi.org/10.1093/cei/uxab003
Advance access publication 5 December 2021
Research Article

Clinical & Experimental IMMUNOLOGY

OXFORD

Research Article

CMTM6: increased circulating level and up-regulated expression in labial salivary glands in patients with primary Sjogren's syndrome

Sirui Qian[1], Jingyi Xu[1], Shan Zhao[1], Pingting Yang[1] and Chunshu Yang[2,*]

[1]Department of Rheumatology and Immunology, First Affiliated Hospital, China Medical University, Shenyang, People's Republic of China
[2]Department of 1st Cancer Institute, First Affiliated Hospital, China Medical University, Shenyang, People's Republic of China

*Correspondence: Chunshu Yang, Department of 1st Cancer Institute, First Affiliated Hospital, China Medical University, Shenyang 110001, People's Republic of China. Email: chunshu28@126.com

Summary

Chemokine-like factor (CKLF)-like MARVEL transmembrane domain containing family member 6 (CMTM6), which is a key regulator of programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) signaling in patients with primary Sjögren's syndrome (pSS). In this study, we analyzed the serum levels of CMTM6, PD-1, and PD-L1 in 50 patients with pSS, 42 patients with non-pSS (simply dry mouth and/or eyes symptoms) and 50 healthy controls (HC). The expression of CMTM6, PD-1, and PD-L1 in labial glands of the same 50 pSS patients and 42 non-pSS patients were assessed by immunohistochemistry (IHC). The clinical significance of CMTM6, PD-1, and PD-L1 were analyzed. We found that levels of CMTM6, PD-L1 as well as PD-1 in sera were all increased significantly in patients with pSS compared with non-pSS controls and HC. Serum CMTM6 level showed significantly correlation with PD-L1, PD-1, as well as clinical laboratory indicators and disease activity of pSS patients. CMTM6, PD-1, and PD-L1 expression in labial glands was also higher significantly in pSS patients than non-pSS controls. pSS patients with higher CM grade or ESSDAI score have higher CMTM6, PD-L1, and PD-1 expression in labial glands. These results suggest that CMTM6 may affect peripheral tolerance and lymphocytes activation by PD-1/PD-L1 pathway in sera and target tissue in pSS.

Keywords: CMTM6, PD-1, PD-L1, Primary Sjögren's syndrome

Abbreviations: CMTM6, Chemokine-like factor-like MARVEL transmembrane domain containing family member 6; PD-1, Programmed death-1; PD-L1, Programmed death ligand-1; pSS, primary Sjögren's syndrome; non-pSS, non-primary Sjögren's syndrome; HC, healthy controls; AECG, American-European Consensus Group; CM, Chisholm-Mason; EULAR, European league against rheumatism; ESSDAI, European league against rheumatism Sjögren's Syndrome Disease Activity Index; IQR, interquartile range.

Introduction

Primary Sjögren's syndrome (pSS) is a diffuse autoimmune disease characterized by dysfunction of the exocrine glands resulting in xerostomia and keratoconjunctivitis sicca [1, 2]. The abnormal activation of lymphocytes is crucial pathological cause in the process of pSS. Typical pathological manifestations in pSS show overactivated and overproliferated lymphocytes infiltrating in inflammatory sites, which should be closely correlated with the tissue and multiple system damage [3].

As known, programmed death ligand 1 (PD-L1) is the major ligand of programmed death-1 (PD-1) in PD-1/PD-L1 signaling which modulates immune cells activities [4]. Ligation of PD-L1/PD-1 inhibits lymphocyte activation, proliferation, and inflammatory cytokine production, participating in the regulation of central and peripheral tolerance [5–8]. Abnormal PD-1/PD-L1 signaling appears to be involved in a variety of autoimmune disorders, including systemic lupus erythematosus and rheumatoid arthritis [9, 10].

Chemokine-like factor (CKLF)-like MARVEL transmembrane domain containing family member (CMTM) 6 is a protein belonging to the CMTM superfamily [11], which is mainly expressed on the cell surface, involving in epigenetic regulation, embryonic development, as well as tumorigenesis [12, 13]. Previous studies have reported that CMTM6 regulates PD-L1 expression by decreasing its ubiquitination and promoting protein half-life.

As far as we know, though previous studies have demonstrated abnormal activation of PD-1/PD-L1 in pSS [14–16], no study has focused on the role of CMTM6 in pSS. In this study, we investigated the level of CMTM6 in sera and its expression in labial glands in patients with pSS, aiming to explore the potential clinical significance of CMTM6.

Materials and methods

Samples

We enrolled 50 pSS patients according to 2002 revised criteria of the American-European Consensus Group (AECG) for pSS [17]. Schirmer's test and salivary gland scintigraphy (SGS) test were used to determine dry eye and dry month respectively.

