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Original Article

Expression of PD-L1 in mononuclear cells, multinucleated cells, and foam cells in tenosynovial giant cell tumors

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Abstract: Tenosynovial giant cell tumor (TGCT) is a rare, proliferative and inflammatory disease with activation of colony stimulating factor 1 (CSF1) expression, and exhibits abnormal proliferation of mononuclear cells, multinucleated cells and foam cells. PD-L1 inhibitors represent a promising strategy in a variety of tumors. However, PD-L1 expression has never been studied in CSF1 activated TGCT. In this study, we determined the expression of programmed cell death ligand 1 (PD-L1) in 40 TGCT cases by immunohistochemistry and evaluated its clinical significance. We found that PD-L1 was positively expressed in 52.5% of all patients, and among them, the mononuclear cells, multinucleated cells, and foam cells with positive PD-L1 expression were observed in 21 (52.5%), 10 (25.0%), and 7 (17.5%) patients, respectively. The mononuclear cells and foam cells exhibited PD-L1 expression on the membrane or in the cytoplasm, and the multinucleated cells showed membranous PD-L1 expression. In addition, the PD-L1-positive mononuclear cells, multinucleated cells, and foam cells co-expressed CD68. Moreover, the patients with positive PD-L1 expression had a larger tumor size than those with negative PD-L1 expression. We further found that the foam cells of human coronary atherosclerosis also exhibited the expression of PD-L1 in two of three patients. These findings provide valuable evidence that PD-L1 is highly positive in CSF1-activated TGCT, and treatment with anti-PD-L1 agents may be a valuable therapeutic option for those diseases with PD-L1 expression on mononuclear cells, multinucleated cells, or foam cells.

Keywords: PD-L1, mononuclear cells, multinucleated giant cells, foam cells, TGCT

Introduction

Tenosynovial giant cell tumor (TGCT) is a locally aggressive and proliferative neoplasm, arising from the synovium of joint, bursa, and tendon sheath, and often resulting in joint impairment or bone destruction [1]. TGCT can be clinically divided into localized and diffuse types. Surgery to remove the tumor is the primary treatment; however, the tumor tends to recur. Studies on the molecular mechanisms of TGCT confirm that a translocation involving colony-stimulating factor 1 (CSF1) and COL6A occurs in a minority of cells, which leads to the activation of CSF1 [2]. Elevated expression of CSF1 is confirmed in all examined TGCT samples [3, 4]. The CSF1 is a cytokine, also known as macrophage colony-stimulating factor, and is involved in the differentiation and proliferation of monocytes and macrophages. The cells with active CSF1 in TGCT recruit CSF1 receptor (CSF1R)-expressing cells of monocyte-macrophage lineage through autocrine and paracrine mechanisms [2]. This is consistent with the studies that treatment of TGCT with a selective CSF1R inhibitor [3] or a novel anti-CSF1R antibody [5] provides valuable therapeutic benefits.

The programmed cell death ligand-1 (PD-L1) plays a crucial role in regulating immune responses; the binding of PD-L1 to its receptor PD-1 transmits an inhibitory signal that attenu-

