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Clinical significance of RCAS1 as a biomarker of uterine cancer

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Abstract

Objectives. Expression of RCAS1 (receptor-binding cancer antigen expressed on SiSo cells) is associated with prognosis of various malignancies including uterine cancer. Proteolytic cleavage of RCAS1 at extracellular domains (ectodomain shedding) yields soluble RCAS1. Although RCAS1 can induce apoptosis in normal peripheral lymphocytes, its biologic function in cancer patients is unclear. Here, we evaluated serum RCAS1 concentrations to clarify its biologic activity in uterine cancer.

Methods. Via ELISA, we measured serum RCAS1 concentrations in samples from 54 healthy blood donors and 113 patients—63 with cervical cancer and 50 with endometrial cancer. We also counted the peripheral lymphocyte number. We correlated via statistical means the RCAS1 values with patients' clinicopathologic variables. We assessed inhibition of growth of K562 cells, which express the putative RCAS1 receptor, via WST-1 assay of serum samples to clarify RCAS1's biologic activity.

Results. Uterine cancer patients had significantly higher serum RCAS1 concentrations than did healthy blood donors ($P<0.05$). Patients with adenocarcinoma had significantly higher RCAS1 concentrations than did those with squamous cell carcinoma ($P=0.0340$). RCAS1 values were also significantly associated with response to treatment ($P<0.001$). FasL and TNF-α serum concentrations were not significantly different for the different groups, however. The WST-1 assay showed that patients' serum induced K562 cell growth inhibition, but this effect partially recovered after immunodepletion of RCAS1. Peripheral lymphocyte number and serum RCAS1 concentration were inversely related ($P=0.0310$).

Conclusion. RCAS1 may be a biomarker of uterine cancer because of its potential to predict results of uterine cancer treatment and inhibit growth of immune cells.

Keywords: Uterine neoplasm; ELISA; Ectodomain shedding; Biologic marker; Apoptosis

Introduction

The discovery of novel biomarkers of cancer to allow early detection and monitoring of disease has been coupled with development of new therapeutic strategies [1,2]. Biomarkers – which are genetic, molecular, or cellular alterations induced by cancer – serve not only as indicators of disease but also as predictors of the effectiveness of medical treatment [3,4]. Several molecules have been assessed and identified as biomarkers of gynecologic malignancies. For example, plasminogen activator inhibitor-1, vascular endothelial growth factor C, and intercellular adhesion molecule-3 were reported to be biomarkers of uterine cervical cancer [5–7]. Urokinase plasminogen activator receptor was found to be a useful prognostic biomarker of endometrial cancer [8]. Transthyretin, hemoglobin, apolipoprotein A1, and transferrin allowed improved detection of early stage ovarian cancer [9]. However, accurate identification of new biomarkers is important for progress in early detection of cancer. In addition, research on such molecules may contribute to development of novel therapies against cancer by means of targeting specific biomarkers.

Cancer cells often produce and secrete such biomarkers. The extracellular domain of a number of membrane proteins can be

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Table 1
Clinicopathologic variables for patients with uterine cancer

Clinicopathologic variable	No. of patients
Cervical cancer	
Age (years; mean±SD)	51±16
Stage	
I	25
II	16
III	18
IV	4
Histologic subtype	
Squamous cell carcinoma	47
Adenocarcinoma	16
Lymph node metastasis	
Negative	52
Positive	11
Endometrial cancer	
Age (years; mean±SD)	56±11
Stage	
I	32
II	3
III	9
IV	6
Histologic subtype	
Endometrioid	41
Serous or clear cell	9
Grade	
1	31
2	11
3	8
Lymph node metastasis	
Negative	43
Positive	7

cleaved proteolytically, which causes release of the protein [10,11]. This proteolytic processing, which is also referred to as ectodomain shedding, is observed for growth factors, growth factor receptors, cell adhesion molecules, extracellular matrix proteins, and other membrane proteins such as RCAS1 (receptor-binding cancer antigen expressed on SiSo cells) [12]. Ectodomain shedding of membrane proteins affects the biologic activities of these proteins. Therefore, ectodomain shedding is an important regulatory step for the functioning of membrane proteins involved in cell–cell communication in development, cell differentiation, and tissue maintenance [13].

