Clinica Chimica Acta 413 (2012) 1848–1854

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Distribution of Th17 cells and Foxp3-expressing T cells in tumor-infiltrating lymphocytes in patients with uterine cervical cancer

Fei Hou [a,c], Zhen Li [b], Daoxin Ma [c], Wenjing Zhang [a,c], Youzhong Zhang [a], Tingguo Zhang [d], Beihua Kong [a], Baoxia Cui [a,*]

[a] Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, China
[b] Oncology Centre, Qilu Hospital of Shandong University, Jinan, China
[c] Haematology Oncology Centre, Qilu Hospital of Shandong University, Jinan, China
[d] Department of Pathology, Qilu Hospital of Shandong University, Jinan, China

ARTICLE INFO

Article history:
Received 28 March 2012
Received in revised form 21 June 2012
Accepted 10 July 2012
Available online 20 July 2012

Keywords:
Uterine cervical cancer
Cervical intraepithelial neoplasia
Th17 cells
Foxp3-expressing T cells

ABSTRACT

Background: Recent studies suggest a potential impact of Th17 cells on tumor. In the present study, we investigated the distribution of Th17 cells in relation to Foxp3-expressing T cells in the tumor-infiltrating lymphocytes (TILs) from patients with uterine cervical cancer (UCC), cervical tissues from patients with cervical intraepithelial neoplasia (CIN) and healthy cervical tissues.
Methods: Th17 cells and Foxp3-expressing T cells were evaluated by immunohistochemical staining. IL-6, TGF-β, IL-17 and IL-10 were detected by enzyme-linked immunosorbent assay (ELISA). Immunohistochemical staining for microvessel density (MVD) was performed in order to assess the association of IL-17 expression with angiogenesis.
Results: Compared with controls, patients with UCC or CIN had a higher proportion of Th17 cells and Foxp3-expressing T cells, when the ratio of Th17/Foxp3-expressing T cells in TILs was decreased in individual cases, it was more markedly decreased in TILs than normal cervical tissues. Meanwhile, the cytokine(IL-6, TGF-β and IL-10) concentrations were significantly higher in UCC patients than those in healthy controls. Interestingly, the levels of intratumoral Th17 cells were positively correlated with MVD in tumors.
Conclusions: The imbalance of Th17/Foxp3-expressing T cells may play critical roles in the development and progression of UCC and Th17 cells may promote tumor progression by fostering angiogenesis.

1. Introduction

Uterine cervical cancer is the second most common cancer in women worldwide, and its incidence is due to the high-risk types of human papillomavirus(HPV), particularly types 16 and 18 [1]. Overall cure rates of early-staged tumors approach 85% [2]. However, a spectrum of relapse risk exists depending on several prognostic factors. The most powerful predictor of outcome in UCC has been reported to be the vascular nature of the tumors, which increases the propensity to spread and invade into neighboring or distant sites [3,4]. Thus far, the only immunologic determinant associated with a better prognosis was a pronounced infiltration of cervical carcinoma by lymphocytes [5,6]. Furthermore, cervical cancer was considered to be an important immunogenic tumor because HPV causes multistep carcinogenesis (cervical intraepithelial neoplasia–carcinoma in situ–invasive cancer–metastatic cancer) in normal cervical cells [7]. Thus, it is important to understand the immunoregulation in cervical cancer, which can help to develop novel treatment strategies or improve the efficacy of standard therapy. A large number of tumor infiltrating lymphocytes (TILs) have been found around the cervical cancer tumor tissues [8–10]. The majority of the cells in the tumor are reactive and predominantly consist of T cells with variable numbers of other inflammatory cells [11]. The presence of TILs correlates with a better prognosis in patients with several types of cancer, and each T lymphocyte subset has a unique role in the antitumor response [12–15].

It has been reported that CD4 + T helper (Th) cells play a central role in orchestrating host immune responses through their capacity to help other cells of the immune systems [4]. Although the balance among the four types of helper T cell (Th1, Th2, Th17 and Treg) was important for the regulation of immune responses in several mouse models, information in humans is still limited and controversial. Th17 cells were characterized as interleukin (IL)-17-producing CD4(+) T cells which also produce IL-21, IL-22, and IL-26 [16]. Moreover, an increase in IL-17-producing cells was also found in both peripheral blood and tumor tissues from patients with advanced stages cancers [16]. Although these data suggests a potential impact of Th17 cells on tumor, the nature and role of Th17 cells in the progression of cervical cancer

* Corresponding author at: Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, #107 Wenhua Xi Road, Jinan 250012, China. Tel.: +86 531 82166671; fax: +86 531 82169201.
E-mail address: cuibaoxia@sdu.edu.cn (B. Cui).

0009-8981/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2012.07.012

remain unknown. Studies have suggested both beneficial and harmful implications of Th17 cells in tumor development [17,18]. IL-17 has been shown to promote tumor growth by increases in angiogenesis and intratumoral infiltration of phagocytes [19].

Foxp3-expressing T cells play a critical role in the maintenance of peripheral self tolerance. Naturally occurring $CD4^{+}CD25^{high}$ Tregs are produced in the thymus and express FoxP3, and CD4 + CD25 + Tregs have been shown to be increased in the peripheral blood and in tumor-draining lymph nodes of cancer patients [20]. These cells can express the transcriptional factor Foxp3 and IL-2 receptor α subunit (CD25),which have the ability to produce IL-2 [21,22]. Foxp3-expressing T cells can suppress CD4 + T cells through competing for IL-2 and transforming growth factor (TGF)-β, meanwhile, these cells can suppress inflammation through secreting IL-10 [23]. It has been reported that Foxp3-expressing T cells expression in the invasive front of cancer might be a very attractive mechanism of tolerance to cervical cancer and the recruitment of Foxp3-expressing T cells may be associated with the invasion and metastasis of cervical cancer [7]. Although the role of Foxp3-expressing T cells in blunting the body's immune response to cancerous cells and preventing autoimmune has been studied in mouse models and human disease, there are few reports on the balance between Foxp3-expressing T cells and Th17 cells in patients with cervical cancer [11].

