原 著

Expression of tumor markers in uterine sarcoma : Immunocytochemical study

Department of Obstetrics and Gynecology, Public Kakunodate Hospital¹⁾, Clinical Laboratory,
Ogachi Central Hospital²⁾, Department of Pathology, Akita Red Cross Hospital³⁾

Shinichi Igarashi¹⁾, Keiji Naito²⁾, Shigeo Sato²⁾

Koichi Nara³⁾, Sadahiro Hosobe³⁾

Expression of the tumor markers in uterine sarcomas was investigated using immunocytochemistry. We found that several tumor markers were expressed heterogeneously in uterine sarcomas as well as in the epithelial tumors. Carcinoembryonic antigen, α -fetoprotein, NCC-CO 450, epithelial membrane antigen and sialyl Tn were expressed in uterine sarcomas significantly higher than controls.

Understanding of the tumor markers in uterine sarcoma may contribute to the detection of the disease at early stage. Immunocytochemistry of tumor markers as functional molecules may be very useful to understand biological behavior of uterine sarcoma.

Key words : Uterine sarcoma—Immunocytochemistry—Tumor marker

I. Introduction

Uterine sarcoma is a rare tumor, which may tend to understand the tumor character insufficiently. Detection of uterine sarcoma at early stage is difficult, and the remedy adequate for the disease is not fully established, which may lead the patients to poor prognosis. To solve such problems,

cytological and immunocytochemical approaches may be beneficial because it has wide information about morphological and chemical character of the tumor¹⁾.

Recent progresses in tumor markers are remarkable. Tumor markers have been essential in cancer detection and/or the treatment. However, most of the tumor markers were found against epithelial tumors as immunogens, while the significance of tumor markers in nonepithelial tumors is not fully discussed^{2,3)}. In the present study, we investigated the significance of tumor markers in uterine sarcoma immunocytochemically.

II. Materials and Methods

Tumors were obtained immediately after excision from patients undergoing routine surgery. The materials from 24 patients with uterine sarcoma and

Expression of tumor markers in uterine sarcoma : Immunocytochemical study

Shinichi IGARASHI¹⁾, M.D., Keiji NAITO²⁾, C.T., J.S.C., Shigeo SATO²⁾, C.T., J.S.C., Koichi NARA³⁾, C.T., I.A.C., Sadahiro HOSOBÉ³⁾, C.T., I.A.C.

¹⁾Department of Obstetrics and Gynecology, Public Kakunodate Hospital, ²⁾Clinical Laboratory, Ogachi Central Hospital, ³⁾Department of Pathology, Akita Red Cross Hospital

論文別刷請求先 ☎ 014-03 秋田県仙北郡角館町岩瀬字上野
18 公立角館病院産婦人科 五十嵐信一

平成5年11月12日受付

平成6年2月18日受理

10 patients with uterine leiomyoma as the controls were obtained and studied retrospectively. Several malignant tumors were included in the study group as follows: 11 endometrial stromal sarcomas, 9 leiomyosarcomas, 3 rhabdomyosarcomas (pleomorphic type) and an osteosarcoma. Study group included 4 mixed Müllerian tumors. However, we used smears from clearly sarcomatous parts, which were histologically defined. In the control group, smears were obtained from myometrium of the uterine leiomyoma, because we thought that the differential diagnosis between uterine sarcoma and leiomyoma was critical problem clinically. Touch smears from the tumors were fixed in 95% alcohol and stained with Papanicolaou and immunocytochemical techniques. In the same part of tumor, histological and immunohistochemical examina-

tions from formalin-fixed tissue sections were performed.

