Original Article CD8⁺ cytotoxic and FoxP3⁺ regulatory T lymphocytes serve as prognostic factors in breast cancer

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Abstract: Background: There is conflicting evidence regarding the prognostic value of cytotoxic T cell infiltration in breast cancer. The aims of this study were to detect the expression levels and localization of FoxP3 and CD8 in invasive ductal carcinoma of the breast and to investigate the correlations among FoxP3⁺ regulatory T cells (Tregs), CD8⁺ cytotoxic T lymphocytes (CTLs), clinicopathological features, and prognosis in patients with breast cancer. Methods: Immunohistochemistry was used to detect the expression levels and localization of FoxP3 and CD8. One-sample t-test, one-way analysis of variance, and Kaplan-Meier log-rank tests were used to analyze correlations between the expression levels of CD8 and FoxP3; Kaplan-Meier Log-rank test was used to analyze clinicopathological features to explore the prognostic significance of CD8 and FoxP3 in patients with breast cancer. Results: FoxP3 expression in the tumor bed was higher than that in the stroma, while CD8 was primarily expressed in the stroma. CD8 expression was associated with favorable prognostic factors. However, FoxP3 expression and an increased ratio of total FoxP3+ Tregs to CD8⁺ CTLs were significantly correlated with unfavorable prognostic factors. Additionally, an increased ratio was associated with molecular subtypes (ER⁺Her2⁺, ER⁺Her2⁺, ER⁺Her2⁺, and ER⁺Her2⁻) of breast cancer. Overexpression of FoxP3 and a high FoxP3⁺/CD8⁺ ratio were correlated with poor overall survival (OS) and disease-free survival (DFS). However, CD8 expression only affected OS in patients with breast cancer. Conclusions: Tumor-infiltrating lymphocytes are localized variously depending on the subtype. CD8⁺ CTLs were associated with a good prognosis, while FoxP3⁺ Tregs were associated with adverse outcomes in patients with breast cancer. CD8⁺ CTLs and FoxP3⁺ Tregs are potential predictive prognostic factors for patients with breast cancer.

Keywords: FoxP3, CD8, breast cancer, tumor bed, tumor stroma

Introduction

Breast cancer is one of the four most common malignant tumors in women, and the incidence and mortality rates of breast cancer continue to increase worldwide. American Cancer Society statistics revealed that breast cancer accounts for approximately 29% (232,670) of new cancer cases (ranked first) and 15% (40,000) of cancer-related deaths (ranked second) [1]. Therefore, it is an urgent for researchers to find new biological prognostic factors for patients with breast cancer, which can predict disease recurrence and therefore, the fundamental for supplemental treatment in breast cancer.

Clinicopathological features, genetic factors, and the cancer stem cell hypothesis are thought

to be the major determinants of prognosis in patients with breast cancer [2]. Recently, the role of immunity in tumors has gained attention, becoming a major topic of debate [3]. Regulatory T lymphocytes (Tregs) and cytotoxic T lymphocytes (CTLs) help maintain homeostasis by immunological surveillance and immune tolerance. Additionally, Tregs and CTLs play important roles in the tumor microenvironment and participate in tumor cell growth, invasion, and metastasis.

FoxP3 is a specific transcription factor that is expressed on the surface of Tregs. FoxP3 belongs to the forkhead/winged-helix family, which is essential for the development and function of Tregs in both humans and mice [4]. Loss of FoxP3 function leads to Treg deficiency, resulting in fatal autoimmune disease; FoxP3 overexpression leads to severe immunodeficiency. FoxP3⁺ Tregs are abundant in tumor infiltrates and peripheral blood from patients with cancer. Studies of many types of cancer have suggested that a high level of tumor-infiltrating Tregs is associated with poor clinical outcomes [5].

CD8⁺ CTLs are the major effector cells for tumor elimination; they specifically recognize tumor-derived antigenic epitopes related to major histocompatibility complex (MHC)-I. Many studies have concluded that tumor-infiltrating CD8⁺ lymphocytes have antitumor activity, as evidenced by its favorable effect on patient survival in colorectal, ovarian, lung, and pancreatic cancers [6, 7]. However, the impacts of lymphocyte infiltrates in breast cancer are numerous and complex. Most studies of breast cancer have revealed that infi-Itration of CTLs is associated with better prognosis, while several studies have demonstrated a negative or nonexistent correlation with prognosis. While in patients with primary invasive ductal carcinoma of the breast, the expression of CD8⁺ TIL and FOXP3⁺ TIL status in tumor bed and stroma has remained unclear.