RCAS1 is a membrane protein that can be detected by 22-1-1 antibody [14]. RCAS1 is released by ectodomain shedding and acts as a ligand for a putative receptor in various human cell lines and on normal peripheral lymphocytes. This protein inhibits in vitro cell growth of receptor-expressing cells and induces apoptotic cell death of lymphocytes around cancer cells in uterine cervical cancer patients [15]. Uterine and ovarian malignancies have reportedly strongly expressed RCAS1 [16,17], with expression being significantly related to progression and invasion of uterine cervical and endometrial cancers [12,18,19]. RCAS1 expression has also been significantly associated with overall survival of patients with uterine cancer [12,20,21].

To clarify whether RCAS1 is indeed a biomarker of uterine cancer, we studied the following: (1) serum RCAS1 concentrations for cancer patients compared with those for healthy blood donors, (2) relationship between serum RCAS1 values and clinicopathologic variables, (3) association between serum RCAS1 values and effects of medical treatment, (4) correlation between serum RCAS1 values and number of peripheral lymphocytes, and (5) in vitro growth inhibition of RCAS1 receptor-expressing cells.

Materials and methods

Patients and serum samples

Serum samples from patients with uterine cervical cancer or endometrial cancer were collected at the Department of Obstetrics and Gynecology, Kyushu University Hospital, between April 2003 and August 2005. Table 1 provides clinicopathologic data for these patients. The staging system – 1994 and 1988 International Federation of Gynecology and Obstetrics criteria, respectively – was used to assign stages to the cervical and endometrial cancers. Slides of both biopsy and hysterectomy specimens were available for evaluation of histologic subtype, grade, and presence of lymph node metastases. Numbers of peripheral blood lymphocytes were also counted.

With regard to treatment, of the 63 patients with cervical cancer, 23 had radical hysterectomy alone; 11 received radiation therapy with or without platinum-based chemotherapy after surgical treatment because of lymph node metastases or involvement of all layers of the uterine cervix; 14 received radiation therapy alone; and 15 had chemoradiation. Of the 50 patients with endometrial cancer, 33 were treated by surgery alone (total abdominal hysterectomy, bilateral salpingo-oophorectomy, pelvic and paraaortic lymphadenectomy). Certain patients received additional treatment after an operation: 11 and 2 cases received platinum-based chemotherapy and radiation therapy, respectively. Two patients received only platinum-based chemotherapy, and two received only radiation therapy.

Serum samples from 54 healthy female blood donors served as controls. The mean age of these donors was 36 years, with a range of 21–69 years.

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

Enzyme-linked immunosorbent assay (ELISA)

We measured serum concentrations of RCAS1, Fas ligand (FasL), and tumor necrosis factor-α (TNF-α) because these molecules are reportedly secreted by

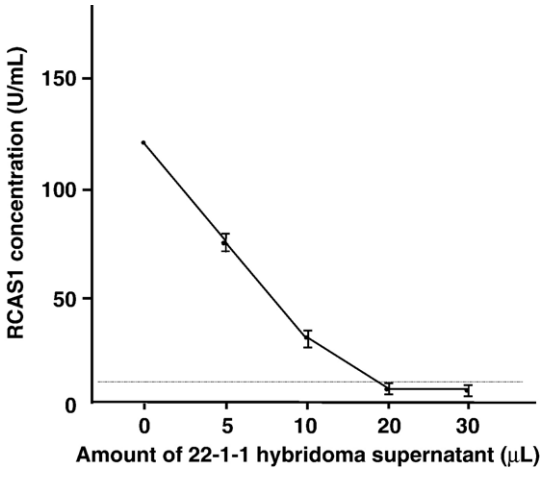

Fig. 1. Immunodepletion of RCAS1 in concentrated SiSo cell culture supernatant. 22-1-1 Supernatant (5–30 μl) was added to 200 μl of SiSo cell culture supernatant in which the RCAS1 concentration was 120 U/ml. Immunoprecipitation by adding 20 μl of anti-RCAS1 antibody efficiently removed RCAS1. The dotted line indicates the mean RCAS1 value in serum from the blood donors.