RAPID COMMUNICATION Jpn. J. Cancer Res. 81, 1206–1209, December 1990

Recognition of the Polypeptide Core of Mucin by Monoclonal Antibody MUSE11 against an Adenocarcinoma-associated Antigen

Yuji Hinoda, Naoaki Nakagawa, Yasuo Ohe, Hideki Kakiuchi, Masayuki Tsujisaki, Kohzoh Imai and Akira Yachi[1]

Department of Internal Medicine (Section 1), Sapporo Medical College, S-1, W-16, Chuo-ku, Sapporo 060

Monoclonal antibody (MAb) MUSE11 detects an adenocarcinoma-associated antigen and is useful for the serodiagnosis of pancreas cancer. We established a sandwich enzyme immunoassay using MAb MUSE11 and MAb DF3 against a breast cancer-associated mucin core protein as a catcher and a tracer, respectively. With this assay system, the binding of the tracer MAb DF3 to an antigen in the human kidney tissue lysate was clearly inhibited by MAb MUSE11. In addition, MAb MUSE11 showed a significant binding activity to the synthetic peptide corresponding to the tandem repeat of a human epithelial mucin core protein. These data suggest that MAb MUSE11 could detect the polypeptide core of a mucin, and may be of use for studying mucin as a gene product.

Key words: Monoclonal antibody — Adenocarcinoma-associated antigen — Mucin core protein — Synthetic peptide

A number of tumor-associated antigens detected by monoclonal antibodies (MAbs)[2] established during the last decade have been identified as mucins.[1-5] Although detailed analyses of the tumor-associated carbohydrate chains of mucin have been reported,[6,7] the polypeptide core structures recognized as epitopes by MAbs remain to be fully elucidated. The MAb MUSE11,[8] prepared against the void fraction of ascites from a gastric cancer patient, was shown immunohistologically to be restricted to adenocarcinoma tissues, and to be of use as a tumor marker in sera of pancreas cancer patients. It has been suggested that the epitope recognized by MAb MUSE11 may be a peptide.[8] This is of interest, since all other pancreas cancer-associated mucin-like antigens which have been revealed to be of serodiagnostic use have carbohydrate epitopes as far as we know.[1,2,4,5,9-12] On the other hand, cDNA clones coding for a mucin core protein have recently been isolated with MAb DF3 against a breast cancer-associated antigen,[13] suggesting that at least a part of the DF3 epitope also is a peptide despite the fact that it was sensitive to neuraminidase.[14] The circulating plasma DF3 antigen was hardly detected in patients with digestive organ cancers,[15] which indicates that it has a different clinical significance from the MUSE11 antigen. However, the facts that both antigens had a molecular weight of 300 kd[8,15] and had a peptide epitope have led us to compare these antigens. In this report, we present data suggesting the recognition of the polypeptide core of a mucin by MAb MUSE11.

To estimate the relationship between the MUSE11 and DF3 epitopes, a sandwich enzyme immunoassay[16] was established with MAb MUSE11 and peroxidase-conjugated MAb DF3 (IMUNOCLONE CA15-3, Toray-Fuji Bionics) as a catcher and a tracer, respectively. By using this assay system, an inhibition test was carried out. After adsorption of the catcher MAb MUSE11 (20 μg/ml) on the beads of the FAST system (Becton Dickinson, CA) overnight at 4°C, the beads were blocked with phosphate-buffered saline (PBS), pH 7.4, containing 3% bovine serum albumin (BSA) at 37°C for 2 h, and then incubated at 37°C for 1 h with the tissue lysate (8 mg/ml) prepared according to Boucher *et al.*[17] from a kidney obtained by autopsy. Following washings with PBS containing 0.05% Tween-20, the beads were incubated with the tracer MAb DF3 (3 μg/ml) and serially diluted with unlabeled MAb MUSE11 or M7-625 (anti-idiotype MAb against anti-CEA MAb MA208) at 37°C for 1 h, then washed. Substrate reaction was performed as described previously.[16] A 24-amino-acid peptide (PDTRPAPGSTAPPAHGVTSAPDTR) corresponding to the core protein of a human polymorphic epithelial mucin gene's 60-nucleotide tandem repeat sequence which has recently been reported by Gendler *et al.*[18] and peptides from carcinoembryonic antigen[19] were produced with a peptide synthesizer at the Central Research Laboratory, Ajinomoto Co., Yokohama. Reactivity of MAb MUSE11 to peptides was examined as follows. The peptides were coated onto 96-well flexible assay plates (Becton

