Tumor-associated Antigen 22-1-1 Expression in the Uterine Cervical Squamous Neoplasias[1]

Kenzo Sonoda,[2] Tsunehisa Kaku, Toshiharu Kamura, Manabu Nakashima, Takeshi Watanabe, and Hitoo Nakano

Department of Gynecology and Obstetrics, Faculty of Medicine [K. S., T. Kak., T. Kam., H. N.], and Department of Molecular Immunology, Medical Institute of Bioregulation [M. N., T. W.], Kyushu University, Fukuoka 812-82, Japan

ABSTRACT

We have reported that a novel tumor-associated antigen (Ag), 22-1-1, was expressed in cancer cells derived mainly from the uterus and ovary [K. Sonoda *et al.*, Cancer (Phila.), *77:* 1501–1509, 1996]. The 22-1-1 Ag existed not only in adenocarcinomas but also in squamous cell carcinomas in the uterine cervix. Here, a relationship between tumor progression and invasion and 22-1-1 Ag expression was investigated in squamous cell neoplasms of the uterine cervix using immunohistochemical staining. The 22-1-1 Ag was not detected in normal uterine cervix (0 of 10 total cases) and dysplasias (0 of 47 total cases). However, 20% of carcinoma *in situ* (4 of 20 total cases) and 16.7% of microinvasive carcinomas (2 of 12 total cases) stained positively for 22-1-1 Ag. Moreover, areas depicting microinvasion on histology in uterine cancers (stage Ia) were more strongly stained than carcinoma *in situ* lesions. 22-1-1 Ag expression was found to be more frequent in invasive squamous cell carcinomas (82.6%; 57 of 69 total cases). The 22-1-1 Ag existed both in the cytoplasm and on the membrane of cancer cells. These findings suggest that 22-1-1 Ag expression might be related to tumor cell progression and invasion in the uterine cervical squamous cell epithelium.

INTRODUCTION

The 22-1-1 MoAb[3] was generated by cell fusion between mouse myeloma cells and spleen cells derived from mice immunized with the human uterine cervical adenocarcinoma cell line, SiSo (1). The tissue distribution and biological properties of 22-1-1 Ag were reported (1, 2). The 22-1-1 Ag is a novel tumor-associated Ag; an immunohistochemical study revealed that 22-1-1 Ag was expressed in 87.5% of uterine cervical adenocarcinomas (56 of 64 total cases), 66% of uterine endometrial adenocarcinomas (68 of 103 total cases), and 58.8% of ovarian carcinomas (10 of 17 total cases). Moreover, 22-1-1 Ag was detected in 87.7% of uterine cervical squamous cell carcinomas (50 of 57 total cases). On the other hand, 22-1-1 Ag was not detected in normal uterine cervical or ovarian tissues, except in uterine endometrial glands, in which its expression was observed at low levels. The 22-1-1 Ag was shown to exist in a comparatively broader and stronger manner in uterine cancers than CA125, CA19-9, and CEA Ags.

In general, neoplasias arising in the uterine cervical squamous cell are thought to progress from dysplasia to invasive carcinoma. Therefore, to examine whether expression of this Ag has a possible relationship to tumor progression and invasion, we investigated the expression of 22-1-1 Ag in dysplasia, carcinoma *in situ*, and invasive carcinoma of the uterine cervix using immunohistochemical analysis.

MATERIALS AND METHODS

Tissue Sections. Tissue sections used in this study included 10 cases of normal uterine cervix, 10 cases of mild dysplasia, 16 cases of moderate dysplasia, 21 cases of severe dysplasia, 20 cases of squamous cell carcinoma *in situ*, 12 cases of microinvasive squamous cell carcinoma, and 69 cases of invasive squamous cell carcinoma (67 cases of large cell nonkeratinizing type and 2 cases of keratinizing type) from pathological specimens at the Kyushu University Hospital. With regard to the clinical staging, all 12 cases of microinvasive carcinoma were stage Ia. The 69 cases of invasive carcinoma included 39 cases of stage Ib, 14 cases of stage IIa, and 16 cases of stage IIb. These stagings were based upon Committee of the International Federation of Gynecology and Obstetrics 1994 classification. The slides of conization specimens of mild dysplasia to carcinoma *in situ* and the slides of hysterectomy specimens of microinvasive and invasive carcinoma were available for this study. All tissue sections were fixed, embedded in paraffin, and diagnosed with H&E staining.