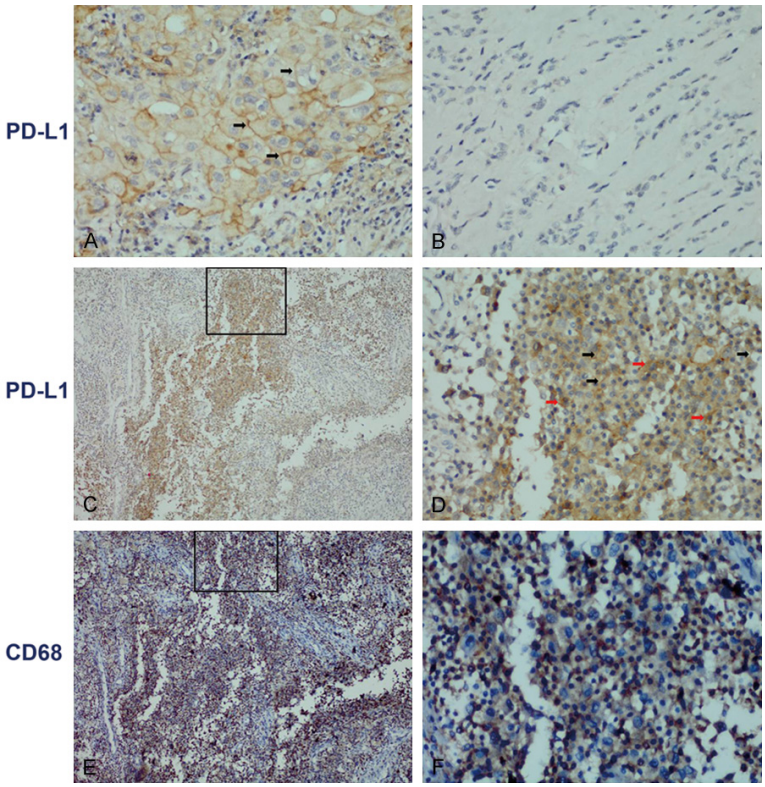

Figure 1. Expression of PD-L1 and CD68 in mononuclear cells in TGCT. (A) Membranous staining of PD-L1 was observed as a positive control in lung cancer, original magnification, ×400. (B) Negative PD-L1 expression was shown in TGCT, original magnification, ×400. (C) Expression of PD-L1 in mononuclear cells in TGCT, original magnification, ×100. (D) Original magnification of the boxed area shown in (C), ×400. Black arrow indicates membranous expression of PD-L1 in mononuclear cells; red arrow indicates cytoplasmic expression of PD-L1 in mononuclear cells. (E) Adjacent serial tissue section of C was stained with CD68; the majority of cells were CD68-positive, original magnification, ×100. (F) Original magnification of the boxed area shown in (E), ×400.

ates lymphocyte activation and favors tumor progression [6]. Overexpression of PD-L1 in various cancers such as urothelial cancer [7], pancreatic cancer [8], ovarian cancer [9], and acute leukemia [10] is associated with unfavorable prognosis. PD-L1 expression may be a predictive biomarker for outcomes of anti-PD-L1 therapy; the response rate to anti-PD-L1 treatment is higher in patients with PD-L1-positive tumors than in those with PD-L1-negative tumors [11]. TGCT is composed predominantly of mononuclear cells, multinucleated cells, and foam cells due to CSF1 activation [2, 12]. Immunophenotyping of TGCT exhibits monocyte-macrophage-like features and myofibroblastic differentiation [13]. Therefore, TGCT can be considered a neoplastic disease model to study the biology of mononuclear cells, multinucleated cells, and foam cells. However, PD-L1 expression in those cells or its relation with clinicopathologic parameters has not been investigated in TGCT.

In this study, we examined PD-L1 expression in 40 TGCT samples by immunohistochemistry (IHC). We found that 21 of 40 patients had positive PD-L1 expression, and PD-L1 protein was detected on the membrane or in the cytoplasm of mononuclear cells and foam cells; whereas, the multinucleated cells showed membranous PD-L1 expression. The PD-L1-positive cells also expressed the monocyte-macrophage lineage marker CD68, and there was a positive correlation between the expression of PD-L1 and tumor size. Our findings support the investigation of immunotherapeutic approaches targeting PD-L1 in diseases with CSF1 activation.

Materials and methods

Human samples

Formalin-fixed paraffin-embedded tumor tissues from 40 patients diagnosed with tenosynovial giant cell tumor between 2012 and 2017 were retrieved from the Pathology Department of Fudan University Shanghai Cancer Center. The 40 study patients were followed up for 7 to 71 months (median, 32 mo) after complete surgical resection in our hospital. Formalin-fixed paraffin-embedded coronary atherosclerosis tissue slides were obtained from the Pathology Department of Changzhou No. 2 People's Hospital. Prior written informed consent was given by each patient, and the study was approved by the Ethical Committee of Fudan University Shanghai Cancer Center.