[1] To whom all correspondence should be addressed.

[2] Abbreviations used are: MAb, monoclonal antibody; EIA, enzyme immunoassay; CEA, carcinoembryonic antigen; cDNA, complementary DNA.

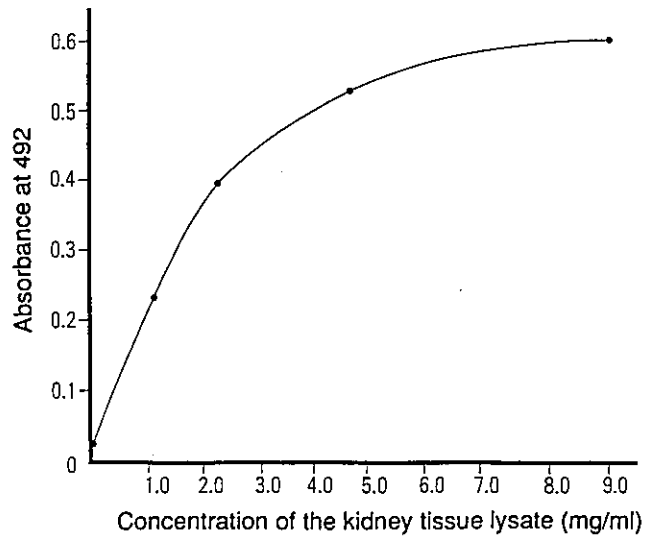

Fig. 1. A sandwich enzyme immunoassay of human kidney tissue lysate. MAbs MUSE11 and peroxidase-conjugated DF3 were used as a catcher and a tracer, respectively.

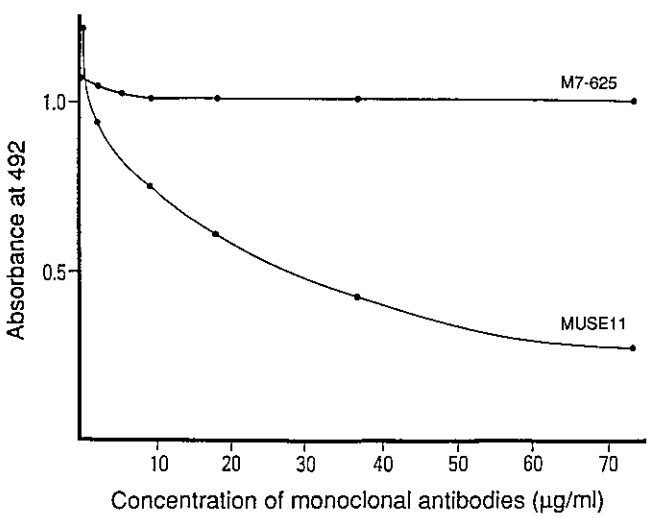

Fig. 2. Inhibitory effect of MAb MUSE11 on the binding of MAb DF3 to an antigen in the kidney tissue lysate. The concentration of the tissue lysate used was 8 mg/ml. Serially diluted MAb MUSE11 or M7-625 was added together with the tracer DF3.

Dickinson) at 100 μg/ml in PBS overnight at 4°C. The plates were washed with PBS, and non-specific binding sites were blocked with 3% BSA at 37°C for 2 h. Purified MAbs (10 μg/ml) were then added and the plates were incubated at 37°C for 2 h or at 4°C overnight. The plates were then incubated with ^{125}I-labeled goat anti-mouse IgG-Fc antibody as described,[19] and washed with PBS. Bound cpm was counted in a γ-scintillation counter.