Here, in this study, we investigated the CD8 expression, FoxP3 expression and the ratio of total FoxP3⁺ Tregs to CD8⁺ CTLs, aiming to evaluate the prognostic value of cytotoxic T cell infiltration in breast cancer and making a development in patients with breast cancer.

Materials and methods

Study patients and specimens

The present study enrolled 122 patients with primary invasive ductal carcinoma of the breast, who received standard surgical treatment. Cancer diagnosis was made by pathologists at Union Hospital, Wuhan, China, from 2004 to 2008. No patient received radiotherapy, neoadjuvant chemotherapy, endocrinotherapy, or targeted therapy. The age range of the patients was 34-79 years (median age: 50 years), and the median follow-up duration was 78 months. Pathology grade: high differentiation (grade II) 49 cases, moderate differentiation (grade III) 33 cases and low differentiation (grade III) 40 cases. All breast cancer specimens were fixed in 10% formalin and embedded in paraffin for histopathological analysis. The clinical and pathological characteristics of the patients, including age, tumor size, lymph node status, pathological grade, Ki-67 expression, and molecular subtype, were routinely assessed. Survival data were maintained prospectively. Survival was defined as the time from the date of the primary surgical treatment to the time of death due to breast cancer. Survival was not included if the patient was lost to follow-up or died due to other causes.

Pathologic examination and immunohistochemistry

The grade of pathological tumor grade was assessed according to the Scarff-Bloom Richardson (SBR) classification modified by Elstonand Ellis [8]. Breast cancer tissues were cut into serial 3-um thick sections and then they were transferred to electrostatic slides for immunochemical analysis. Briefly, these specimens were heated at 65°C for 30 min, deparaffinized in xylene, and rehydrated in a graded ethanol series. The endogenous peroxidase activity was blocked by 3% H₂O₂ for 10 min. Next, 5% bovine serum albumin/1 × tris-buffered saline and Tween-20 were used to reduce nonspecific background staining. A primary antibody (FoxP3, dilution 1:100, eBioscience, San Diego, CA; CD8, DAKO, Glostrup, Denmark, ready-to-use) was applied for 2 h at 37°C. After rinsing three times with phosphate-buffered saline (PBS), the bound primary antibody was detected by using the secondary antibody (DAKO, Carpinteria, CA) and the mixture was incubated at 37°C for 30 min. After a series of PBS rinses, the bound antibody was visualized with a 10-min incubation with by 3,3'-diaminobenzidine in buffered substrate (DAKO) at room temperature. The specimens were counterstained with hematoxylin, polarized with 1% hydrochloric acid alcohol solution, and incubated with the bluing agent lithium carbonate.

Assessment of immunohistochemical staining

The tumor bed was distinguished from the stroma using hematoxylin and eosin staining. Immunohistochemical staining revealed the level and localization of FoxP3 and CD8 expression. Under light microscopy, 10 representative

	Tumor	nest	Tumor	$D_{v}(D_{v},O,O,E)$	
	Case number	Mean	Case number	Mean	P-value ($P < 0.05$)
CD8 ⁺ cells	118	8.48±0.32	116	12.53±0.91	0.002
FoxP3 ⁺ cells	118	9.89±0.21	114	7.02±0.18	0.005
FoxP3 ⁺ /CD8 ⁺ ratio	116	2.69±0.61	116	0.96±0.03	0.036

Table 1. The average number of CD8⁺ and FoxP3⁺ cells in tumor nest and stroma

fields were chosen in the tumor bed and stroma for each slide [10]. Cell Sens Entry microscopy imaging software was used to count the positive cells in each field and obtain images. The number of positive cells on each slide is reported as a mean value of 10 fields.

Statistical analysis

All data are expressed as the mean \pm standard deviation. Statistical analyses were performed using SPSS 17.0. One-sample *t*-tests and oneway analysis of variance (ANOVA) were applied to analyze the correlations between FoxP3⁺ Tregs or CD8⁺ CTL expression levels and clinical and pathological features of patients with breast cancer. The correlations between the expression levels of FoxP3⁺ Tregs or CD8⁺ CTLs and patient survival was calculated by the Kaplan-Meier method and analyzed by the log-rank test. A *P*-value of < 0.05 was considered statistically significant.