Immunohistochemical Examination. For immunohistochemical analysis, one or two representative sections for each case were selected, and streptavidin-biotin methodology (Histofine SAB-PO kit; Nichirei, Tokyo, Japan) was used for formalin-fixed, paraffin-embedded specimens (3). Four-μm sections were cut from paraffin-embedded blocks. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol for 30 min. Slides were then washed twice in PBS and incubated with normal rabbit serum diluted in PBS for 15 min. The 22-1-1 hybridoma culture supernatant fluid diluted 1:20 in PBS was applied, and the slides were incubated in a humid chamber for

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[2] To whom requests for reprints should be addressed, at Department of Gynecology and Obstetrics, Faculty of Medicine, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-82, Japan. Phone: 81-92-642-5395; Fax: 81-92-642-5414.
[3] The abbreviations used are: MoAb, monoclonal antibody; Ag, antigen; CEA, carcinoembryonic antigen; CIN, cervical intraepithelial neoplasia; EGF, epidermal growth factor.

Table 1 22-1-1 monoclonal antibody reactivity against normal uterine cervix and squamous cell neoplasms

Pathological diagnosis	No. of cases	No. of positive cases[a]
Normal cervix	10	0 (0.0%)[b]
Mild dysplasia	10	0 (0.0%)
Moderate dysplasia	16	0 (0.0%)
Severe dysplasia	21	0 (0.0%)
Squamous cell carcinoma *in situ*	20	4 (20.0%)
Microinvasive squamous cell carcinoma	12	2 (16.7%)
Invasive squamous cell carcinoma	69	57 (82.6%)

[a] For immunohistochemical analysis, streptoavidin-biotin methodology was used for formalin-fixed, paraffin-embedded specimens. Tissue sections with >5% reactive cells were defined as positive.

[b] Numbers in parentheses are the percentages of positive cases.

30 min. After two additional washes, sections were incubated with biotinylated rabbit antimouse antibody for 30 min. Sections were washed three times in PBS and incubated with avidin-biotinylated peroxidase complex for 30 min. After three additional washes in PBS, 3,3′-diaminobenzine tetrahydrochloride working solution was applied. Sections were then counterstained in hematoxylin or methyl green and mounted in permount. The entire procedure was performed at room temperature. Negative controls were treated in the same way, but the 22-1-1 MoAb was replaced by mouse IgM. Tissue sections with <5% reactive tumor cells were considered negative, and those with >5% reactive cells were defined as positive.

RESULTS

The 22-1-1 Ag was not found in the uterine cervical normal tissues (0 of 10 total cases) and squamous dysplasias (0 of 47 total cases; Table 1 and Fig. 1). However, 22-1-1 Ag was detected in the squamous carcinomas: 20% of carcinoma *in situ* (4 of 20 total cases), 16.7% of microinvasive carcinomas (2 of 12 total cases), and 82.6% of invasive carcinomas (57 of 69 total cases). The 22-1-1 Ag was expressed diffusely both in the cytoplasm and on the membrane of squamous carcinoma cells. In carcinoma *in situ*, 22-1-1 Ag was stained weakly, whereas it was strongly stained in the invasive carcinomas. In microinvasive carcinomas, cancer cells were more strongly stained compared with adjacent carcinoma *in situ* lesions in areas depicting microinvasion (Fig. 2). Both cases of invasive keratinizing squamous cell carcinoma were also stained strongly. Concerning the clinical staging, 22-1-1 Ag was stained more strongly and frequently in cancer patients who were diagnosed as stage Ib or more (Table 2). However, any significant differences concerning 22-1-1 Ag expression were not detected among stage Ib–IIb patients.