2. Materials and methods

2.1. Patients and controls

Forty-six consecutive patients with cervical cancer (age range 22–79 years median 45 years) and 28 CINIII patients (age range 25–61 y, median 39 y) were enrolled in the present study. Individuals with concurrence of autoimmune disease, HIV and syphilis were excluded and none of the patients received radiotherapy, chemotherapy, or other medical interventions before the study. The characteristics of the UCC patients are summarized in Table 1. The clinical stage of UCC patients was based on the FIGO (International Federation of Gynecology and Obstetrics) 2000. Histological specimens of normal cervices from women (n = 18; age range 31–60 years, median 46 years) who underwent hysterectomies for benign uterine diseases with no cervical abnormalities served as controls. These specimens were obtained as Paraffin-embedded blocks from the Department of Pathology, Qilu Hospital of Shandong University. All samples were taken in a blinded manner for clinical information. Individuals gave written, informed consent. Ethical approval for the study was obtained from the Medical Ethical Committee of Qilu Hospital of Shandong University.

2.2. Immunohistochemistry

2.2.1. Immunohistochemistry detection of Th17 cells

For immunohistochemistry double staining of Th17 cells, Paraffin-embedded slides were deparaffinized and rehydrated, followed by microwave antigen retrieval in EDTA (PH 9.1). The sections were dealt with Doublestain system (kit-9999). The following 2 antibodies were used: rabbit anti-human IL-17antibody (1:700 v/v; Abcam, Cambridge, MA) and mouse anti-human CD4 antibody (1:30 v/v; Abcam).

Table 1
Clinical characteristics of UCC patients.

Characteristic	Category	N = 46 (%)
FIGO stage	I	38(82.61)
	II	8(17.39)
Tumor differentiation	Well- moderate	24(52.17)
	Poor	22(47.83)
Lymph node metastasis	Positive	8(17.39)
	Negative	38(82.61)
Tumor size (cm)	<4	27(58.70)
	≥4	19(41.30)
Vasoinvasion	Yes	6(13.04)
	No	40(86.96)

Abbreviation: FIGO, International Federation of Gynecologists and Obstericians.

2.2.2. Immunohistochemistry detection of Foxp3-expressing T cells and microvessel density (MVD)

Paraffin-embedded slides were deparaffinized and rehydrated, followed by microwave antigen retrieval in EDTA (PH 9.1). The sections were blocked with blocking solution (serum albumin) diluted in phosphate buffer saline (PBS) for 5 min and 3 times, and then incubated with each primary antibody in a moist chamber at 4 °C overnight. The following antibodies were used in this study: CD34 and FoxP3 (neat and 1:700 (v/v), respectively; Abcam). After washing three times with PBS for 5 min each, horseradish peroxidase (HRP) polymer-linked secondary antibody was added and incubated for 15 min at 37 °C. The sections were then visualized with diaminobenzidine (DAB) and counterstained with hematoxylin.

Negative control staining was performed with isotype control, PBS instead of the primary antibody.

2.3. Enzyme linked immunosorbent assay (ELISA)

The serum concentrations of IL-6, TGF-β, IL-17 and IL-10 were measured by ELISA (Multisciences, Biotech Co., Ltd, Norman, OK), following the manufacturer's instructions. All samples were measured in duplicate.

2.4. Statistical analysis

MVD was quantified by counting the vessels per 10 randomly selected high-power fields (HPFs). The number of Foxp3 + cells and IL-17 + CD4 + cells were counted in 10 high-power fields and the average number of cells in each HPF was calculated. All data were summarized as median, minimum–maximum, and were analyzed with SPSS software (version 13.0). Differences between groups were examined by Student's *t*-test. Correlations between variables were determined using linear regression analysis. Results were considered significant when $P<0.05$.

3. Results

3.1. Th17 cells and Foxp3-expressing T cells significantly increased in UCC and CIN

To analyse the distribution of Th17 cells on the local scale, TILs of tumor tissues in 46 UCC patients and cervical tissues in 28 CIN patients were characterized by immunohistochemical double staining. We detected a substantial amount of Th17 cells in both tumor tissues and CIN tissues, and a remarkable proportion of IL-17 + cells were CD4 negative cells (Fig. 1). As shown in Fig. 2A, the level of Th17 cells increased significantly in tumor tissues compared with that in normal cervical tissues. (5.59 ± 2.78 cells/HPF vs. 1.43 ± 0.37 cells/HPF, $P<0.0001$), particularly during lymph node metastases (6.38 ± 1.56 cells/HPF). Moreover, the level of Th17 cells in cervical tissues from CIN patients was also detected significantly higher than in normal cervical tissues (3.38 ± 0.98 cells/HPF, $P<0.0001$). The proportion of Th17 cells and CD4 + cells was shown in Fig. 2C. (UCC: 30.51 ± 8.38%; CIN: 23.43 ± 3.31%; Control: 15.11 ± 1.48%).

The expression of Foxp3-expressing T cells was shown in Fig. 3. Immunoassaying revealed that Foxp3-expressing T cells increased synchronically following the disease development. (UCC: 256.02 ± 68.46 cells/HPF; CIN: 139.81 ± 27.65 cells/HPF; Control: 42.3 ± 17.72 cells/HPF, $P_{UCC}<0.0001$, $P_{CIN}<0.0001$) (Fig. 2B).