Immunohistochemical staining

Formalin-fixed paraffin-embedded tumor tissues were cut into 4-μm sections. IHC staining was done according to standard procedures.

Table 1. Relation between PD-L1 expression and clinicopathologic features

Clinicopathologic feature	Number of cases	PD-L1-positive cases	PD-L1-negative cases	*P-value*
Gender				0.721†
Male	10	6	4	
Female	30	15	15	
Age (y)				0.666*
≤50	26	13	13	
>50	14	8	6	
Status at admission				0.457†
First recurrence	9	6	3	
New diagnosis	31	15	16	
Bone erosion				0.962*
Yes	17	9	8	
No	23	12	11	
Diameter (cm)				0.028*
≤5	18	6	12	
>5	22	15	7	
Relapse				0.664†
Yes	6	4	2	
No	34	17	17	
Histology				0.119*
Localized type	18	7	11	
Diffuse type	22	14	8	
Overall survival				1.000†
Alive	37	19	18	
Dead	3	2	1	

*: Chi-square test. †: Fisher's exact test.

Table 2. PD-L1 expression in 40 cases of TGCT

	Negative	Weak	Moderate	Strong
Cases	19 (47.5%)	4 (10.0%)	7 (17.5%)	10 (25.0%)

Heat-induced epitope retrieval in ethylenediamine tetraacetic acid (EDTA) buffer was performed. Following incubation with protein blocking, slides were incubated at 4°C overnight with PD-L1 monoclonal rabbit antibody (1:200, E1L3N, Cell Signaling Technology) or CD68 monoclonal mouse antibody (1:200, ab955, Abcam). The negative control samples were treated identically but without the addition of primary antibody. Formalin-fixed paraffin-embedded human lung cancer tissues sectioned at 4-μm from Fuzhou Maixin Biotechnology (Fujian, China) were used as a positive control. The staining underwent signal amplification using IHC Detection Kit (KIT-5920, Fuzhou Maixin Biotechnology, Fujian, China). PD-L1 expression was classified as positive if a distinct membranous or cytoplasmic staining with 5% or greater expression was observed [6, 14], we defined samples with 5% to 10% of positive cells, 11% to 50% of positive cells, and greater than 51% of positive cells as weak, moderate, and strong expression, respectively. All slides were reviewed by two experienced pathologists.

Statistical analysis

The categorical parameters between the PD-L1-positive and PD-L1-negative groups were compared with the chi-square test or the Fisher exact test, where appropriate. *P*-values <0.05 were considered statistically significant.

Results

Expression of PD-L1 on mononuclear cells, multinucleated cells, and foam cells in TGCT

The immune checkpoint regulator PD-L1 has not been determined in TGCT. To evaluate the biological and clinical significance of PD-L1 expression on TGCT, we performed IHC analysis on 40 TGCT samples. PD-L1-positive lung cancer slides were used, and obvious membranous staining of PD-L1 as a positive control was observed (**Figure 1A**). Nineteen of 40 (47.5%) samples exhibited negative PD-L1 expression using 5% as a cut-off value (**Figure 1B**; **Tables 1** and **2**). Four patients had weak expression, 7 patients had moderate expression, and 10 patients showed strong expression of PD-L1 (**Table 2**). The mononuclear cells, multinucleated cells, and foam cells with positive PD-L1 expression were observed in 21 (52.5%), 10 (25.0%), and 7 (17.5%) patients, respectively (**Table 3**). Mononuclear cells are the major cell type involved in TGCT. Interestingly, we found

Table 3. PD-L1 expression in different cell types

Cell types	PD-L1-positive cases	PD-L1-negative cases
Mononuclear cells	21 (52.5%)	19
Multinucleated cells	10 (25.0%)	30
Foam cells	7 (17.5%)	33