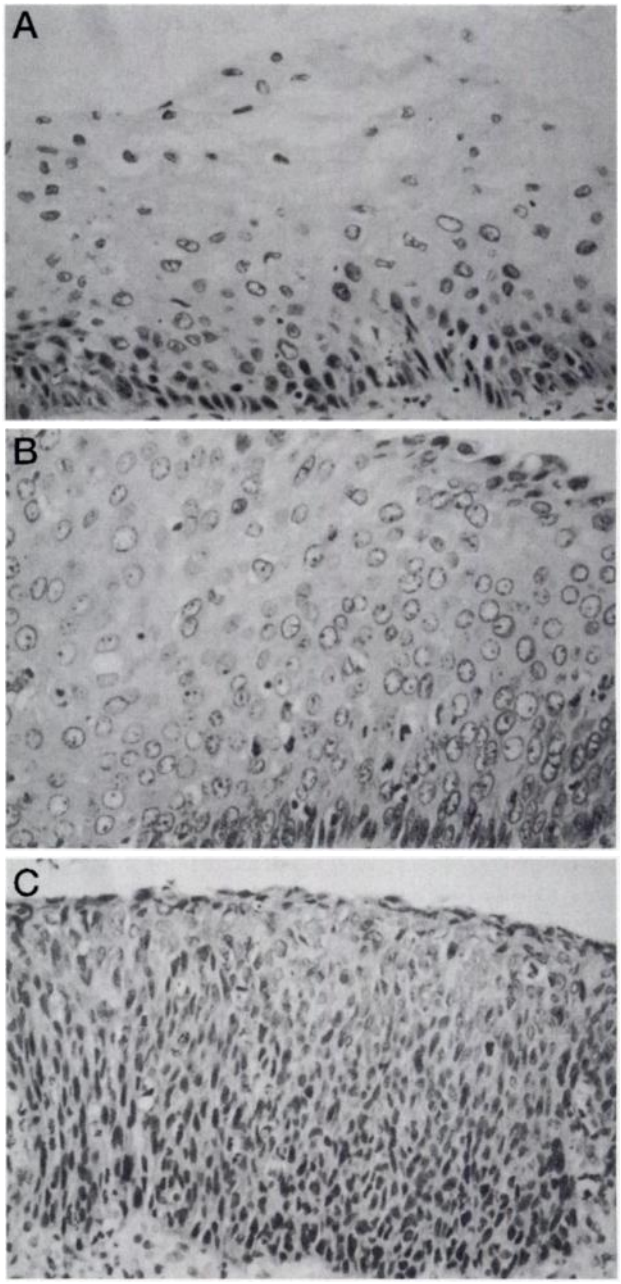

Fig. 1 Immunohistochemical analysis of the uterine cervical dysplasias by 22-1-1 MoAb. 22-1-1 Ag was negative in the cervical dysplasias. These sections were counterstained with hematoxylin. *A*, mild dysplasia; *B*, moderate dysplasia; *C*, severe dysplasia. Original magnification, ×200.

DISCUSSION

The 22-1-1 MoAb was produced from mice immunized with SiSo cells derived from uterine cervical adenocarcinoma. The 22-1-1 Ag was distinct from the known tumor-associated Ags that have been reported thus far (1, 2). An immunohistochemical study revealed that 22-1-1 Ag was expressed in uterine and ovarian carcinomas. In nongynecological cancers, 22-1-1 Ag was detected in esophageal squamous cell carcinomas (two of two total cases), gastric adenocarcinomas (three of three total cases), colon adenocarcinomas (three of three total cases), and pancreatic adenocarcinomas (two of two total cases). These findings indicated that 22-1-1 Ag exists widely in various cancer tissues. The 22-1-1 Ag was secreted into the vaginal discharges of uterine cervical carcinoma patients but not into those of normal healthy donors, indicating that 22-1-1 MoAb can be used in the diagnosis of uterine cancer with vaginal discharge. The