Results

Average numbers of CD8⁺ and FoxP3⁺ cells in the tumor bed and stroma

The average number of CD8⁺ cells in the tumor bed (8.48) was less than that in the tumor stroma (12.53, P = 0.002). However, the average number of FoxP3⁺ cells in the tumor bed (9.89) was greater than that in the tumor stroma (7.02, P = 0.005). The FoxP3⁺/CD8⁺ ratio was 2.69 in the tumor bed and 0.96 in the tumor stroma (P = 0.036) (**Table 1** and **Figure 1**).

Correlations of the expression levels of CD8 and FoxP3 with clinicopathological features

The positive expression of CD8 in the tumor bed was associated with negative lymph node metastasis, while the expression of FoxP3 was associated with lymphatic metastasis, a higher pathological grade (grade III), and Ki-67 \geq 14%. Additionally, a higher FoxP3⁺/CD8⁺ ratio was correlated with a higher pathological grade (III, P = 0.025; Table 2).

In the tumor stroma, a high CD8 expression level was correlated with a low pathological grade (grade I/II, P = 0.025), and high FoxP3 expression was associated with a high pathological grade (P < 0.001) and Ki-67 $\ge 14\%$ (P = 0.001, Table 3).

Furthermore, a greater total number of CD8positive cells was associated with negative lymph node metastasis (P = 0.028) and a low pathological grade (P = 0.047), while a greater total number of FoxP3 cells and a greater FoxP3⁺/CD8⁺ ratio were both correlated with lymphatic metastasis, high pathological grade, and Ki-67 \ge 14% (**Table 4**).

Relationship of the expression levels of CD8 and FoxP3 with molecular subtypes of breast cancer

Breast cancer is classified into four molecular subtypes, namely, ER⁺Her2⁺, ER⁺Her2⁻, ER⁻ Her2⁺, and ER⁻Her2⁻, based on the ER and Her2 expression profile. One-way ANOVA was used to assess the correlation of the expression levels of CD8 and FoxP3 with these four molecular subtypes. Only FoxP3 expression was associated with the four molecular subtypes (**Table 5**).

The correlations between the FoxP3 expression level and molecular subtypes were further evaluated in the tumor bed and stroma. Significant differences in FoxP3 levels in the tumor stroma were detected between the ER⁺Her2⁺ and ER⁻Her2⁻ subtypes and between the ER⁺Her2⁻ and ER⁻Her2⁻ subtypes. However, in terms of the level in the tumor bed and the total expression level, FoxP3 also exhibited a marked, statistically significant difference between ER⁺Her2⁺ and ER⁻Her2⁺, ER⁺Her2⁺ and ER⁻Her2⁻, ER⁺Her2⁻ and ER⁻Her2⁺, and ER⁺Her2⁻ and ER⁻Her2⁻.



Figure 1. The expression level of CD8 and FoxP3 in tumor nest and stroma of breast cancer tissues. (A-C) are the expression level of CD8 in breast cancer tissues (A is total level of tumor nest and stroma, 100×, B is tumor nest, 400×, C is tumor stroma, 400×); (D-F) are expression level of FoxP3 in breast cancer tissues (D is total level of tumor nest and stroma, 100×, E is tumor nest, 400×, F is tumor stroma, 400×).

Correlation of CD8 and FoxP3 levels with overall survival (OS) and disease-free survival (DFS)

Based on a pairwise over strata comparison, in the tumor bed, the DFS of patients with a high CD8 expression level was obviously greater than that in patients with a low CD8 expression level of (P = 0.021). There was no correlation between DFS of patients and CD8 expression level in the tumor stroma and in the entire tumor (**Figure 2**). However, OS was not correlated with CD8 expression level. For the tumor bed, the tumor stroma, and the entire tumor,

Cliniconothological factures	Case	CD8 ⁺ cells		FoxP3 ⁺ cells		FoxP3 ⁺ /CD8 ⁺ ratio	
	number	Mean	P value	Mean	P value	Mean	P value
Patient age							
< 50	60	7.633±0.483	0.462	9.540±0.722	0.973	3.464±0.814	0.532
≥ 50	62	8.752±0.725		9.594±0.648		1.971±0.631	
Tumor size (cm)							
≤2	54	8.511±0.150	0.717	9.033±0.92	0.550	3.784±0.348	0.251
> 2	68	7.956±0.174		9.991±0.298		1.921±0.115	
Lymph node status							
Negative	46	11.157±0.177	0.002	6.883±0.726	0.007	0.874±0.010	0.073
Positive	76	6.413±0.211		11.192±0.703		3.803±0.079	
Pathology grade							
1/11	64	9.178±0.052	0.175	6.031±0.267	< 0.001	0.935±0.137	0.025
III	58	7.124±0.301		13.469±0.394		4.449±0.289	
Ki-67 value							
< 14%	46	9.749±0.161	0.110	4.756±0.750	< 0.001	0.679±0.072	0.055
≥ 14%	76	7.266±0.056		12.474±0.183		3.8357	