Antibodies raised against the following antigens was used (Table 1): Carcinoembryonic antigen⁴⁾ (CEA, Dako Co., Santa Barbara, CA, USA); α -fetoprotein⁵⁾ (AFP, Nichiray Co., Tokyo, Japan); basic fetoprotein⁶⁾ (BFP, Nihonkayaku Co., Tokyo, Japan); CA 125⁷⁾ (Dako Co.); CA 19-9⁸⁾ (Dako Co.); CA 72-4⁹⁾ (Toray Fuji Bionics Co., Tokyo, Japan); NCC-CO 450¹⁰⁾ (Nihonkayaku Co., Tokyo, Japan); NCC-ST 439¹¹⁾ (Nihonkayaku Co.); epithelial membrane antigen¹²⁾ (EMA, Dako Co.) and sialyl Tn¹³⁾ (STN, Otsuka Assay Inst., Tokushima, Japan). All antibodies were diluted in phosphate-buffered saline (pH 7.4) with 1% bovine serum albumin. The antigen-antibody reaction was then visualized employing the avidin biotin

Table 1 Properties and immunogens of the tumor markers

Tumor marker	Antibody	Property	Immunogen
CEA	A 5 B 7	glycoprotein M.W. 180 kDa E.M. β carbohydrate 40-60% oncofetal antigen	fetal intestine
AFP	292161	glycoprotein M.W. 38 kDa E.M. α_1 carbohydrate 4% oncofetal antigen	hepatoma
BFP	K-1	protein M.W. 55 kDa E.M. γ pI=9.3 oncofetal antigen	fetal serum, colon, brain
CA 125	OC 125	glycoprotein M.W. 550 kDa	ovarian carcinoma
CA 19-9	NS 19-9	glycolipid M.W. 36 kDa monosialoganglioside	colon carcinoma
CA 72-4	B 72.3	glycoprotein M.W. 1000 kDa sialyl-sugar residue+	breast carcinoma
NCC-CO 450	NCC-CO 450	glycoprotein M.W. >400 kDa no sialic acid mucinous type sugar chain+	colon carcinoma
NCC-ST 439	NCC-ST 439	glycoprotein M.W. >1000 kDa sialyl-sugar residue+	gastric carcinoma
EMA	E 29	glycoprotein M.W. 3000 kDa	milk fat globule
STN	TKH-2	glycoprotein M.W. >1000 kDa sialyl-sugar residue+ onco-developmental antigen	sheep mandibular gland

M.W. : molecular weight

E.M. : electrophoretic mobility (globulin region)

Table 2 Incidence of expression of tumor markers in uterine sarcoma

Tumor Marker	ESS	LMS	RMS	OS	Total	Control	p value
CEA	5/9	2/8	1/3	0/1	8/21(38.1)	1/10	<0.03
AFP	4/9	2/8	2/3	1/1	9/21(42.9)	1/10	<0.02
BFP	4/10	1/9	2/3	0/1	7/23(30.4)	1/10	n.s.
CA 125	1/10	1/9	0/2	0/1	2/22(9.1)	1/10	n.s.
CA 19-9	1/8	1/8	0/2	0/1	2/19(10.5)	1/10	n.s.
CA 72-4	5/10	2/8	1/3	1/1	9/22(40.9)	1/10	<0.02
NCC-CO 450	7/10	3/8	2/3	1/1	13/22(59.1)	1/10	<0.01
NCC-ST 439	2/11	2/8	2/3	0/1	6/23(26.1)	1/10	n.s.
EMA	5/10	4/7	1/1	0/1	10/19(52.6)	1/10	<0.01
STN	2/7	1/4	2/2	0/1	5/14(35.7)	1/10	<0.05

Each value was compared to that in the control group. Figures in parentheses indicate percentage.

ESS : endometrial stromal sarcoma, LMS : leiomyosarcoma, RMS : rhabdomyosarcoma, OS : osteosarcoma, n.s. : not significant

Fig. 1 Diffuse cytoplasmic reactivities were found in leiomyosarcoma cell against NCC-CO 450 (left) and in rhabdomyosarcoma cell against CA 72-4 (right). (ABC method, $\times 40$)

Fig. 2 Marginal reactivities were found in endometrial stromal sarcoma cell against EMA (left) and in rhabdomyosarcoma cell against STN (right). (ABC method, $\times 40$)

Fig. 3 Granular cytoplasmic reactivities were found in endometrial stromal sarcoma cell against CEA (left) and in leiomyosarcoma cell against AFP (right). (ABC method, $\times 40$)

complex (ABC) method using a kit (Vectastain ; Mountain View, CA, USA), and diaminobenzidine as chromogen. A sample of appropriate positive controls, cytological materials of ovarian carcinoma were stained in a parallel fashion with the tumor tissues. Negative controls, substituting nonimmune mouse serum samples for the primary antibody, were used to assess quality of the immunoperoxidase staining.

