

A Preliminary Study of the Immunohistochemical Detection of a Novel Tumour Marker, 22-l-l Antigen, in Gynaecological Cancer Specimens

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Abstract

A novel tumour associated antigen, 22-l-l, has been recently described in association with a cervical adenocarcinoma cell line. The aims of this paper were to study the tissue distribution of this antigen in sections of gynaecological cancer specimens and to compare it with negative controls. Six cases of cervical cancers, 5 cases of endometrial cancers, 4 cases of ovarian cancers and 5 cases each of normal endometrium and cervix were studied. Immunohistochemical staining using streptavidin-biotin methodology was used for each tumour specimen. This revealed positive staining for the 22-l-l antigen in 5 out of 6 cases of cervical cancer, 3 out of 5 cases of endometrial cancers, and all 4 cases of ovarian mucinous cystadenocarcinomas. Importantly, the antigen was expressed in the cytoplasm, cell membrane and glandular lumen of adenocarcinoma cells. The 22-l-l antigen was not detected in normal uterine tissues except in uterine cervix, in which its expression was observed at low levels. This study shows that the 22-l-l antigen was expressed in cancer cells derived from the uterus, cervix and the ovary and may be a potential tumour marker in the management of gynaecological cancer patients.

Ann Acad Med Singapore 1999; 28:392-4

Key words: Adenocarcinoma, Immunohistochemistry, Monoclonal antibody, Squamous cell carcinoma

Introduction

Monoclonal antibodies expressed against specific tumour cells can play a useful role in the study and management of such cancers. Such antibodies have been useful in diagnosis and treatment of certain cancers (as tumour markers) and many such antibodies have now been described.^{1,2} Others have been useful in studying the pathophysiology of tumour cells.^{3,4} A novel human uterine cervical adenocarcinoma cell line, SiSo, has been established and characterised.⁵ Subsequently, the 22-l-l monoclonal antibody (MoAb) was established by cell fusion between mouse myeloma cells and spleen cells derived from mice immunized with the human uterine cervical adenocarcinoma cell line, SiSo.⁶ This was used to study the tissue distribution and biologic characteristics of the 22-l-l antigen (Ag). Initial data showed 22-l-l Ag expression in uterine cervical adenocarcinomas, endometrial adenocarcinomas and ovarian carcinomas. The 22-l-l Ag was secreted into cell culture supernatant fluids and was also detected in the vaginal discharges of uterine cervical carcinoma patients. The antigenic epitope

of this antigen is a protein with a molecular weight of 78 kilodaltons.⁶

The purpose of this paper was to validate the data from the initial study in Japan by examining paraffin-embedded specimens of gynaecological cancers for 22-l-l antigen, obtained from the Department of Pathology, National University Hospital, Singapore.

Materials and Methods

Paraffin sections of 16 gynaecological cancers were used for this study. Paraffin sections of 5 normal tissues each of the cervix and the endometrium were used as controls. For immunohistochemical analysis, streptavidin-biotin methodology was used for formalin-fixed and paraffin-embedded specimens.⁷ The 22-l-l MoAb was derived from culture supernatant fluid of growing hybridoma colonies that were produced after inoculation of nude mice with SiSo cells.⁶ An initial optimisation study was performed to determine the minimum concentration of antibody required for the immunohistochemical analysis. Antibody dilutions of

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x500, x1000, x2000 and x4000 were used and the dilution of 1 in 2000 was found to give optimal specific staining. Tissue sections with less than 5% reactive tumour cells were considered negative whilst those with more than 5% reactive cells were defined as positive. Sections with greater than 50% to tumour cells staining positively were considered to be strongly stained for the antigen. For each histological slide that was stained with the 22-l-l MoAb, another slide from the same specimen was also used as a negative control where the 22-l-l MoAb was replaced by mouse IgM. All tissue sections used in this study were diagnosed with haematoxylin and eosin staining.

Results

Three out of 5 cases of endometrial adenocarcinoma stained positively for 22-l-l Ag (Fig. 1a) while all 4 cases of ovarian mucinous cystadenocarcinoma stained strongly for this antigen (Fig. 1b). Of the 7 cases of cervical cancers studied, 5 stained positively for the 22-l-l Ag (Figs. 1c & 1d). Of these 7 cases, there were 3 cases each of adenocarcinoma (2 out of 3 stained positively) and squamous cell carcinoma (2 out of 3 stained posi-

tively) and one case of adenosquamous carcinoma (both the squamous and adenocarcinoma component stained positively). The results are summarised in Table I. The 22-l-l Ag was expressed in both the cytoplasm and on the membrane of the tumour cells. Moreover, it was also detected in the glandular lumen of some of the adenocarcinoma cells, indicating that the 22-l-l Ag was secreted. The 22-l-l Ag was not detected in the 5 normal uterine endometrial tissue slides but weak expression was noted in the 5 cases of normal uterine cervix. The results are summarised in Table I.