Received 29 May 2021; Revised 2 October 2021; Accepted for publication 14 October 2021

Forty-two patients with dry mouth and dry eyes not meeting the criteria of pSS (non-pSS controls).

Labial gland samples were obtained from the pSS patients and non-pSS contols. Peripheral blood samples were acquired from the 50 pSS patients, 42 non-SS controls as well as another 50 healthy volunteers. All samples were initially diagnosed at the Department of Rheumatology and Immunology of the First Affiliated Hospital of China Medical University from August 2019 to December 2020 without any treatment. Donors carrying other definitively diagnosed autoimmune diseases, infectious disease, or tumor were excluded. All the patients and controls were informed and consented the study which was approved by the Medical Ethics Committee (No. 2019-216).

Labial gland biopsies were fixed in 10% buffered formalin and embedded in paraffin using the standard protocol. Six micron sections were processed through dewaxing, antigen retrieval, and blocking of endogenous peroxidase. Serum samples were obtained from all peripheral blood samples by means of centrifugation for 10 minutes, 400 g, room temperature, and were immediately stored at −80°C. Labial gland biopsy specimens were calculated with Chisholm-Mason (CM) grade [18]. Disease activity was scored based on the European league against rheumatism (EULAR) Sjögren's Syndrome Disease Activity Index (ESSDAI) [19].

Reagents

CMTM6 antibody (NBP1-31183) and CMTM6 human ELISA kit (NBP2-75298) were purchased from NOVUS; PD-1 antibody (66220-1-Ig), PD-L1 antibody (66248-1-Ig), CD8 antibody (66868-1-AP), CD20 antibody (24828-1-AP), and PD-L1 human ELISA kit (KE00074) were commercially from Proteintech; CD4 antibodies were purchased from Immunolway (YT0762); PD-1 human ELISA kit was bought from Invitrogen (BMS2214). Kit of Immunohistochemistry (IHC) and 3,3′-Diaminobenzidine tetrahydrochloride (DAB) were from Maixin Biotechnologies (Fuzhou, China).

Salivary gland scintigraphy (SGS)

SGS is a dynamic scintigraphy performed after intravenous injection of 370 MBq (10 mCi) of 99mTc. After 20 minutes of injection, 100 mg vitamin C was administered to stimulate the salivary glands with acid. Total number and residual count were measured before and after collection. The time–activity curves of bilateral parotid gland and submandibular gland were generated and region of interests (ROI) were drawn. On the basis of this ROI counts, uptake ratio (UR) and excretion fraction (EF%) were calculated for all salivary glands. The normal UR of parotid gland ranged from 1.48 to 3.88‰, while that of submandibular gland ranged from 1.72 to 3.38‰. The normal EF% range of the parotid gland was 74.18–87%, while the normal EF% range of the submandibular gland was 52.32–76.46%.

ELISA

Serum levels of CMTM6, PD-1, and PD-L1 were measured using commercially available ELISA kits. All steps were performed in accordance with the instructions provided by the manufacturer of the kits.

Immunohistochemistry

The expression of PD-1, PD-L1, and CMTM6 in the labial gland tissue was examined by the IHC method. Tissues were incubated with primary antibody (CMTM6 antibody at 1:300, PD-1 antibody at 1:100, PD-L1 antibody at 1:300, CD4 antibody at 1:100, CD8 antibody at 1:500, CD20 antibody at 1:50) overnight at 4°C, followed by secondary anti-rabbit antibody (CMTM6, CD20) or anti-mouse antibody (PD-1, PD-L1, CD4, CD8) for 10 minutes at room temperature. DAB staining was performed after PBS wash and color was developed under microscope. Hematoxylin counterstaining was performed followed by dehydrating, cover slipping, and fixing tissues.

The expression of CMTM6, PD-L1, and PD-1 in labial gland was decided by IHC score system, which was calculated by the staining intensity and the percentage of positive cells. Specifically, the staining intensity was divided into four grades based on the color in the cells: no expression = 0 point; weak expression = 1 point; medium expression = 2 points; strong expression = 3 points. The percentage of positive cells was scored into four categories: positive cells ≤5% = 0 point, 6–25% = 1 point, 26–50% = 2 points, 51–75% = 3 points, >75% = 4 points. Sum of above formula scores was used as the final staining score. ≥3 points was considered as positive expression, otherwise taken as negative expression.

Statistical analysis

Statistical analysis was performed using the SPSS 24.0 software. Measurement data are expressed as mean ± standard deviation (X ± SD), and non-normally distributed data is represented by median and interquartile range (IQR). Student *t*-test or Mann–Whitney *U*-test was used between two groups. ANOVA or Kruscal–wallis test followed by Bonferroni correction was used among three groups. If there were significant differences, Student *t*-test or Mann–Whitney *U*-test was used. Categorical variables were described as number (percentage), tested by Chi-square test and Bonferroni correction (among three groups). Correlation analysis was analyzed using Spearman or Pearson analysis. $P < 0.05$ was defined as statistically significant.