 Table 2. The relationship between expression level of CD8 and FoxP3 and clinicopathological features in tumor nest

 Table 3. The relationship between expression level of CD8 and FoxP3 and clinicopathological features in tumor stroma

Oliniaan athalagiaal faatuwaa	Case CD8+		ls	FoxP3 ⁺ cells		FoxP3 ⁺ /CD8 ⁺ ratio	
	number	Mean	P value	Mean	P value	Mean	P value
Patient age							
< 50	60	12.740±0.143	0.444	6.027±0.467	0.410	1.097±0.824	0.448
≥ 50	62	11.116±0.399		7.070±0.509		0.832±0.032	
Tumor size (cm)							
≤ 2	54	10.548±0.908	0.249	6.663±0.058	0.882	1.154±0.111	0.376
> 2	68	13.000±0.773		6.473±0.765		0.839±0.539	
Lymph node status							
Negative	46	13.030±0.725	0.413	5.283±0.289	0.114	0.886±0.008	0.727
Positive	76	11.240±0.327		7.328±0.763		1.012±0.093	
Pathology grade							
1/11	64	14.134±0.448	0.025	4.540±0.354	< 0.001	0.710±0.025	0.142
III	58	9.466±0.154		8.783±0.563		1.218±0.009	
Ki-67 value							
< 14%	46	14.126±0.298	0.101	3.990±0.322	0.001	0.810±0.026	0.488
≥ 14%	76	10.576±0.270		8.111±0.457		1.059±0.305	

there was no statistically significant correlation between CD8 expression level and OS of patients with breast cancer based on both pairwise over strata and pooled over strata comparisons (**Figure 2**).

In terms of expression in the tumor nest and entire tumor, patients with low expression of FoxP3 had a higher DFS and OS than those of subjects with high expression of FoxP3 (all P < 0.05). However, in the tumor stroma, the FoxP3 expression level was not significantly correlated with DFS and OS in patients with breast cancer (**Figure 3**).

The correlation between DFS and FoxP3⁺/CD8⁺ ratio was statistically significant (P < 0.01), and further analysis revealed that patients with a

Clinicopathological Case		CD8⁺ cel	ls	FoxP3⁺ ce	ells	FoxP3 ⁺ /CD8 ⁺ ratio	
features	number	Mean	P value	Mean	P value	Mean	P value
Patient age							
< 50	60	20.373±0.357	0.863	15.567±0.542	0.988	1.246±0.161	0.976
≥ 50	62	19.868±0.494		15.528±0.532		1.234±0.096	
Tumor size (cm)							
≤ 2	54	19.059±0.107	0.520	15.696±0.631	0.916	1.606±0.003	0.116
> 2	68	20.956±0.083		15.429±0.682		0.989±0.010	
Lymph node status							
Negative	46	24.187±0.342	0.028	12.165±0.108	0.033	0.585±0.023	0.008
Positive	76	17.653±0.406		17.594±0.602		1.629±0.033	
Pathology grade							
1/11	64	23.969±0.810	0.047	10.571±0.117	< 0.001	0.794±0.046	0.017
III	58	17.176±0.127		21.038±0.159		1.700±0.039	
Ki-67 value							
< 14%	46	23.004±0.510	0.307	8.755±0.140	< 0.001	0.665±0.080	0.020
≥ 14%	76	19.368±0.281		19.658±0.315		1.582±0.017	

 Table 4. The relationship between expression level of CD8 and FoxP3 and clinicopathological features in total level

 Table 5. The difference between expression level of CD8 and FoxP3 and the molecular subtype of breast cancer