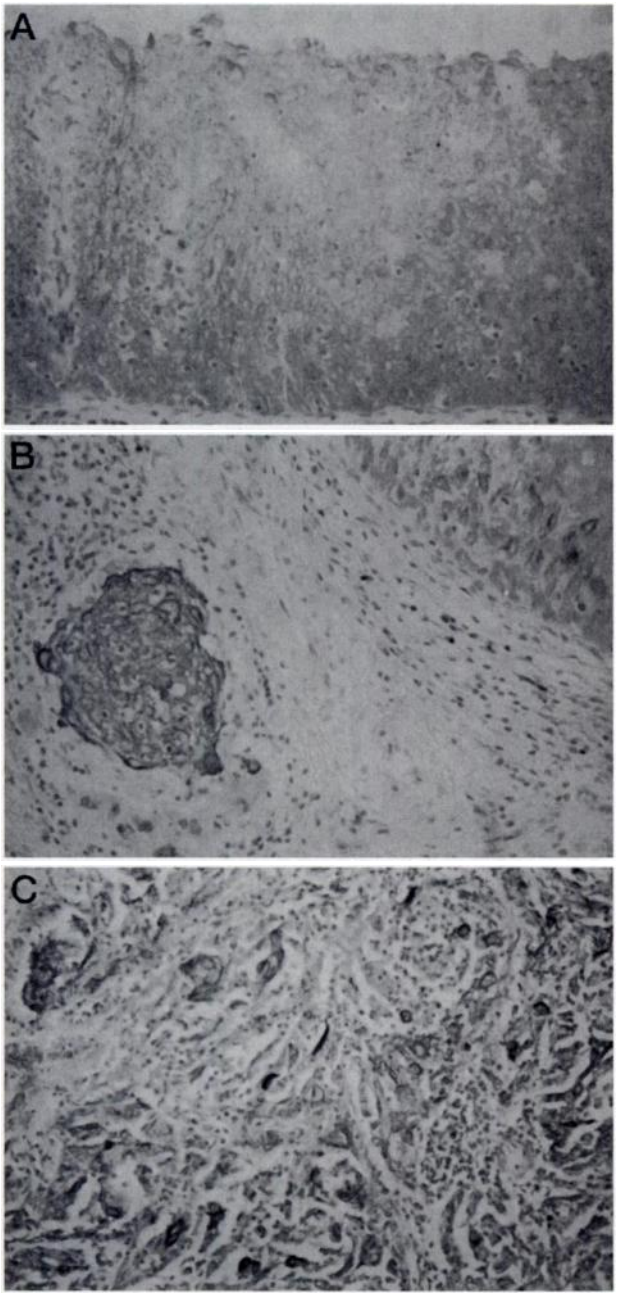

Fig. 2 22-1-1 Ag was shown to exist in the uterine cervical squamous carcinomas by immunohistochemical analysis. These sections were counterstained with methyl green. *A*, carcinoma *in situ* was stained weakly. *B*, in the microinvasive carcinoma, cancer cells in areas of invasion were more strongly stained than carcinoma *in situ* lesion. *C*, 22-1-1 Ag was expressed more intensely both in the cytoplasm and on the membrane of invasive squamous carcinoma cells. Original magnification, ×200.

clinical application of 22-1-1 MoAb as a tumor marker for cancer patients is now under investigation. The antigenic epitope of 22-1-1 Ag was shown to be a protein with M_r 78,000 using SDS-PAGE analysis (2).