Results

Participants characteristics

The demographic and clinical characteristics of pSS patients, non-pSS, and HC were given in Tables 1 and 2. As given in the tables, there were no significant differences among the three groups for age and sex ratio. The CM grade is significant higher in pSS group than non- pSS group.

Levels of CMTM6, PD-1, and PD-L1 in sera

As shown in Fig. 1, there were significant differences for the serum levels of CMTM6, PD-L1, and PD-1 among pSS, non-pSS controls, and HC (all $P < 0.01$). The three indicators were all increased significantly in patients with pSS when compared with non-pSS controls and HC. Correlation analysis results showed significantly positive correlations between serum levels of CMTM6 and PD-L1 ($r = 0.402$, $P = 0.004$), CMTM6 and PD-1 ($r = 0.297$, $P = 0.036$), as well as PD-L1 and PD-1 ($r = 0.337$, $P = 0.017$).

Correlations between serum CMTM6 and clinical indicators

Our results showed that the serum level of CMTM6 was negatively correlated with the number of lymphocytes, CD3+,

Table 1. Participants' characteristics

	HC (n = 50)	pSS (n = 50)	non-pSS (n = 42)	P
Age,years,median(range)	52 (29–72)	56 (21–75)	53 (32–74)	ns
Sex,women/men	40:10	40:10	36:6	ns
Disease duration, M[Q1,Q3], years	-	1 [0.50, 3]	1 [0.50, 2.50]	ns
Schirmer's test, mm/5 min	-	2 [0.25, 3.75]	10.5 [8, 12.5]	<0.001
Anti-Ro/SSA positivity, n (%)	-	42 (84%)	7 (16.7%)	<0.001
Anti-La/SSB positivity, n (%)	-	16 (32%)	0 (0%)	<0.001
WBC (X ± SD,10^9/l)	5.91 ± 1.52	5.34 ± 1.67	5.79 ± 1.43	ns
HGB (X ± SD, g/l)	131.77 ± 10.31	123.12 ± 11.24	129.46 ± 12.57	0.012
LY (X ± SD, 10^9/l)	1.32 ± 0.23	1.41 ± 0.34	1.30 ± 0.26	ns
CD3+T cell (X ± SD, 10^6/l)	-	870.98 ± 229.16	850.24 ± 214.57	ns
CD4+T cell (X ± SD, 10^6/l)	-	499.34 ± 139.28	474.41 ± 139.60	ns
CD8+T cell (X ± SD, 10^6/l)	-	326.04 ± 100.77	319.1 ± 107.61	ns
ESR (M[Q1, Q3], mm/h)	-	24 [11, 50]	12 [8, 17]	<0.001
IgG (M[Q1, Q3], g/l)	-	18.13 [12.94, 23.01]	12.57 [8.66, 16.41]	<0.001
IgA (M[Q1,Q3], g/l)	-	2.84 [1.99, 3.82]	1.87 [1.05, 2.79]	<0.001
IgM (M[Q1,Q3], g/l)	-	1.18 [0.85, 1.60]	1.08 [0.69, 1.15]	0.005
IL-6 (pg/ml)	-	27.25 ± 32.51	5.01 ± 1.69	<0.001
TNF-α (pg/ml)	-	22.71 ± 20.28	13.98 ± 4.77	0.005
CM grade, median (range)	-	4(1–4)	0 (0–2)	<0.001
ESSDAI, median (range)	-	9 (3–23)	-	-

Data are presented as mean (SD), median (IQR), number (percent) and median (range).
CM grade, Chisholm-Mason grade; ESSDAI, EULAR Sjögren's syndrome disease activity index; ESR, erythrocyte sedimentation rate; LY, lymphocyte; ns, not statistical significant.

Table 2. Salivary gland scintigraphy findings between pSS and non-pSS group

	pSS (n = 50)	non-pSS (n = 42)	P
Abnormal SGS, n (%)	37 (74%)	8 (19%)	<0.001
UR(‰)			
Right parotid gland	0.75 [0, 1.52]	1.85 ± 0.56	<0.001
Left parotid gland	0.62 [0, 1.49]	1.78 ± 0.47	<0.001
Right submandibular gland	0.65 [0, 1.74]	2.14 ± 0.50	<0.001
Left submandibular gland	0.58 [0, 1.73]	2.03 ± 0.51	<0.001
EF%			
Right parotid gland	42.13 [0, 74.52]	79.95 ± 7.21	<0.001
Left parotid gland	40.55 [0, 74.29]	77.13 ± 6.31	<0.001
Right submandibular gland	28.77 [0, 52.62]	59.98 ± 8.37	<0.001
Left submandibular gland	25.98 [0, 53.11]	58.55 ± 7.63	<0.001

Data are presented as mean (SD), median (IQR) and number (percent).

