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Prognostic effect of stromal lymphocyte infiltration in thrymic carcinoma

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ABSTRACT

Background: The purpose of this study was to clarify the relationship between the tumor infiltrating lymphocytes and clinical outcome in patients with thymic carcinoma.
Methods: Tissue specimens from 32 patients who underwent surgical resection for thymic carcinoma were immunohistochemically analyzed for CD4, CD8 and CD20 expression.
Results: Tumor-infiltrating lymphocytes were generally more abundant in the stroma. The patients with low CD4+ lymphocytes ($p = 0.037$) and low CD20+ lymphocytes ($p = 0.045$) within tumor stroma showed poor survival. Furthermore, concurrent low levels of CD4+ and CD20+ ($p = 0.014$), CD8+ and CD20+ ($p = 0.025$), and, CD4+, CD8+, and CD20+ ($p = 0.025$) in tumor stroma were significantly associated with poor prognosis when compared to the others group.
Conclusion: Our results indicate that infiltrating CD4+, CD8+, and CD20+ lymphocytes in cancer stroma may cooperate to suppress cancer progression and their presence together appear to be prognostic factor in thymic carcinoma.

1. Introduction

Primary thymic carcinomas are rare [1], and already have invaded neighboring organs, pleural or pericardial dissemination, and lymph node metastasis or distant metastasis at diagnosis. There are few studies to determine prognostic factors for patients with thymic carcinoma due to the rarity of this tumor. Some studies suggest that tumor completeness of resection, tumor stage, tumor histology grade, great vessel invasions, or postoperative radiotherapy have an impact on prognosis of thymic carcinoma [1,2].

Although the specific functions of tumor infiltrating lymphocytes are poorly understood, tumor infiltrating lymphocytes are found in a variety of solid cancer tissues and have been considered important in anticancer immunomechanisms [3]. Activation of the adaptive immune system may suppress malignant cells, whereas activation of various types of innate immune cells may promote tumor growth [4]. The adaptive immunity is orchestrated by antigen-specific T and B lymphocytes and inhibits tumor growth through direct killing by cytotoxic T lymphocytes as well as a combination of cytokine- and antibody-mediated tumor cell lysis [4]. Most CD8+ T lymphocytes are cytotoxic T lymphocytes that recognize particular tumor-associated antigens presented on MHC (major histocompatibility complex) class I molecules at the cancer cell surface and possess the ability to destroy cancer cells directly. CD4+ T lymphocytes play a central role in orchestrating the immune response to cancer, the main role of CD4+ T lymphocytes in the immune response to cancer is to prime CD8+ lymphocytes and maintain their proliferation [5]. B lymphocytes (CD20+) are precursors of antibody-mediated immunity, which may contribute in tumor eradication [6]. The presence of these tumor infiltrating lymphocytes has been suggested to be associated with prognosis in a variety of cancers, including colorectal cancer [17], esophageal cancer [8], pancreatic cancer [9] and non small lung cancer [10]. There has been no report regarding the prognosis with lymphocyte infiltration in thymic malignancy. To elucidate the prognostic significance of lymphocyte infiltrate in thymic cancer, we analyzed the degree of infiltration of CD4+, CD8+, and CD20+ lymphocytes in thymic cancer in both tumor nest and stromal compartments and studied their relationship to prognosis.

2. Methods

2.1. Patients and tissue specimens

Tissue specimens from 32 patients who underwent surgical resection for thymic carcinoma between 1994 and 2008 were used in this retrospective study. Cases with WHO classification type B3 thymoma (well-differentiated thymic carcinoma) and thymic

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Table 1
Clinicopathologic variables.

Variables	No. of patients (%)
Age (median, range)	57.0(33–77)
Sex	
Male	23(71.9)
Female	9(28.1)
Histology subtype	
Squamous cell carcinoma	24(75.0)
Basaloid carcinoma	1(3.1)
Mucoepidermoid carcinoma	1(3.1)
Lymphoepidermoid-like carcinoma	2(6.3)
Adenocarcinoma	1(3.1)
Undifferentiated carcinoma	3(9.4)
Masaoka stage	
I	2(6.3)
II	7(21.9)
III	10(31.3)
IVa	7(21.9)
IVb	6(18.8)

neuroendocrine tumors were excluded in this study because they showed a different prognosis and different lymphocyte infiltration compared to thymic carcinoma.

The tumors were staged according to Masaoka's staging system. The patients included 23 men and 9 women with a median age of 57 years (range, 33–77). The median follow-up was 38 (range, 8–187) months. Demographic, clinical, and histopathologic variables are summarized in Table 1. The Institutional Review Board of Yonsei University College of Medicine approved this retrospective study. The need for individual consent of patients whose records were evaluated was waived because individuals were not identified within the study.