Discussion

The results show that a novel tumour associated antigen, the 22-l-l antigen, was expressed in cancer cells from the cervix, endometrium and ovary. It was not detected in significant amounts in the normal uterine cervix or endometrial tissues. These data validate the results of original work. It also shows that 22-l-l expression is almost the same despite racial differences between the population in this study and the original data from Japan. The fact that the 22-l-l MoAb shows a marked differential staining between normal cells and

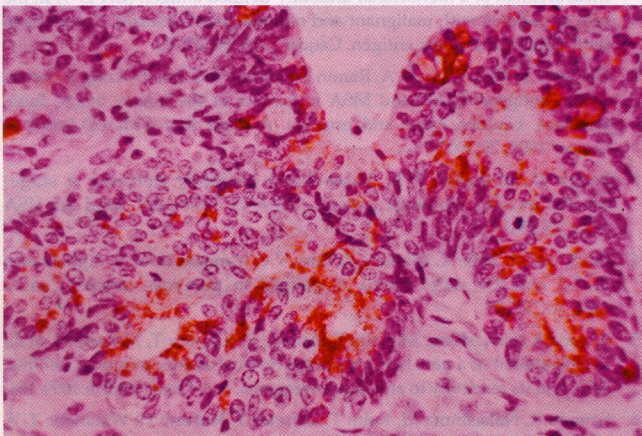


Fig 1a. Uterine endometrial adenocarcinoma stained for 22-l-l Ag (brown) (H&E x120)

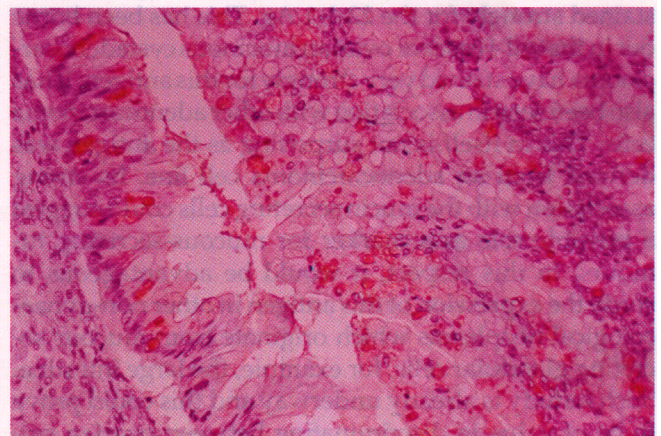


Fig. 1b. Ovarian mucinous cystadenocarcinoma. (H&E x60)

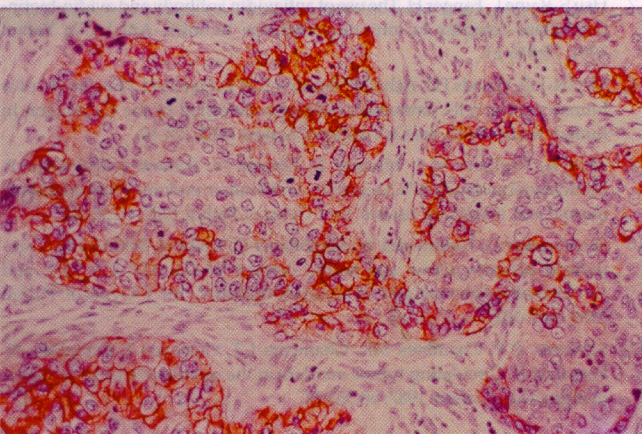


Fig. 1c. Squamous cell carcinoma of the uterine cervix. (H&E x60)

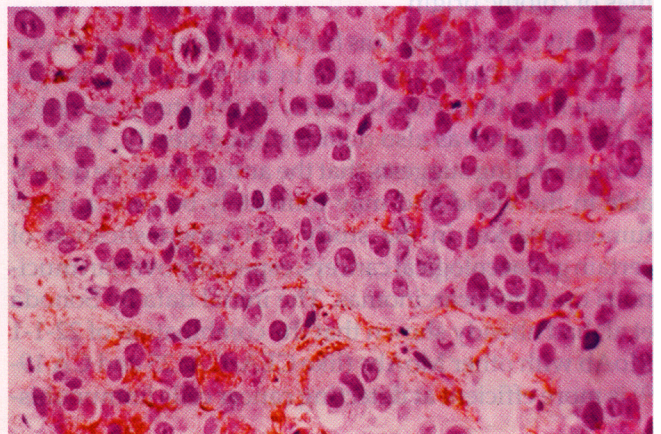


Fig 1d Adenocarcinoma of the uterine cervix (H&E x120)

Fig. 1. Immunohistochemical analysis of female genital tract neoplasm. The 22-l-l Ag existed in both the cytoplasm and membrane of cancer cells

TABLE 1: REACTIVITY OF 22- 1- 1 MONOCLONAL ANTIBODY AGAINST VARIOUS NEOPLASMS AND NORMAL TISSUES OF THE FEMALE GENITAL TRACT