CD4+, CD8+T cells and positively correlated with the level of IgG, ESR, and ESSDAI scores. PD-1 and PD-L1 both showed the similar correlation relationship with the clinical indicators as CMTM6 (Table 3).

The expression of CMTM6 and PD-L1/PD-1 in labial gland tissue

It has been demonstrated that PD-L1/PD-1 activation plays an important role in lymphocytes infiltration in labial gland tissues of pSS. We then explored the CMTM6, PD-L1, and PD-1 expression in labial gland tissues of pSS patients and non-pSS controls. IHC results showed CMTM6 and PD-L1 were primarily expressed on epithelial cells of dual, but PD-1 was more frequently expressed on infiltrating lymphocytes (Fig. 2). Our results showed that positive expression rates of CMTM6, PD-L1 as well as PD-1 were significantly higher in pSS patients than non-pSS controls (Table 4).

Furthermore, we determined whether the expression of CMTM6, PD-1, and PD-L1 in the labial gland correlated with the CM grade and ESSDAI scores. Our results showed that patients with higher CM grades and ESSDAI scores had higher CMTM6 expression. For PD-1 and PD-L1, significant differences only found between the CM grade = 3 group and grade = 4 group, and between ESSDAI (5–13) group and ESSDAI (>=14) group (Fig. 3).