Evaluation of the staining was conducted by two independent investigators, and subsequent diagnosis was reviewed by a panel that included the authors. Staining was judged as positive if the frequency of positive cells was $>25\%$ in cytology as well as in histology. The incidence of positive patients in

each tumor marker was compared with the controls by Fisher's exact test.

III. Results

The incidence of positive cases in tumor markers is shown in Table 2. No reactive staining against the studied tumor markers was observed either in cytologically defined endometrial stromal cells or in muscle cells in leiomyomas. The expression of CEA, AFP, CA 72-4, NCC-CO 450, EMA or STN in uterine sarcomas was higher than in the controls. However, the expression of BFP, CA 125, CA 19-9 or NCC-ST 439 showed no significant increase. Strong cytoplasmic reactivity against NCC-CO 450 in the endometrial stromal sarcoma cells was detected. No reactivity against CA 125 or CA 19-9 was found in rhabdomyosarcoma cells. In the immunohistochemical analysis done in parallel, similar pattern was seen.

Various staining patterns were observed against several tumor markers. Diffuse cytoplasmic (Fig. 1) or marginal reactivity (Fig. 2) in tumor cells, or granular cytoplasmic reactivity (Fig. 3) was found.

IV. Discussion

Recent progress in tumor markers has been contributing in cancer detection and treatment. The tumor markers are classified into onco-fetal (developmental) antigens, cancer associated antigens, hormones, isoenzymes and the others. Most of the cancer associated antigens are glycoproteins. Several tumor marker proteins have been clarified to have important functions in oncogenesis, cell differentiation, cell adhesion and immunological recognition. However, most of the tumor markers were found against epithelial tumors as immunogens, while the significance of tumor markers in nonepithelial tumors is not fully discussed^{2,3}. Miettinen et al.¹⁴ have discovered epithelial markers in sarcoma. Mills et al.¹⁵ noted no EMA or keratin positivity in leiomyosarcomas from various organs. We found that the tumor

markers including EMA are expressed in nonepithelial tumors as well as in epithelial tumors.

In the process of oncogenesis lacking differentiation in uterine sarcoma, gene mutations may lead expression not only of oncogene products but also of epithelial tumor markers. Some of the sarcomas may have potentiality to make epithelial tumors. Analysis of expression of tumor markers may provide new knowledge of uterine sarcoma.

Tumor microenvironment may affect the nature of sarcoma as well as carcinoma. Chung¹⁶ reported that microenvironmental factors may have an influence on the regulation of genes of molecular markers. A certain microenvironmental factor may induce change in the nature of the neoplastic cells, resulting in the appearance of positivity against tumor markers. In the metastatic process of the malignant tumors, it is very interesting to know whether tumor marker antigens have a role in tumor behavior. Some of the antigens may have function as adhesion molecules.

Within the limits of neoplasm, a new panel of tumor markers for differentiation epithelial elements from nonepithelial elements is required¹⁷. Further, new combination of tumor markers useful in uterine sarcoma is necessary, especially for detection at early stage and of the recurrence. Indeed, tumor markers in sera were decreased by hysterectomy in a few evaluable cases. But many cases had no positive serum marker. A heterogeneity in expression of tumor markers is a very important, but difficult problem in the treatment.

In conclusion, tumor markers were expressed not only in the epithelial tumors but also in the uterine sarcomas heterogeneously. Understanding of the tumor markers as functional molecules can contribute to detection of uterine sarcomas at early stage and to know the biological behavior.