Pathologic diagnosis	No. of cases	22-l-l Ag staining characteristics		
		Negative	Weak	Strong
Normal Tissues				
Uterine cervix	5	3	2	0
Uterine endometrium	5	5	0	0
Carcinoma Tissues				
Cervix squamous cell	3	1	1	1
Cervix adenocarcinoma	3	1	2	0
Cervix adenosquamous	1	0	1	1
Uterine adenocarcinoma	5	2	3	0
Ovarian mucinous cystadenocarcinoma	4	0	0	4

cancerous cells from the gynaecologic tract requires further study. If similar results are obtained when comparing benign and malignant gynaecologic neoplasms, then it could be another immunohistologic marker for discrimination of benign versus malignant cells in histologic preparations.^{8,9}

A novel finding is that mucous-producing cells have stained strongly for the 22-l-l Ag. This has been borne out in that all 4 cases of ovarian mucinous cystadenocarcinoma have stained very strongly for this antigen. It has also been seen in association with other adenocarcinoma cells. This in itself is not surprising as the antigen was derived from an adenocarcinoma cell line. Its strong association with the mucin secreting cells could suggest a possible role as a marker for mucous-secreting tumours. In this respect, it would be advantageous to study the expression of this antigen in other non-gynaecological neoplasms which originate from mucous secreting glandular cells, for example, the large intestine. Such a study is already underway and this could prove to be of value in discriminating between ovarian primary adenocarcinomas and metastatic adenocarcinomas of colonic origin.

Thirdly, this antigen has been previously been shown to be secreted and was found in the vaginal discharges of patients with cervical cancers.⁶ In this study, secretion of the antigen was also demonstrated clearly. This important finding suggests that the antigen may be detectable in the blood and may ultimately become a novel tumour marker in the study and prognostication of certain gynaecological cancers, especially ovarian mucinous cystadenocarcinomas. The initial data by Sonoda et al⁶ had already shown that the combination of 22-l-l MoAb with other MoAbs (like CEA, CA125 and CA 19-9) is more efficient at detecting cancer cells immunohis-

tologically than with any MoAb used alone. If 22-l-l Ag expression can be determined in human sera, then a comparison with other tumour markers^{2,10,11} would be needed to be done to establish its sensitivity and specificity when used alone or in combination for the management of gynaecological cancers. This would ultimately determine the role of this novel tumour associated antigen in the management of gynaecological neoplasms.

Acknowledgements

This study is funded by a grant from the Medical Faculty Research Committee, National University of Singapore and the Singapore Cancer Society.

The authors would like to thank Mr Ow Cheok Kee and staff from the Pathology laboratory for technical assistance rendered, and to Mr Tan Tee Chok and Mr Anthony Khoo for help rendered with photomicrography.

REFERENCES

- Gupta M K, Arciaga R, Bocci L, Tubbs R, Bukowski R, Deodhar S D. Measurement of a monoclonal-antibody-defined antigen (CA 19-9) in the sera of patients with malignant and nonmalignant diseases. Comparison with carcinoembryonic antigen. *Cancer* 1985; 56:277-83.
- Devine P L, McGuckin M A, Ramm L E, Ward B G, Pee D, Long S. Serum mucin antigens CASA and MSA in tumors of the breast, ovary, lung, pancreas, bladder, colon and prostate. A blind trial with 420 patients. *Cancer* 1993; 72:2007-15.
- Solter D, Knowles B B. Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc Natl Acad Sci USA* 1978; 75:5565-9.
- Itai S, Arai S, Tobe R, Kitahara A, Kim Y C, Yamabe H, et al. Significance of 2-3 and 2-6 sialyl Lewis A antigen in pancreas cancer. *Cancer* 1988; 61:775-87.
- Sonoda K, Nakashima M, Saito T, Amada S, Kamura T, Nakano H, et al. Establishment of a new human uterine cervical adenocarcinoma cell line, SiSo, and its reactivity to anti-cancer reagents. *Int J Oncol* 1995; 6:1099-104.
- Sonoda K, Nakashima M, Kaku T, Kamura T, Nakano H, Watanabe T. A novel tumor-associated antigen expressed in human uterine and ovarian carcinomas. *Cancer* 1996; 77:1501-9.
- Hsu S, Raine L, Fanger H. The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase techniques. *Am J Clin Pathol* 1981; 75:816-21.
- McGee J O, Woods J C, Ashall F, Bramwell M E, Harris H. New marker for human cancer cells. Immunohistochemical detection of the Ca antigen in human tissues with the Ca 1 antigen. *Lancet* 1982; 2:7-10.
- Thor A, Viglione M J, Murano R, Ohuchi N, Schlom J, Goldstein F. Monoclonal antibody B72.3 reactivity with human endometrium: a study of normal and malignant tissues. *Int J Gynecol Pathol* 1987; 6:235-47.
- Breitenecker G, Neunteufel W, Bieglmayer C, Kolbl H, Schieder K. Comparison between tissue and serum content of CA 125, CA 19-9, and carcinoembryonic antigen in ovarian tumours. *Int J Gynecol Pathol* 1989; 8:97-102.
- Schwartz P E, Chambers S K, Chambers J T, Gutmann J, Katopodis N, Foemmel R. Circulating tumor markers in the monitoring of gynecologic malignancies. *Cancer* 1987; 60:353-61.