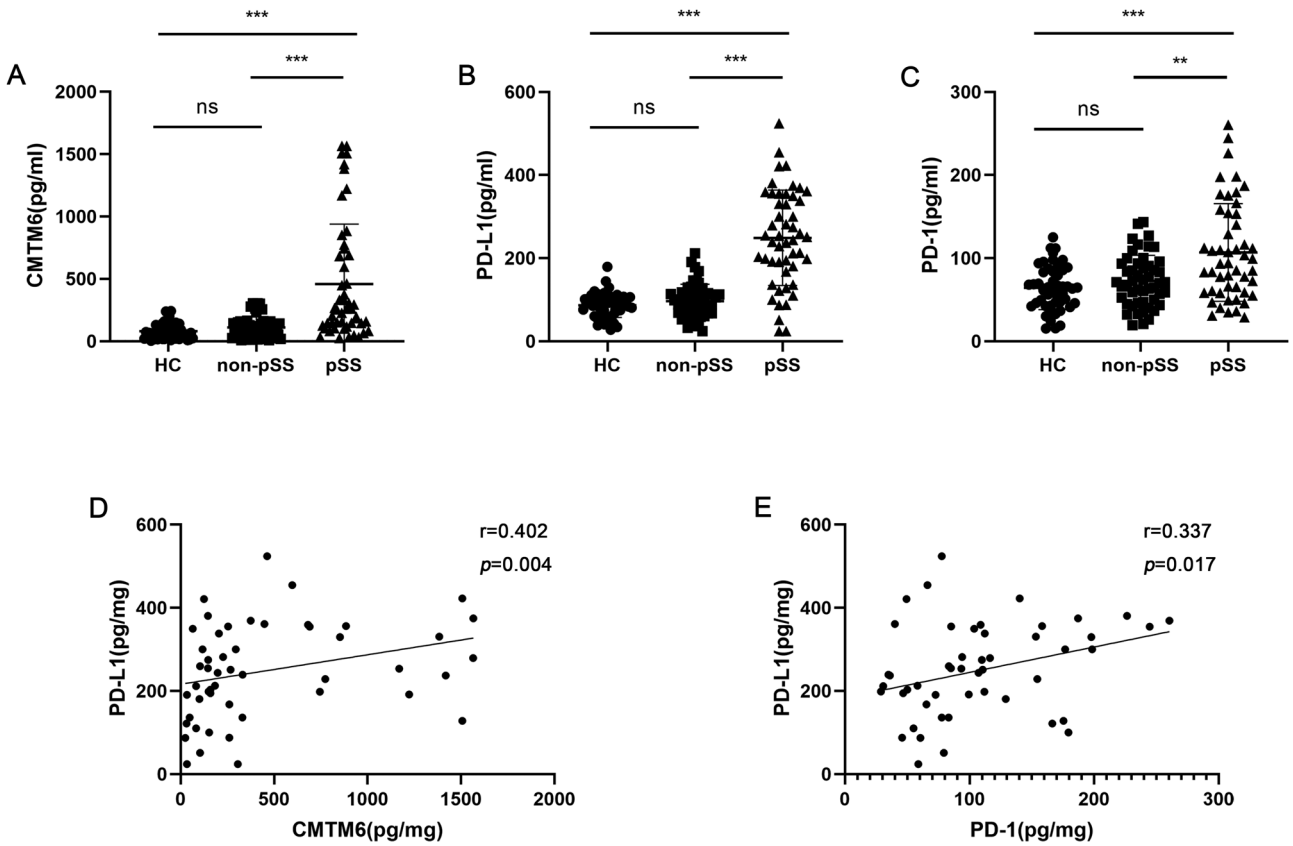

Fig. 1 Expression level of serum CMTM6, PD-L1, and PD-1 in pSS patients, non-pSS and HC. (A) Serum CMTM6 levels were significantly higher in pSS patients (257.04 [123.22, 704.41] pg/ml) compared to those in HC (65.02 [29.59, 121.35] pg/ml). (B) Serum PD-L1 levels in HC (86.51 ± 29.26 pg/ml) were also decreased than those in pSS patients (248.94 ± 114.73 pg/ml). (C) Serum PD-1 levels in HC (64.35 ± 26.33 pg/ml) were lower than those in pSS patients (93.72 [58.83, 153.62] pg/ml). (D–E) Correlation between CMTM6 and PD-L1 level ($r = 0.402$, $P = 0.004$) and correlation between PD-1 and PD-L1 level ($r = 0.337$, $P = 0.017$). Results are presented as the mean (SD) and median (IQR). Mann–Whitney *U*-test and Student *t*-test; Spearman Correlation. $^{**}P < 0.01$, $^{***}P < 0.001$, ns: not statistical significant.

Table 3. Correlations between serum CMTM6, PD-L1, and PD-1 levels and clinical parameters

	CMTM6		PD-L1		PD-1	
	r	*P*	*r*	*P*	*r*	*P*
LY (X ± SD, 10^9/l)	−0.341	0.015	−0.295	0.038	−0.318	0.024
CD3+T cell (X ± SD, 10^9/l)	−0.397	0.004	−0.239	0.094	−0.358	0.011
CD4+T cell (X ± SD, 10^9/l)	−0.350	0.013	−0.148	0.304	−0.355	0.018
CD8+T cell (X ± SD, 10^9/l)	−0.349	0.013	−0.312	0.027	−0.438	0.001
IgG (g/l)	0.349	0.013	0.359	0.010	0.44	0.001
IgA (g/l)	0.187	0.194	0.142	0.325	0.345	0.014
IgM (g/l)	−0.167	0.246	0.186	0.195	0.022	0.878
ESR (mm/h)	0.359	0.010	0.351	0.013	0.319	0.024
ESSDAI	0.386	0.006	0.315	0.026	0.335	0.017

Spearman Correlation and Person Correlation were used to access *P* value.

Discussion

In this study, we reported significantly increased peripheral CMTM6 level and up-regulated expression of CMTM6 in labial glands in pSS patients. Our results also showed significant positive correlation between CMTM6 and ESSDAI scores either at the serum level or labial glands expression in pSS patients.

Dysfunction of the salivary glands and lymphocytes abnormal activation is a prominent feature of immune abnormalities in pSS patients. It was reported that PD-1 was overexpressed on T and B lymphocytes, while PD-L1 was highly expressed on epithelial cells in patients with pSS [14–16, 20]. Because PD-L1/PD-1 signaling plays negative regulatory role in autoimmune reaction, it is believed that increased PD-L1/PD-1 might restrict active CD4+T, CD8+T cells, or B cells through exhaustion of PD-1+ T cells or B cells to limit inflammation and tissue damages [21–23].