要 約

免疫細胞化学を用い、子宮肉腫における腫瘍マーカーの発現を検討した。多くの腫瘍マーカーは、上皮性腫瘍同様に、子宮肉腫でも不均一ながら発現がみられた。Carcinoembryonic

antigen, α -fetoprotein, NCC-CO 450, epithelial membrane antigen, sialyl Tn が良性コントロールに比し, 子宮肉腫で有意に高頻度に発現していた。

子宮肉腫における腫瘍マーカーに関して多くの情報が得られれば, 早期発見や治療に有用と思われる。また機能分子としての腫瘍マーカーに関する, 免疫細胞化学的検討は, 子宮肉腫の生物学的動態の把握に有力な武器となると思われる。

References

- 1) Flens, M.J., Valk, P., Tadema, T.M., et al. : The contribution of immunocytochemistry in diagnostic cytology, Comparison and evaluation with immunohistology, *Cancer*, 65 : 2704~2711, 1990.
- 2) Dabbs, D.J., Silvermann, J.F., Geisinger, K.R. : Immunohistochemical study of uterine stromal sarcoma and rhabdomyosarcoma, *Arch. Pathol. Lab. Med.*, 113 : 1151~1154, 1989.
- 3) Fisher, C. : The value of electronmicroscopy and immunohistochemistry in the diagnosis of soft tissue sarcomas : a study of 200 cases, *Histopathology*, 16 : 441~454, 1990.
- 4) Gold, P., Freedman, S.O. : Demonstration of tumor-specific antigens in human colonic carcinoma by immunological tolerance and absorption techniques. *J. Exp. Med.*, 121 : 439, 1965.
- 5) Abelev, G.I., Perova, S., Khramkova, N. : Production of embryonal α -globulin by transplantable hepatomas. *Transplantation*, 1 : 174, 1963.
- 6) Ishii, M. : A new carcinoembryonic protein characterized by basic property. *Scand. J. Immunol.*, 8(suppl. 8) : 611, 1978.
- 7) Bast, R.C.Jr., Feeney, M., Lazarus, H., et al. : Reactivity of a monoclonal antibody with human ovarian carcinoma. *J. Clin. Invest.*, 68 : 1331, 1981.
- 8) Koprowski, H., Steplewski, Z., Mitchell, K. : Colorectal carcinoma antigens detected by hybridoma antibodies. *Somatic Cell Genet.*, 5 : 952, 1979.
- 9) Colcher, D., Hand, P.H., Nuti, M., et al. : A spectrum of monoclonal antibodies reactive with human mammary tumor cells. *Proc. Natl. Acad. Sci. USA*, 78 : 3199~3203, 1981.
- 10) Sakurai, Y., Hirohashi, S., Shimosato, Y., et al. : Selection of monoclonal antibody reactive with high-molecular-weight glycoprotein circulating in the body fluid of gastrointestinal cancer patients. *Cancer Res.*, 48 : 4053~4058, 1988.
- 11) Hirohashi, S., Watanabe, M., Shimosato, Y., et al. : A monoclonal antibody reactive with the sialyl-sugar residue of a high molecular glycoprotein in sera of cancer patients, *Gann*, 75 : 485~488, 1984.
- 12) Ormerod, M.G., Steele, K., Westgood, J.H., et al. : Epithelial membrane antigen : Partial purification assay and properties. *Br. J. Cancer*, 48 : 533~541, 1983.
- 13) Kjeldsen, T., Clausen, H., Hirohashi, S., et al. : Preparation and characterization of monoclonal antibodies directed to the tumor-associated o-linked sialosyl-2-6 α -N-acetylgalactosaminyl (sialosyl-Tn). *Cancer Res.*, 48 : 2211~2220, 1988.
- 14) Miettinen, M. : Immunoreactivity for cytokeratin and epithelial membrane antigen in leiomyosarcoma, *Arch. Pathol. Lab. Med.*, 112 : 637~640, 1988.
- 15) Mills, S.E., Bova, S., Young, R.H. : Leiomyosarcoma of the urinary bladder, *Am. J. Surg. Pathol.*, 13 : 480~489, 1989.
- 16) Chung, M.T., Mukai, K., Teshima, S., et al. : Expression of various antigens by different component of uterine mixed Müllerian tumors. An immunohistochemical study, *Acta. Pathol. Jpn.*, 33 : 35~45, 1988.
- 17) Ridley, P.D., Braimbridge, M.V. : Primary sarcoma of the lung : potential dangers of immunocytochemistry, *Br. J. Clin. Pract.*, 44 : 685~688, 1990.