	ER ⁺ Her2 ⁺	ER⁺Her2 ⁻	ER ⁻ Her2⁺	ER Her2	P value
CD8 ⁺ cells					
Tumor nest	7.363±0.265	7.856±0.217	9.387±0.035	8.631±0.859	0.849±0.013
Tumor stroma	16.038±0.546	11.244±0.344	11.407±0.062	13.562±0.414	0.659±0.279
Total level	23.400±0.226	19.100±0.842	20.793±0.032	22.192±0.685	0.846±0.226
FoxP3 ⁺ cells					
Tumor nest	4.700±0.325	7.396±0.080	10.807±0.701	13.359±0.002	0.015±0.001
Tumor stroma	4.988±0.390	5.160±0.481	8.453±0.901	8.462±0.695	0.040±0.004
Total level	10.888±0.659	12.556±0.385	19.260±0.915	21.821±0.792	0.009±0.021
FoxP3 ⁺ /CD8 ⁺ ratio					
Tumor nest	1.286±0.485	1.638±0.661	5.024±0.076	2.735±0.687	0.345±0.077
Tumor stroma	0.301±0.008	0.978±0.116	1.025±0.225	1.305±0.114	0.419±0.101
Total level	0.491±0.037	1.238±0.014	1.416±0.520	1.522±0.121	0.449±0.071

low FoxP3⁺/CD8⁺ ratio had a higher DFS than patients with a high FoxP3⁺/CD8⁺ ratio, for both the levels in the entire tumor and levels in the tumor bed. However, there was no statistically significant correlation between FoxP3⁺/CD8⁺ ratio in the tumor bed, tumor stroma, or entire tumor and OS in patients with breast cancer (**Figure 4**).

Relationship between CD8 and FoxP3 expression levels and 5-year DFS and OS

In the tumor bed, the 5-year DFS and OS of patients with low levels of FoxP3 expression

and low FoxP3⁺/CD8⁺ ratios were 86.7% and 90.0%, and 82.8% and 82.8%, respectively. And in the tumor stroma, the 5-year DFS and OS of patients with low levels of FoxP3 expression and low FoxP3⁺/CD8⁺ ratio were 83.3% and 90.0%, and 86.2% and 86.2%, respectively. These values were all significantly higher than those in patients with a high FoxP3 expression and a high FoxP3⁺/CD8⁺ ratio. However, FoxP3 expression level and FoxP3⁺/CD8⁺ ratio in the tumor stroma had no effect on 5-year DFS and OS. CD8 expression in both the tumor bed and stroma similarly had no impact











Figure 3. The relationship between expression level of FoxP3 and DFS and OS of breast cancer patients. (A and B) are the relationship between expression level of FoxP3 in tumor nest and DFS and OS of breast cancer patients. (C and D) are the relationship between expression level of FoxP3 in tumor stroma and DFS and OS of breast cancer patients. (E and F) are the relationship between expression level of FoxP3 in total level and DFS and OS of breast cancer patients.







	5 years DFS (%)	P value	5 years OS (%)	P value
Tumor nest				
CD8 low expression	56.7	0.189	66.7	0.520
CD8 high expression	73.3		73.3	
FoxP3 low expression	86.7	< 0.001	90.0	0.002
FoxP3 high expression	44.8		62.1	
FoxP3 ⁺ /CD8 ⁺ low ratio	82.8	0.002	82.8	0.014
FoxP3 ⁺ /CD8 ⁺ high ratio	44.8		55.2	
Tumor stroma				
CD8 low expression	63.3	0.817	70.0	0.828
CD8 high expression	70.0		70.0	
FoxP3 low expression	75.9	0.078	82.8	0.173
FoxP3 high expression	56.7		70.0	
FoxP3 ⁺ /CD8 ⁺ low ratio	69.0	0.274	75.9	0.214
FoxP3 ⁺ /CD8 ⁺ high ratio	57.1		60.7	
Total level				
CD8 low expression	56.7	0.257	66.7	0.608
CD8 high expression	73.3		73.3	
FoxP3 low expression	83.3	0.001	90.0	0.001
FoxP3 high expression	50.0		53.3	
FoxP3 ⁺ /CD8 ⁺ low ratio	86.2	0.001	86.2	0.007
FoxP3 ⁺ /CD8 ⁺ high ratio	44.8		55.2	

Table 6. The relationship between expression level of CD8and FoxP3 and 5 year DFS and 5 year OS

on DFS and OS in patients with breast cancer (Table 6).

Discussion

Immune surveillance and immune tolerance are important mechanisms for the maintenance of homeostasis. However, in tumor tissues, this balance is often disrupted and defective allowing tumor cells to evade monitoring by the immune system, resulting in continuous proliferation and growth, with the potential for invasion and metastasis [8]. In the tumor microenvironment, cancer cells induce the infiltration of various immune cells, predominantly CD8⁺ T lymphocytes and FoxP3⁺ Tregs, which are two types of tumor-infiltrating lymphocytes. CD8⁺ T lymphocytes and FoxP3⁺ Tregs are the main mediators of immune surveillance and immune tolerance, respectively; they play key roles in destroying tumor cells and abrogating the process of continuous proliferation and growth after tumor immune escape [10, 11].