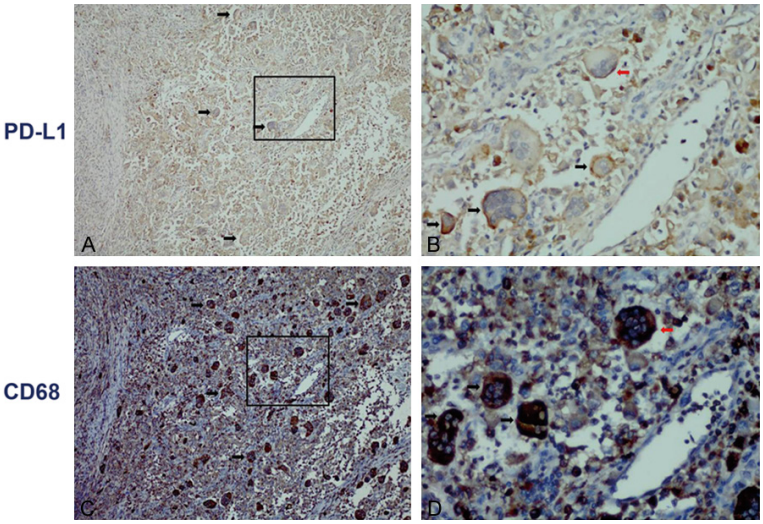

Figure 2. Expression of PD-L1 and CD68 in multinucleated cells in TGCT. (A) Membranous staining of PD-L1 was shown in multinucleated cells, original magnification, ×100. (B) Original magnification of the boxed area shown in (A), ×400. (C) Adjacent serial tissue section of A was stained with CD68, original magnification, ×100. (D) Original magnification of the boxed area shown in (C), ×400. Red arrow indicates the same multinucleated cell in adjacent serial sections, showing membranous staining of PD-L1 (B) and cytoplasmic staining of CD68 (D).

that mononuclear cells showed positive staining for PD-L1 either on the membrane or in the cytoplasm (**Figure 1C** and **1D**). Cytoplasmic expression may represent intracellular stores of PD-L1, which may be deployed to the membrane depending on appropriate stimulation [15]. Multinucleated cells result from cell membrane fusion of mononucleated stromal cells in TGCT [16]. In this study, we found that multinucleated cells exhibited membranous PD-L1 expression (**Figure 2A** and **2B**). PD-L1 was expressed on the membrane or in the cytoplasm of foam cells (**Figure 3A** and **3B**).

PD-L1-positive cells co-express the monocyte-macrophage lineage marker CD68

TGCT is a rare and neoplastic disease with CSF1 (also known as macrophage colony-stimulating factor) activation, which leads to the differentiation and proliferation of monocytes and macrophages [2]. IHC was performed with the monocyte-macrophage lineage marker CD68 on adjacent serial tissue sections; the results showed that the majority of mononuclear cells, multinucleated cells, and foam cells with positive PD-L1 expression co-expressed the CD68 protein (**Figures 1**-**3**). Monocytes circulate in the bloodstream and move into particular tissues where they then differentiate into macrophages. Most of the mononuclear cells in TGCT showed strong positive staining for CD68 (**Figure 1E** and **1F**), indicating that most of the mononuclear cells are macrophages. Interestingly, almost every multinucleated cell in TGCT was CD68-positive, and multinucleated cells had a stronger expression for CD68 than did mononuclear cells (**Figure 2C** and **2D**). CD68 was also strongly positively expressed in the majority of foam cells (**Figure 3C** and **3D**).