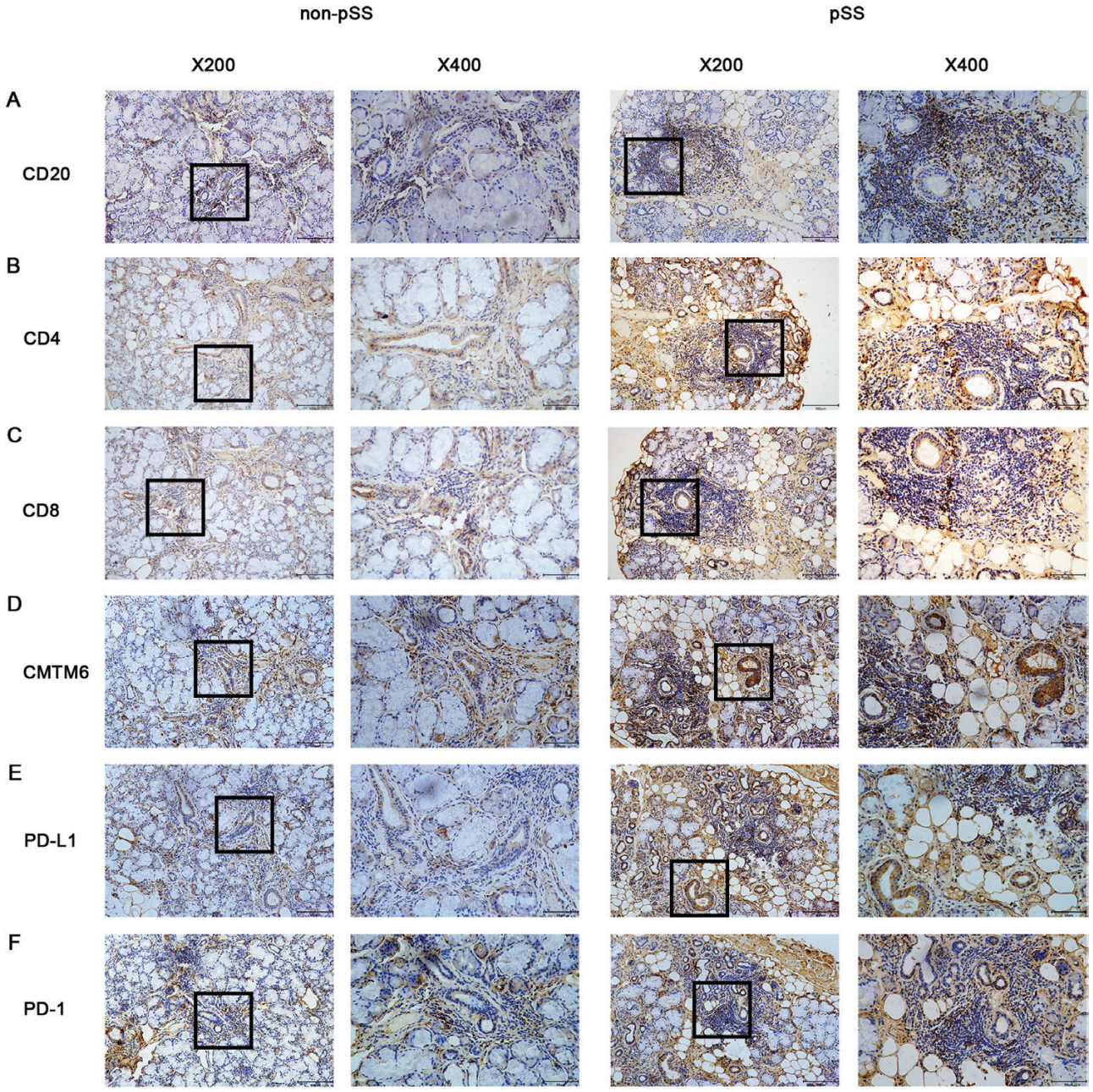

Fig. 2 Immunohistochemical staining(brown) of B, T Cells marker, CD20 (A), CD4 (B), and CD8 (C) in infiltrating cells and CMTM6 (D), PD-L1 (E), PD-1 (F) expression in minor salivary gland pSS patients (right) and non-pSS patients (left). Boxed areas represented the sites of the zoomed -in images in the right. All sections are shown at 200× (left) and 400× (right) magnifications. (A–C) The expressions of CD20, CD4, and CD8 in the minor salivary glands of pSS patients and non-pSS controls. (D) The expressions of CMTM6 tended to highly expressed on dual cells both in pSS and non-pSS and CMTM6 showed much stronger expression in ductal epithelial cells around periphery of lymphocytic foci of pSS patients. (E) The PD-L1 preotein expressed both in epithelia cells and periphery of lymphocytic foci as well as had higher expression in the glands of pSS patients. (F) The PD-1 strongly expressed in lymphocytic foci and showed higher expression in the glands of pSS. The PD-1-positve staining cells also located in the sites with CD20, CD4, and CD8-positive staining sites.

Table 4. CMTM6, PD-L1, and PD-1 expression on labial gland

	pSS	Non-pSS	*P*
CMTM6 positive	38 (76%)	9 (21.4%)	<0.001
PD-L1 positive	35 (70%)	13 (31%)	<0.001
PD-1 positive	32 (64%)	14 (33.3%)	0.004

Chi-square test.

CMTM6, which was reported to regulate PD-L1 expression in tumor cells by reducing PD-L1 ubiquitination, has been shown important roles in inhibiting CD8+T cell activation in tumor. To our knowledge, it is the first time that CMTM6 is focused on in autoimmune diseases. Our findings showed significantly increased serum CMTM6 levels, which were positively correlated with PD-L1, PD-1 and disease activity index in pSS.

