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Association between RCAS1 expression and microenvironmental immune cell death in uterine cervical cancer

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Abstract

Objective. The presence of regional lymph node metastasis is one of the prognostic factors for uterine cervical cancer. The development of metastasis requires that cancer cells avoid lymphocyte attack. Impaired lymphocyte function is mediated by apoptotic factors including receptor-binding cancer antigen expressed on SiSo cells (RCAS1), Fas ligand (FasL), and tumor necrosis factor-α (TNF-α). Our aim was to evaluate the association between expression of these factors and microenvironmental lymphocyte apoptosis in this disease.

Methods. Immunohistochemical methods were used to determine the relationship between the expression of RCAS1, FasL, and TNF-α, and the number of apoptotic lymphocytes in primary lesions and metastatic lymph nodes in patients with cervical cancer.

Results. Expression of these apoptosis-inducing molecules was quite different in primary tumors and metastatic lymph nodes: RCAS1 expression in lymph nodes was significantly higher than that in primary lesions ($P < 0.0001$), whereas FasL and TNF-α expressions at these two locations were not significantly different. The number of cells with positive expression of RCAS1, but not of FasL or TNF-α, was significantly correlated with the number of apoptotic lymphocytes in uterine cervix and metastatic lymph nodes ($P < 0.0001$ for both).

Conclusion. RCAS1 expression may be related to tumor cell evasion of immune surveillance via induction of lymphocyte apoptosis in primary lesions and metastatic lymph nodes in uterine cervical cancer.

Keywords: RCAS1; Uterine cervical cancer; Lymphocyte apoptosis

Introduction

Invasive cancer of the uterine cervix is the major cause of death resulting from gynecologic cancer worldwide, with reported incidence rates in developing countries much higher than those in developed countries [1]. Clinical studies have demonstrated that regional lymph nodes play a pivotal role in diagnosis, staging, and management of many human cancers [2]. Tumor extension occurs via lymph vessels after stromal invasion [3]. The presence of metastatic tumor in regional lymph nodes is the most important prognostic factor for patients with malignant tumors of epithelial origin [4,5], and lymph node metastasis has been reported to be one of the prognostic factors specifically for uterine cervical cancer [6–9]. Lymph node metastasis develops as the result of lymphangiogenesis and tumor cell migration as cells avoid immune surveillance [10]. T lymphocytes are an essential feature of the host defense system against the neoplastic process [11], and several apoptosis-inducing molecules, including FasL, TNF-α, and RCAS1, have been implicated in suppression of T lymphocyte function [12–14]. It is plausible that these apoptosis-inducing molecules are involved in tumor development by means of suppression of immune surveillance in the microenvironment of human cancers.

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RCAS1, which is a type II membrane protein that forms oligomers via a C-terminal coiled-coil structure [14], is secreted and acts as a ligand for a putative receptor in human cell lines and on normal peripheral lymphocytes. RCAS1 acts to inhibit the in vitro growth of these receptor-expressing cells and induce apoptotic cell death. RCAS1 expression was associated with clinical outcome in several human cancers and was significantly related to overall survival of patients with uterine cervical adenocarcinoma and those with lung, gallbladder, bile duct, pancreas, or esophageal carcinoma [15–22]. RCAS1 may play a key role in the aggressive behavior of cervical cancer: it was strongly expressed in uterine cervical cancer [23,24], with its expression correlated with the invasive tendency of this cancer [25]. Because RCAS1 can induce apoptosis of T lymphocytes, RCAS1-induced death of immune cells surrounding a tumor may contribute to development of uterine cervical cancer.

To investigate this immune suppression mediated by apoptotic factors in the tumor microenvironment, we examined, by immunohistochemical means, the association between expression of three apoptosis-inducing molecules–RCAS1, FasL, and TNF-α–and the number of apoptotic lymphocytes in primary lesions and metastatic lymph nodes from patients with uterine cervical cancer.

Materials and methods

Patients and surgical specimens

All patients had had surgery between March 1976 and July 2003 at the Department of Obstetrics and Gynecology, Kyushu University Hospital. The patients' mean age was 49.7 years (range, 25–75 years; Table 1). The mean duration of follow-up was 77.9 months, with a range of 3–316 months. Clinical staging of the 120 invasive cancers (histologic subtypes: squamous cell carcinoma and adenocarcinoma) revealed that the greatest number of patients (63) had stage Ib cancer (Table 1). Specimens were graded according to the 1994 International Federation of Gynecology and Obstetrics criteria. Fifty-five patients had lymph node metastases.

Table 1
Characteristics of patients with uterine cervical cancer

Variable	Total number (%)	Number (%) of patients with squamous cell carcinoma	Number (%) of patients with adenocarcinoma
Number of patients	120	66	54
Age (years)			
Mean ± SD	49.7 ± 11.7	52.2 ± 12.3	46.9 ± 10.4
Range	25–75	25–75	25–72
Cancer stage			
Ia	7 (6%)	3 (5%)	4 (8%)
Ib	63 (53%)	32 (49%)	31 (57%)
IIa	19 (16%)	13 (21%)	6 (11%)
IIb	29 (25%)	16 (25%)	13 (24%)
IIIb	2 (2%)	2 (3%)	0 (0%)
Lymph node metastases			
Negative	65 (54%)	34 (52%)	31 (58%)
Positive	55 (46%)	32 (48%)	23 (42%)

Treatment of 48 of the 113 patients with stage Ib or advanced disease consisted of radical hysterectomy alone. Sixty-three of the 113 patients received postoperative radiation therapy, platinum-based chemotherapy, or combination therapy because of lymph node metastases or the presence of tumor cells in all tissue layers of the uterine cervix. Two patients received only radiation therapy.

Both biopsy and hysterectomy specimens were evaluated. All specimens were fixed, embedded in paraffin, and stained with hematoxylin and eosin. We used histologic methods to determine histologic subtype of the cancer and the presence or absence of metastases in lymph nodes. Tissue specimens of lymph nodes without metastases (controls) were also obtained from 18 cervical cancer patients at surgery.

Specimens of normal uterine cervical tissue were also removed from 30 other Japanese patients (15 premenopausal and 15 postmenopausal) during a surgical procedure.

Informed consent was obtained from all patients. The study protocol was approved by the Ethical Committee of Kyushu University.

Immunohistochemistry

We used the immunohistochemical streptavidin-biotin method [26] to analyze formalin-fixed, paraffin-embedded specimens (4-μm-thick sections cut from specimen blocks); duplicates of one or two representative slides for each case were evaluated. After endogenous peroxidase was blocked for 5 min with 3% hydrogen peroxide, slides were washed twice in Tris-buffered saline (TBS). Slides were then incubated for 30 min with normal goat serum diluted in a cell-staining buffer (TBS containing 0.1% bovine serum albumin and 0.01% sodium azide). Mouse antihuman RCAS1 monoclonal antibody (MBL, Nagoya, Japan) was applied, after which slides were incubated for 30 min in a moist chamber. Slides were washed two more times in the cell-staining buffer and were incubated for 30 min with biotinylated second antibody (goat anti-mouse immunoglobulins; DAKO, Glostrup, Denmark). Slides were then washed three times in cell-staining buffer and were incubated with avidin-biotinylated peroxidase complex (Strept ABC complex/horseradish peroxidase; DAKO) for 30 min. A 3,3′-diaminobenzidine tetrahydrochloride (DAB) working solution was applied after three more washes in the cell staining buffer. Slides were then counterstained with hematoxylin and mounted in Permount. The entire procedure was performed at room temperature.