PD-L1 expression is positively associated with tumor size

The relation between the PD-L1 expression and clinicopathologic parameters of TGCT is summarized in **Table 1**. The results showed that no significant correlations were observed between the PD-L1 expression and gender, age, status at admission (first recurrence vs. new diagnosis), bone erosion, relapse, histology type, and overall survival (**Table 1**). However, we found that patients with positive PD-L1 expression had a larger tumor size than patients with negative PD-L1 expression (**Table 1**), suggesting that PD-L1 may play an important role in immune regulation by delivering inhibitory signals to maintain the tumor growth in TGCT. During follow-up, 3 of 40 patients died. One patient who had been diagnosed with TGCT for 20 years died of spinal metastasis with the pathological diagnosis of malignant TGCT. Another patient diagnosed with malignant TGCT

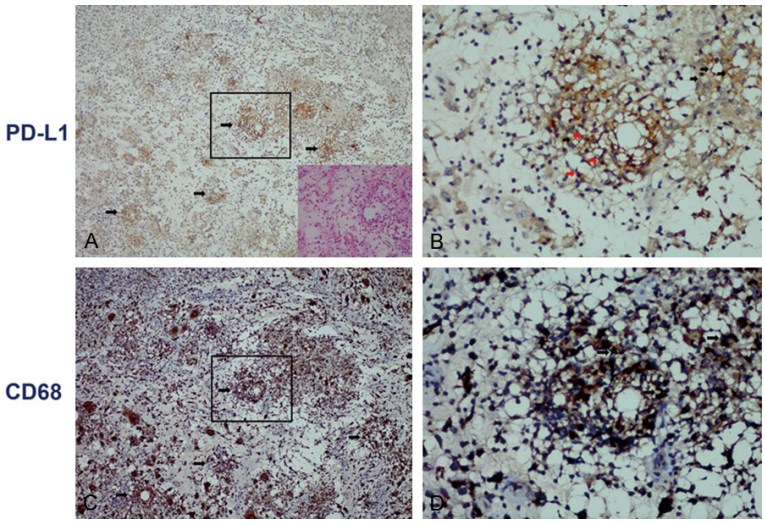

Figure 3. Expression of PD-L1 and CD68 in foam cells in TGCT. (A) Membranous or cytoplasmic staining of PD-L1 is shown in foam cells, original magnification, ×100. Hematoxylin and eosin staining of the adjacent section of the boxed area is shown in the bottom right corner. (B) Original magnification of the boxed area shown in (A), ×400. Black arrow indicates membranous expression of PD-L1 in foam cells; red arrow indicates cytoplasmic expression of PD-L1 in foam cells. (C) Adjacent serial tissue section of A was stained with CD68, original magnification, ×100. (D) Original magnification of the boxed area shown in (C), ×400.

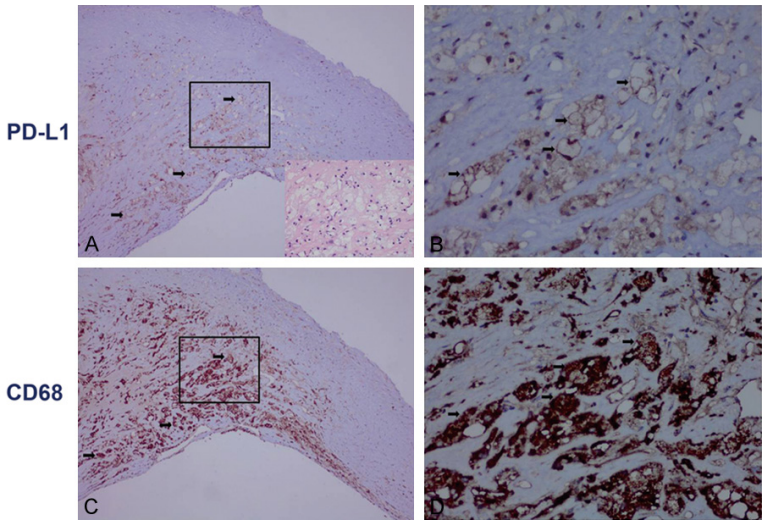

Figure 4. Expression of PD-L1 and CD68 in foam cells in human coronary atherosclerosis. (A) Membranous staining of PD-L1 was observed, original magnification, ×100. Hematoxylin and eosin staining of the adjacent section of the boxed area was shown in the bottom right corner. (B) Original magnification of the boxed area shown in (A), ×400. (C) Adjacent serial tissue section of A was stained with CD68, original magnification, ×100. (D) Original magnification of the boxed area shown in (C), ×400.

died of lung metastasis. Both of the patients had positive staining for PD-L1. The third patient with negative PD-L1 expression in TGCT died of a second primary pancreatic cancer.