Here, the relationship between tumor progression and invasion and 22-1-1 Ag expression was investigated with respect to uterine cervical squamous cell carcinomas. In uterine cervical squamous cell carcinomas, tumor progression had been clearly distinguished from dysplasia to invasive cancer. Human papilloma virus infection had been reported to promote this process (4–8). Immunohistochemical study showed that 22-1-1 Ag was not found in normal uterine cervical tissues and squamous dysplasias. However, 22-1-1 Ag was detected in squamous carcinomas. It was expressed in 20% of carcinoma *in situ* (4 of 20 total cases), 16.7% of microinvasive carcinomas (2 of 12 total cases), and 82.6% of invasive carcinomas (57 of 69 total cases). The 22-1-1 MoAb reacted weakly with carcinoma *in situ*, but it strongly stained areas where invasion had occurred.

Table 2 22-1-1 antigen expression in uterine cervical squamous cancer patients

Clinical stage	No. of cases	No. of positive cases
0	20	4 (20.0%)[a]
Ia	12	2 (16.7%)
Ib	39	34 (87.2%)
IIa	14	10 (71.4%)
IIb	16	13 (81.3%)

[a] Numbers in parentheses are the percentages of positive cases.

Some Ags have been reported to correlate with tumor progression. C-*erb* B-2 is among the >50 different proto-oncogenes that code for proteins that function as growth factors, growth factor receptors, and cytoplasmic second messengers. C-*erb* B-2 is believed to play an important role in carcinogenesis of the breast and ovary. This proto-oncogene is negative or only rarely positive in normal ovary. Strongly positive c-erb B-2 protein was reported in ovarian cancers (9). In cases of endometrial adenocarcinomas, high expression of c-erb B-2 was found in 27% of patients with metastatic disease compared with 4% of patients with disease confined to the uterus, suggesting that positive c-erb B-2 protein is associated with more aggressive biological behavior (10). CAM5.2 detects low molecular weight keratins (keratin 8, 18, and 19) and CAM5.2 is positive in invasive squamous cell carcinoma but rarely so in CIN 3 and not in CIN 1 and CIN 2 (11). CA125 is a tumor-associated glycoprotein that is frequently positive in normal secretory endometrium and also positive in atypical hyperplasia and endometrial carcinomas. High CA125 expression by endometrial adenocarcinomas is reportedly associated with increased metastatic potential (12). CEA is a high molecular weight glycoprotein that was found to be positive in many epithelial malignancies. It was reported that CEA was negative in normal uterine endometrium but positive in cystic hyperplasia (10.5%; 2 of 19 total cases), atypical hyperplasia (100%; 11 of 11 total cases), and endometrial carcinomas (62.5%; 10 of 16 total cases; Ref. 13). However, there was no correlation with the progression of CIN (14). MSN-1 antibody, raised against a human endometrial cancer cell line SNG-II, was reported to be useful in distinguishing between atypical endometrial hyperplasia and those without atypia. However, MSN-1 antibody could not be used to discriminate between atypical hyperplasia and carcinoma (15). The loss of blood group carbohydrate isoantigens A, B, and H was associated with a higher grade of CIN, suggesting that the loss

of isoantigen may indicate greater risk of disease progression (16). It was reported that, although normal cervical epithelium did not show appreciable staining for EGF receptor, predominant staining for the receptor was observed in most dysplastic epithelia and carcinomas. This suggests that the elevated expression of EGF receptor may be involved in the initial stage of carcinogenesis of cervical squamous epithelium (17).

The 22-1-1 Ag is a different molecule from the proto-oncogenes or tumor-associated Ags mentioned above. This study suggests that 22-1-1 Ag correlates with the progression and invasion in uterine cervical squamous neoplasms. The gene cloning of 22-1-1 Ag and further investigations concerning about the functions of 22-1-1 Ag are now in progress. Additionally, it is important to elucidate whether the expression of 22-1-1 Ag in cervical squamous neoplasia is associated with the amplification of other molecules, including c-erb B-2 and EGF receptor.

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