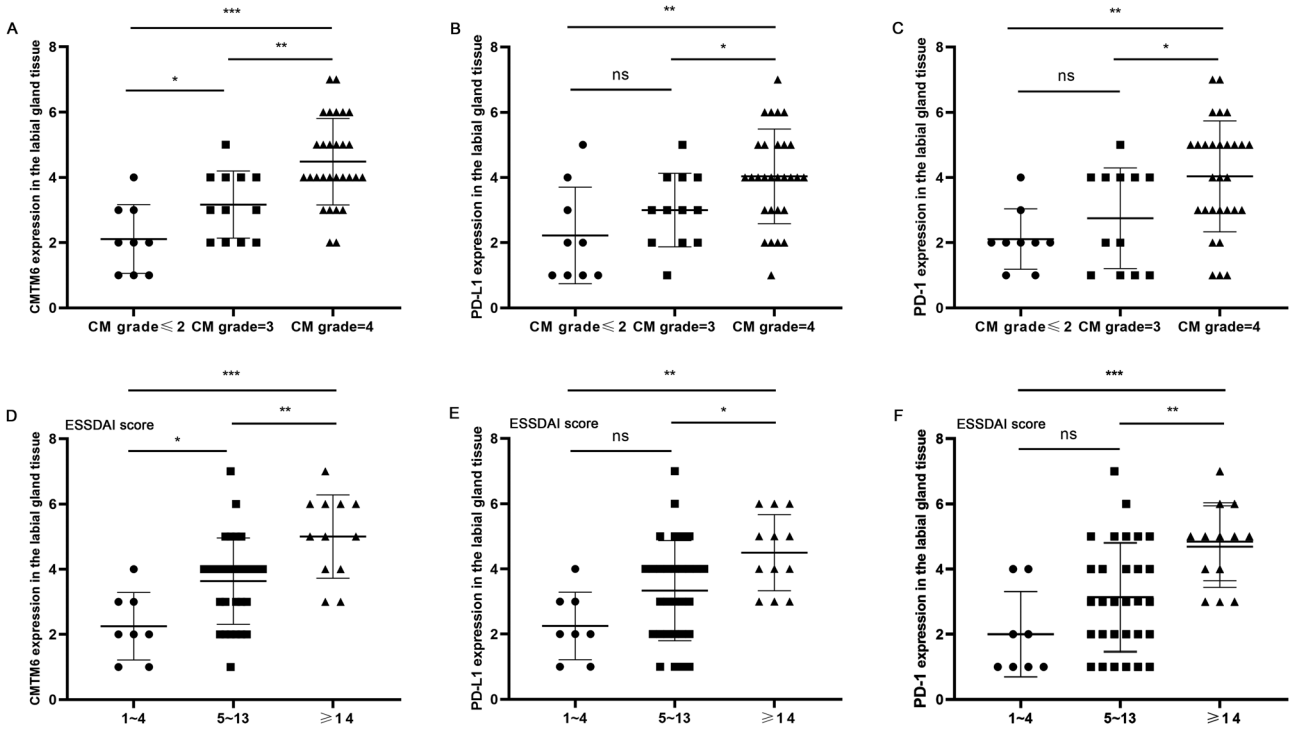

Fig. 3 CMTM6, PD-L1, and PD-1 expression in labial gland of pSS patients with different CM grades and ESSDAI scores. (A) CMTM6 expression in CM = 4 (n = 29) group was significantly higher than those in CM<2 (n = 9) and CM = 3 (n = 12) groups. (B) PD-L1 expression in CM = 4 group was higher than in those in CM<2 and CM = 3 groups but there was no expressional difference between GM<2 and GM = 3 groups. (C) PD-1 expression show significance between CM = 4 group and CM = 3 group but there was no difference between CM<2 and CM = 3 groups. (D) CMTM6 expression with ESSDAI scores>14 showed significance compared to those with ESSDAI scores>14 or 5<ESSDAI<13. (E) There was difference between PD-L1 expression with ESSDAI scores>14 and those with 5<ESSDAI<13. (F) pSS patients with ESSDAI scores>14 showed higher PD-1 expression than those patients with 5<ESSDAI<13. Mann–Whitney test. *P < 0.05, **P < 0.01, ***P < 0.001, ns: not statistical significant.

To explore the local role of CMTM6, we compared its expression in labial glands between pSS patients and those with simply dry mouth who did not meet the criteria of pSS. IHC results showed CMTM6 and PD-L1 were primarily expressed on epithelial cells, whereas PD-1 was more frequently expressed on infiltrating lymphocytes and IHC results showed CD4+ T cells, CD8+ T cells, and B cells in lymphocyte infiltrating area of labial gland tissue are positively expressed. In addition, the expression of CMTM6 and PD-L1/PD-1 in the labial gland had significantly positive correlation with CM grades and ESSDAI scores in pSS patients. Given that the inhibiting role of PD-1/PD-L1 signaling pathway on the continuous activation and proliferation of lymphocytes, we think the increased CMTM6 has the potential compensatory and protective effect in the local labial glands of patients with pSS.

In summary, our work provides clues that CMTM6 may affect peripheral tolerance and lymphocytes activation by PD-1/PD-L1 inhibitory functions in sera and target tissue in pSS. The significant correlation between CMTM6 and diseased activity also suggests its potential role of reflecting disease activity. CMTM6 may be an important up-stream player and therapeutic target in pSS. It is worth to further study on how CMTM6 affects the development of pSS.

Acknowledgements

We thank Pingting Yang for the excellent technical assistance, and the donors for kindly accepting their essential collaboration.