Immune cells located and distributed in different sites playing various roles. For example, CD8⁺ CTLs possess prognostic significance due to its antitumor effects [12]. FoxP3⁺ Treg infiltration in tumors mainly functions to suppress the local anti-tumor immune response [2]. The expression levels of CD8+ CTLs in pericancerous tissues are significantly higher than those in cancer beds, when the distance from nests increases, the rate of CD8⁺ CTLs increases [13]. In contrast to the average number of CD8⁺ T cells in tumor beds, that in pericancerous tissues was 12.53 which significantly higher than that in tumor beds. Interestingly, nests exhibited greater FoxP3⁺ Treg infiltration than that in pericancerous tissues (Table 1 and Figure 1). Due to the immunosuppressive effects of FoxP3⁺ Tregs in the anti-tumor immune response, breast cancer cells cou-Id escape from the immune response and rapidly proliferate.

Generally, prognosis in patients with breast cancer is most highly correlated with cancer tissue pathological grade, lymph node status, hormone receptor status, epidermal growth

factor status, and the expression level of the cell proliferation factor Ki-67 [14, 15]. In our experimental studies, CD8⁺ T cells were related to favorable prognostic factors, such as carcinoma without lymph node metastasis and a low tumor grade, indicating that CD8⁺ T lymphocytes have a dominant antitumor function in low-grade breast invasive ductal carcinoma (Table 2). However, CD8 expression levels had no influence on the 5-year DFS and OS rates of patients with breast cancer (Table 6). In addition, increased FoxP3 expression levels in both cancer beds and peritumoral tissues were closely associated with lymph node metastasis, high Ki-67 expression levels, and breast invasive ductal carcinomas of pathological grade III (Table 2); these findings were consistent with results from previous studies [16, 17].

Furthermore, it is concluded that FoxP3⁺/CD8⁺ ratio was correlated with unfavorable prognostic factors and the state of FoxP3 expression. In most cancer beds of grade III breast cancer, the ratio of FoxP3⁺ Tregs to CD8⁺ T cells was always greater than that in grade I/II breast cancer (**Table 2**). The role of FoxP3⁺ Tregs in promoting breast cancer progression is wellestablished. According to a recent study, the number of Tregs that infiltrate tumors is closely related to the formation and progression of breast cancer [18]. Also, from normal breast tissues to ductal carcinomas *in situ* and invasive ductal cancer, the average ratio of FoxP3⁺ T lymphocytes gradually increases from 0.005 and 0.019 to 0.030 along with the malignant progression of tissues.

In addition, among the four types of breast cancers, namely, ER⁺Her2⁺, ER⁺Her2⁻, ER⁻Her2⁺, and ER⁻Her2⁻, ER⁺Her2⁺ breast cancers consistently exhibited the lowest FoxP3 expression levels in both the cancer bed and pericancerous tissues, while ER⁻Her2⁻ breast cancer was associated with the highest FoxP3 expression levels (P < 0.05) (**Table 3**). FoxP3 expression level was positively correlated with recurrence rate in patients with breast cancer and negatively correlated with the 5-year DFS rate [19-22]. The 5-year DFS and OS rates for patients with breast cancer with low FoxP3 expression could exceed 80%. However, the latter value was only approximately 50%, or even less than 50% in patients with high FoxP3 expression. Conversely, patients with a high FoxP3⁺/CD8⁺ ratio exhibited a significantly lower OS, DFS, and 5-year OS or DFS than those in patients with a low ratio (Table 4), demonstrating that FoxP3 expression is associated with poor prognosis in patients with breast cancer.

In conclusion, we demonstrated that increases in FoxP3⁺ Treg cells and the Foxp3⁺ Treg/CD8⁺ T cell ratio are correlated with more aggressive tumor characteristics, whereas increases in CD8⁺ T cells are correlated with favorable clinicopathologic features. Prognostic significance according to molecular subtypes and balance of tumor-infiltrating lymphocytes remains unclear, and future studies of the immunologic microenvironment with respect to the biological subtype of breast cancer are essential.

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Disclosure of conflict of interest

None.

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