2.2. Immunohistochemistry

Formalin fixed, paraffin-embedded samples of tumor were selected for immunohistochemical staining. In brief, 3 μm-thick sections were cut, deparaffinized, rehydrated, and treated with 3% hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was performed by immersing slides in citrate buffer at pH 6.0 and microwaving at a high power for 25 min. The sections were incubated with primary monoclonal antibodies to CD4 (Dako, Clone 4B12, 1:80 dilution), CD8 (Dako, Clone C8/144B, 1:100 dilution), and CD20 (Dako, Clone L26, 1:200 dilution). The antibodies visualized using the Dako EnVision™ kit and diaminobenzidine as a chromogen. The sections were counterstained with Mayer hematoxylin.

2.3. Evaluation of immunohistochemistry

The numbers of lymphocytes were counted with a magnification of 200× (Olympus Optical Co., Ltd, Tokyo, Japan). Scoring was assessed according to a previous report [10]. By light microscopy, the tissue sections were scored for the degree of infiltration of CD4+, CD8+, and CD20+ lymphocytes. The percentages of lymphocytes compared with the total amount of nucleated cells in the nest and stromal compartments were assessed. For CD4+ lymphocytes, the nest infiltrate was scored as a low ≤5% or a high >5% in the whole surface area of the tumor and was scored as a low ≤25% or a high >25% of the total nucleated cells in the whole surface area of the stroma. CD8+ lymphocytes were scored as high if >5% or >50% of the total nucleated cells in the nest and stromal areas, respectively. CD20+ lymphocytes were scored as low if they represent <1% of the nucleated cells or if absent, or high otherwise, in both nest and stromal compartments. All samples were scored blind to the clinical data and outcomes. Examples of a high and a low lymphocyte infiltration in stroma are shown in Fig. 1.

2.4. Statistical analysis

The Chi-square test or Fisher's exact test were used to examine the association between the density of the lymphocyte infiltrates and various clinicopathologic variables. Survival analyses were done by using the Kaplan–Meier method, and statistically significant differences between survival curves were assessed by the log-rank test. Overall survival was determined from the date of surgery to the time of death or date of the last follow-up. A p-value of less than 0.05 was considered significant. Data were analyzed using SPSS for Windows (Statistical Package for Social Science, SPSS Inc. Chicago, Illinois).

3. Results

3.1. Lymphocyte infiltration

Tumor-infiltrating lymphocytes were observed in both tumor and stromal compartments and were generally more abundant in the stroma. The mean number of infiltrating CD4+ lymphocytes in the cancer nest and stroma was 18.1 ± 33.0 and 85.9 ± 90.5 ($p < 0.001$). That of CD8+ in the cancer nest and stroma was 44.9 ± 47.1 and 90.6 ± 79.9 ($p = 0.007$). That of CD20+ in the cancer nest and stroma was 5.7 ± 7.6 and 64.5 ± 78.8 ($p < 0.001$).

3.2. Kaplan–Meier survival analysis with lymphocytes infiltration

The survival of patients according to the levels of lymphocyte infiltration in the tumor stroma and nest are summarized in Table 2. A low level of stromal CD4+ lymphocytes ($p = 0.037$) and stromal CD20+ lymphocytes ($p = 0.045$) were significantly associated with poor survival. The level of stromal CD8+ lymphocytes failed to show prognostic significance. Regarding the lymphocyte in the cancer nest, there was no survival difference according to the level of lymphocyte infiltration by CD4+, CD8+, and CD20+.

Concurrent infiltration of lymphocyte in the tumor stroma was analyzed in Table 3. Concurrently low levels of CD4+ and CD20+ ($p = 0.014$), CD8+ and CD20+ ($p = 0.025$), and, CD4+, CD8+, and

Table 2
Nest and stroma lymphocyte subsets and their prediction for survival in thymic carcinoma.

Marker expression	Patients, n (%)	5-y survival (%)	p value
CD4			
Tumor			
Low	26(18.8)	70.6	0.402
High	6(81.3)	83.3	
Stroma			
Low	17(53.1)	55.8	0.037
High	15(46.9)	93.3	
CD8			
Tumor			
Low	21(65.6)	58.7	0.186
High	11(34.4)	100	
Stroma			
Low	22(68.8)	66.7	0.909
High	10(31.3)	90.0	
CD20			
Tumor			
Low	27(84.4)	71.7	0.988
High	5(15.6)	80.0	
Stroma			
Low	19(59.4)	58.5	0.045
High	13(40.6)	92.3	