Foam cells not only from TGCT but also from human coronary atherosclerosis may express PD-L1

Foam cells are derived from macrophages, endothelial cells, or vascular smooth muscle cells with lipid accumulation, and they are the hallmark of atherosclerosis [17]. We found PD-L1 expression on foam cells in TGCT (**Figure 3A** and **3B**). To confirm whether the foam cells from coronary atherosclerosis similarly express the PD-L1, we next determined the expression of PD-L1 on foam cells in three coronary atherosclerosis patients and found that the foam cells exhibited membranous expression of PD-L1 in two of three patients (**Figure 4A** and **4B**). Foam cells were fat-laden macrophages, as they showed ectopic fat deposition (**Figure 4A**) and strong CD68 expression in serial section samples (**Figure 4C** and **4D**). To our knowledge, this is the first study to report PD-L1 expression in foam cells, suggesting that PD-L1 may play an important role in foam cells of TGCT and atherosclerosis.

Discussion

Immunotherapy holds substantial promise for anti-tumor treatment [6]. TGCT is characterized by CSF1 activation [2]. In this study, we explored the PD-L1 expression in TGCT, and found that PD-L1 was positively expressed in 53% of all patients. Mononuclear cells and foam cells exhibited PD-L1 expression on the membrane or in the cytoplasm; multinucleated cells showed membranous PD-L1

expression. The majority of PD-L1-positive cells co-expressed CD68. Furthermore, patients with positive PD-L1 expression had a larger tumor size than those patients with negative PD-L1 expression. To our knowledge, this is the first study to explore the PD-L1 expression in the CSF1-activated disease, and our results support the investigation of immunotherapeutic approaches targeting PD-L1 in diseases with CSF1 activation to liberate antitumor immunity.

CSF1 is a hematopoietic growth factor that has significant impact on the proliferation, differentiation, and survival of monocytes and macrophages. The op/op mice with an inactivating mutation in the CSF1 have a severe deficiency of mononuclear phagocytes. Moreover, the mice display defects in skeletal abnormalities, a low body weight, impaired fertility, and no teeth. Interestingly, administration of human recombinant CSF1 can restore the partial defects [18], indicating that CSF1 has important physiologic roles. The effect of CSF1 is mediated through its receptor (CSF1R), which is expressed on mononuclear phagocytes, osteoclasts, and tumor cells. The abnormally activated CSF1/CSF1R signaling pathway plays critical roles during cancer progression and metastasis in breast cancer [19], lung cancer [20], glioma [21], and pancreatic neuroendocrine tumor [22]. Overexpression of CSF1 in the mammary tumors of PyMT mice accelerates tumor development, progression, and pulmonary metastasis. Conversely, the absence of CSF1 delays tumor progression and decreases lung metastasis [23]. Clinically, elevated expression of CSF1 is confirmed in all examined TGCT samples [3, 4]. Administration of the small-molecule inhibitor PLX3397 [3] or the monoclonal antibody emactuzumab [5] against CSF1R to TGCT patients leads to impressive response rates. Based on these encouraging data, a variety of small molecules and monoclonal antibodies directed against CSF1 or its receptor CSF1R are being investigated in solid tumors [24]. TGCT can be considered a disease model with proliferation of mononuclear cells, multinucleated cells, foam cells, and other inflammatory cells due to CSF1 activation [2, 12]. In our study, we found that PD-L1 was expressed on those cells. Tumor PD-L1 expression is an effective predictor of the outcomes of cancer immunotherapy [11]. PD-L1 not only on malignant cells but also on immune cells is involved in the mechanism of cancer immunotherapy [25]. Therefore, anti-PD-L1 in combination with other molecular targeted therapy may improve the management of tumors with activated CSF1/CSF1R signaling.

