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SCIENCE DIRECT

Gynecologic Oncology 99 (2005) 189 – 198

Gynecologic Oncology

www.elsevier.com/locate/ygyno

Invasive potency related to RCAS1 expression in uterine cervical cancer

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Received 20 October 2004
Available online 19 August 2005

Abstract

Objectives. RCAS1 expression is significantly associated with clinical prognosis in various human cancers, which suggests that RCAS1 may be involved in acquisition of malignant phenotypes. To investigate the relationship between RCAS1 and one such characteristic, tumor invasiveness, we examined RCAS1 expression in cervical neoplasms ranging from the precancerous state to invasive cancer.

Methods. RCAS1 expression was studied retrospectively via immunohistochemical methods. Samples consisted of biopsy tissue from 90 patients with intraepithelial neoplasia and resected tumor tissue from 154 patients with invasive cancer. Statistical analysis was done to correlate RCAS1 expression and clinicopathologic variables in patients with a depth of cancer cell invasion into stromal tissue of >5 mm.

Results. RCAS1 expression was detected in patients with intraepithelial cancer and invasive cancer but not in patients with dysplasia. The occurrence and degree of RCAS1 expression increased with the depth of invasion. In patients with invasive cancer, RCAS1 overexpression was significantly correlated with invasion of the lymph-vascular space, lymph node metastasis in two or more sites, and tumor volume; RCAS1 expression was not associated with histologic subtype. Overall survival rates for patients with RCAS1 overexpression were significantly shorter than those for patients without RCAS1 overexpression. In connective tissue surrounding tumor cells, the number of cells expressing vimentin significantly decreased in relation to RCAS1 expression level. Moreover, significant associations between expression levels of RCAS1 and those of MMP-1 and laminin-5 were found.

Conclusion. RCAS1 may contribute to acquisition of malignant uterine cervical phenotypic characteristics including invasion, metastasis, and tumor growth via connective tissue remodeling.

Keywords: RCAS1; Uterine cervical cancer; Immunohistochemistry; Prognostic factors; Connective tissue remodeling

Introduction

Uterine cervical cancer is one of the most common malignancies affecting women worldwide, predominantly in developing countries, with approximately 500,000 new cases being diagnosed annually [1]. The best prophylaxis for uterine cervical cancer and its precursors is thought to be early detection. Until the early 1970s, approximately 75% to 80% of cervical cancer cases in the United States were at the invasive stage at the time of diagnosis [2]. Tumor stage, lymph-vascular space invasion, lymph node metastasis, histologic type, and tumor volume have been reported as prognostic factors in surgically treated cervical cancer [3–6]. In uterine cervical cancer, tumor extension is mediated through lymph vessels after stromal invasion [7]. Therefore, invasion of stromal tissue is a crucial step during carcinogenesis in this disease. Several molecules, including matrix metalloproteinase-1 (MMP-1), laminin-5, thrombospondin, and tetranectin, have been reported as involved in tumor invasion [8–12]. However, the properties of invasion in cervical cancer have not yet been clarified.

RCAS1 (receptor-binding cancer antigen expressed on SiSo cells) is a membrane protein that can form oligomers through a coiled-coil structure in its C-terminal portion [13].

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0090-8258/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2005.06.061

RCAS1 acts as a ligand for a putative receptor present in various human cell lines and on normal peripheral lymphocytes. It inhibits in vitro growth of receptor-expressing cells and induces apoptotic cell death. In different human cancers, RCAS1 expression has been associated with clinical outcome. RCAS1 was strongly expressed in uterine and ovarian malignancies [14–16], and RCAS1 expression was significantly related to overall survival of patients with uterine cervical adenocarcinoma and patients with lung, gallbladder, bile duct, pancreas, or esophageal carcinoma [17–24]. RCAS1 expression has also been reported to correlate with tumor progression or the invasive tendency of uterine endometrial, gastric, skin, hepatocellular, breast, and thyroid carcinomas [25–32]. Thus, RCAS1 may play a pivotal role in aggressive behavior of several tumors.