Funding

This work was supported by the following grants: foundation from the Program of Distinguished Professor of Liaoning Province, Rheumatology (28020 to P.Y.), the Program of Platform Construction of Shenyang, Liaoning, China (19-109-4-15 to P.Y.), foundation from the Clinical Medical Research Center of Shenyang, Liaoning, China (18009-4-03 & 20-204-4-43 to P.Y.).

Conflict of interest

The authors have declared no conflicts of interest.

Author contributions

C.Y. designed the experiments. S.Q. performed the major experiments, analyzed the data, and prepared the manuscript. P.Y. revised the manuscript. J.X. and S.Z. performed the experiments. All authors read and approved the manuscript prior to submission.

Ethical approval

This study was approved by the ethics committee of the First Affiliated Hospital of China Medical University (No. 2019-216).

Patient consent statement

All patients enrolled gave their consent to be included in the study.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Ramos-Casals M, Brito-Zerón P, Sisó-Almirall A, Bosch X. Primary Sjogren syndrome. *BMJ* 2012, 344, e3821.
2. Mariette X, Criswell LA. Primary Sjögren's syndrome. *N Engl J Med* 2018, 379, 97.
3. Christodoulou MI, Kapsogeorgou EK, Moutsopoulos HM. Characteristics of the minor salivary gland infiltrates in Sjögren's syndrome. *J Autoimmun* 2010, 34, 400–7.
4. Gianchecchi E, Delfino DV, Fierabracci A. Recent insights into the role of the PD-1/PD-L1 pathway in immunological tolerance and autoimmunity. *Autoimmun Rev* 2013, 12, 1091–100.
5. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* 2008, 26, 677–704.
6. Collins M, Ling V, Carreno BM. The B7 family of immune-regulatory ligands. *Genome Biol* 2005, 6, 223.
7. Francisco LM, Salinas VH, Brown KE, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med* 2009, 206, 3015–29.
8. Thibult ML, Mamessier E, Gertner-Dardenne J, et al. PD-1 is a novel regulator of human B-cell activation. *Int Immunol* 2013, 25, 129–37.
9. Zamani MR, Aslani S, Salmaninejad A, Javan MR, Rezaei N. PD-1/PD-L and autoimmunity: a growing relationship. *Cell Immunol* 2016, 310, 27–41.
10. Dai S, Jia R, Zhang X, Fang Q, Huang L. The PD-1/PD-Ls pathway and autoimmune diseases. *Cell Immunol* 2014, 290, 72–9.
11. Li M, Luo F, Tian X, Yin S, Zhou L, Zheng S. Chemokine-like factor-like MARVEL transmembrane domain-containing family in hepatocellular carcinoma: latest advances. *Front Oncol* 2020, 10, 595973.
12. Mezzadra R, Sun C, Jae LT, et al. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. *Nature* 2017, 549, 106–10.
13. Burr ML, Sparbier CE, Chan YC, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature* 2017, 549, 101–5.
14. Legány N, Berta L, Kovács L, Balog A, Toldi G. The role of B7 family costimulatory molecules and indoleamine 2,3-dioxygenase in primary Sjögren's syndrome and systemic sclerosis. *Immunol Res* 2017, 65, 622–9.
15. Kobayashi M, Kawano S, Hatachi S, et al. Enhanced expression of programmed death-1 (PD-1)/PD-L1 in salivary glands of patients with Sjögren's syndrome. *J Rheumatol* 2005, 32, 2156–63.
16. Li P, Yang Y, Jin Y, et al. B7-H3 participates in human salivary gland epithelial cells apoptosis through NF-κB pathway in primary Sjögren's syndrome. *J Transl Med* 2019, 17, 268.
17. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002, 61, 554–8.
18. Chisholm DM, Mason DK. Labial salivary gland biopsy in Sjögren's disease. *J Clin Pathol* 1968, 21, 656–60.
19. Seror R, Ravaud P, Bowman SJ, et al.; EULAR Sjögren's Task Force. EULAR Sjogren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjogren's syndrome. *Ann Rheum Dis* 2010, 69, 1103–9.
20. Szabo K, Papp G, Dezso B, Zeher M. The histopathology of labial salivary glands in primary Sjögren's syndrome: focusing on follicular helper T cells in the inflammatory infiltrates. *Mediators Inflamm* 2014, 2014, 631787.
21. Willemsen M, Melief CJM, Bekkenk MW, Luiten RM. Targeting the PD-1/PD-L1 axis in human vitiligo. *Front Immunol* 2020, 11, 579022.
22. Nishimura H, Honjo T. PD-1: an inhibitory immunoreceptor involved in peripheral tolerance. *Trends Immunol* 2001, 22, 265–8.
23. Wang J, Yoshida T, Nakaki F, Hiai H, Okazaki T, Honjo T. Establishment of NOD-Pdcd1-/- mice as an efficient animal model of type I diabetes. *Proc Natl Acad Sci USA* 2005, 102, 11823–8.