Many studies confirm that PD-L1 is expressed in tumor cells and tumor-infiltrating immune cells in various cancers [6]. However, which types of immune cells express PD-L1 is not well clarified. In this study, we found that most of the cells were macrophages, as they showed CD68 expression (**Figures 1**-**3**), which is consistent with CSF1 activation in TGCT [2]. We further found that most of the PD-L1-positive cells co-expressed the CD68 protein by IHC on adjacent serial tissue sections (**Figures 1**-**3**), indicating the PD-L1 positive cells are macrophages or originated from macrophages. Macrophages play important roles in the immune system, especially in phagocytosis, antigen presentation, and cytokine production. In malignant diseases, increasingly, studies demonstrate that macrophages actively mediate tumor metastasis [26], angiogenesis [27], drug resistance [28], and immune impairment [29]. Strikingly, Germano *et al* report that trabectedin, an approved antitumor agent, induces apoptosis exclusively in mononuclear phagocytes of blood and tumor tissues, and its cytotoxicity on mononuclear phagocytes is a key component of antitumor activity [30]. A recent study found that specific inhibition of the PD-1/PD-L1 axis in tumor-associated macrophages increases macrophage phagocytosis, inhibits tumor growth, and prolongs the survival of mice in mouse models of cancer [31]. Together with our findings, these results suggest macrophages with PD-L1 expression might be suitable targets for antitumor therapy.

There are few research studies about PD-L1 expression in multinucleated cells and foam cells, which play important roles in certain diseases such as bone destruction and atherosclerosis. Multinucleated cells result from the cell membrane fusion of mononucleated stromal cells [16], and they express phenotypic features of osteoclasts in TGCT [32]. Osteoclasts are also multinucleated cells that are crucial to biomaterial degradation during bone resorption or destruction. In cell experiments, An *et al*

found that osteoclasts protect multiple myeloma cells against T-cell-mediated cytotoxicity via upregulating expression of PD-L1, Galectin-9, and HVEM [33]. The results together with our findings suggest that multinucleated cells expressing PD-L1 may have important roles in the immune escapes in TGCT patients. Targeting multinucleated cells through PD-L1 may have the potential to inhibit bone destruction. But, we did not find a relation between PD-L1 expression and bone erosion in our study; this may be not only due to PD-L1, but also due to other genes that are involved in bone destruction. The role of foam cells in TGCT has not been clarified. In atherosclerosis [17], foam cells are derived from macrophages, endothelial cells, or vascular smooth muscle cells; the formation of foam cells plays a central role in the development of atherosclerosis, as they accumulate large amount of lipids. Foam cells are an indication of plaque build-up, which is closely associated with myocardial infarction and stroke. To our knowledge, this is the first study to find the positive expression of PD-L1 on foam cells not only from TGCT but also from coronary atherosclerosis. Although only one study shows that tissue-resident macrophages express PD-L1 through all stages of atherosclerosis, whether the foam cells with ectopic fat deposition express the PD-L1 was not further clarified in this study [34]. Our results suggest that anti-PD-L1 treatment may have a therapeutic potential to target the foam cells, especially in atherosclerosis; further validation of this hypothesis is needed. It is also noteworthy that the PD-L1-positive foam cells may be targeted when cancer patients with coronary atherosclerosis receive the anti-PD-L1 treatment.

In this study, we found that PD-L1 was positively expressed in mononuclear cells, multinucleated giant cells, and foam cells in TGCT. The expression of PD-L1 was associated with large tumor size. In addition, we found that the foam cells of human coronary atherosclerosis also exhibited the expression of PD-L1. Our findings open a new opportunity to target the mononuclear cells, multinucleated giant cells, and foam cells with anti-PD-L1 immunotherapy in those malignant or benign diseases, but this needs to be further investigated.

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Disclosure of conflict of interest

None.

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