To clarify whether RCAS1 is involved in invasion by cervical cancer, including squamous cell carcinoma and adenocarcinoma, we examined the following: (1) the presence of RCAS1 expression in lesions from a precancerous state to invasive cancer, (2) the relationship between the degree of RCAS1 expression and the depth of tumor invasion, (3) the relationship between RCAS1 expression and clinicopathologic variables in particular cancerous states, and (4) the contribution of RCAS1 expression to remodeling of stromal tissue in tumors.

Materials and methods

Patients and surgical specimens

All the patients with uterine cervical cancer in this study had undergone surgery between April 1977 and August 2003 at the Department of Obstetrics and Gynecology, Kyushu University Hospital. The mean age of the patients was 49.1 years, with a range of 25–75 years. The mean duration of follow-up for all patients was 70.3 months, with a range of 3–316 months. The histologic subtypes were 65 cases of dysplasia (20 mild, 20 moderate, 25 severe), 12 cases of squamous cell carcinoma in situ (CIS), 81 cases of invasive squamous cell carcinoma, 13 cases of adenocarcinoma in situ (AIS), and 73 cases of invasive adenocarcinoma. The 154 invasive cancers were classified as follows: 57 cases: stage Ia; 58 cases: stage Ib; 16 cases: stage IIa; and 23 cases: stage IIb. The specimens in this study were graded according to the 1994 International Federation of Gynecology and Obstetrics criteria. Slides of both biopsy and hysterectomy specimens were available for this study. All specimens were fixed, embedded in paraffin, and stained with hematoxylin and eosin (HE). Histologic methods were used to determine histologic subtype, invasion of lymphvascular space, and lymph node metastases. Forty-six patients had lymph node metastases; 22 of these had metastases at two or more sites.

For the 97 patients with stage Ib or advanced disease, 49 were treated by radical hysterectomy alone, and 48 received radiation therapy after surgical treatment because of lymph node metastases or involvement of all layers of the uterine cervix. Overall survival time was defined as the time from the surgery date to the final date of observation in this study (August 31, 2004) or the time from the surgery date to the date at death caused by the cancer.

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

Immunohistochemistry

For immunohistochemical analyses, one or two representative samples selected for each case were fixed with formalin, embedded in paraffin, and analyzed by means of the streptavidin–biotin method [33]. Sections 4 μm thick were cut from the paraffin-embedded blocks. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 5 min. The slides were washed twice in Tris-buffered saline (TBS) and were 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 anti-human RCAS1 monoclonal antibody (MBL, Nagoya, Japan) was then applied, and slides were incubated in a moist chamber for 30 min. After two additional washes in the cell-staining buffer, the slides were incubated for 30 min with biotinylated second antibody (goat anti-mouse immunoglobulins; DAKO, Glostrup, Denmark). The slides were again washed three times in the cell-staining buffer and were incubated for 30 min with avidin-biotinylated peroxidase complex (Strept ABC complex/horseradish peroxidase; DAKO). After three additional washes in the cell-staining buffer, a 3,3′-diaminobenzidine tetrahydrochloride (DAB) working solution was applied. The slides were then counterstained and mounted in Permount.

In patients who had a depth of cancer cell invasion into stromal tissue of more than 5 mm, the expression of RCAS1 and the expression of molecules involved in tumor invasion were analyzed. Monoclonal antibodies for MMP-1 (Daiichi Fine Chemical, Toyama, Japan), laminin-5 (Chemicon, Temecula, CA), thrombospondin (Oncogene, Darmstadt, Germany), and tetranectin (Novocastra Laboratories, Newcastle upon Tyne, UK) were used in the same manner as mentioned above. Positive control samples used in this study were as follows: for RCAS1: uterine cervical adenocarcinoma, which was used for manufacture of anti-RCAS1 antibody; for laminin-5: skin; for MMP-1: colon cancer; and for thrombospondin and tetranectin: breast cancer. We also used normal ovary as a negative control tissue and mouse IgM as a negative control antibody.

Measurement of cells expressing vimentin in connective tissue

Paraffin-embedded uterine cervical cancer specimens were simultaneously stained with anti-RCAS1 and anti-