Table I. Binding Activity of MAb MUSE11 to Synthetic Peptides[a]

	Binding activity (cpm)[b] to peptides	
	Mucin core protein[c]	CEA-P3[d]
MUSE11	9543±53	2335±65
P1-255[e]	1870±150	1088±10

a) Concentrations of peptides and MAbs used were 100 μg/ml and 10 μg/ml, respectively. Values are mean±SD of triplicate determinations.
b) Detected with an ^{125}I-labeled anti-mouse IgG-Fc antibody.
c) A peptide consisting of one repeat and 4 amino acids from the tandem repeat region of mucin core protein.
d) A 22-amino-acid peptide from the B1 domain of CEA (amino acid numbers 202–221).
e) A MAb against CEA-P1 peptide corresponding to 22 amino acids from the A1 domain of CEA (amino acid numbers 119–140).

In the first place, the kidney tissue lysate was examined by using a sandwich EIA system with MAbs MUSE11 and DF3, since the renal tubules are immunohistochemically positive for both MAbs MUSE11[8] and DF3 (data not shown). A typical curve obtained by titrating the lysate is shown in Fig. 1. With this assay system, an inhibition test was then performed. As depicted in Fig. 2, MAb MUSE11 clearly inhibited the binding of the tracer MAb DF3 to an antigen caught by MAb MUSE11 in a dose-dependent manner. No inhibitory effect was observed when MAb M7-625 was used.

These data suggest that the epitopes recognized by MAbs MUSE11 and DF3 may be coexpressed on the same mucin molecule, as described in the case of CA 19-9 and the DU-PAN-2 antigen,[20] and that they may share a common antigenic structure. To elucidate the MUSE11 epitope structure, we then synthesized a 24-amino acid peptide as described above, and examined the binding activity of MAb MUSE11 to it. MAb MUSE11 showed a significant binding activity to this peptide compared with that to the CEA peptide (P3). In addition, MAb P1-255[19] against another CEA peptide (P1) did not have any binding activity to these peptides (Table I). This finding was further substantiated by the fact reported by Siddiqui *et al.*[13] that the cDNA clone obtained by antibody screening of a breast cancer cell cDNA library with MAb DF3 encoded the mucin core protein, and has almost an identical tandem repeat sequence with Gendler's cDNA clones.[21] Thus, it is likely that MAb MUSE11 directly detects a mucin core protein, and this MAb should be useful for evaluation of mucin as a gene

product. Our previous data based on the effect of tunicamycin on cultured pancreas cancer cell line Panc-1, suggesting that the MUSE11 antigen may have N-glycosidically linked carbohydrate chains, should be corrected, although the reproducibility of the experiments is being further evaluated.

By means of measurement of binding activity to synthetic peptides, more than 10 MAbs have been identified to react with mucin core protein.[22,23] It is of interest that all of them are MAbs established against a breast cancer-associated antigen. Before these studies, Abe and Kufe[24] had shown that MAb F36/22 prepared against the MCF-7 breast cancer cell line completely inhibited MAb DF3 binding in competitive blocking assays, suggesting that it may also recognize the same kind of mucin core protein as MAb DF3 does. MAb F36/22 showed a wide reactivity to epithelial carcinomas[25] in addition to the breast carcinoma as in the case of MUSE11,[8] but it is different from MAb MUSE11 in its sensitivity to neuraminidase.[24] In this context, it appears that MAb MUSE11 is the first example of an MAb recognizing a mucin core protein among MAbs which have been revealed to be of practical use for detecting a circulating pancreas cancer-associated antigen. Some other MAbs may also recognize a polypeptide core antigen of mucin, and comparative studies will be required to clarify the specificity of each one. It is noteworthy in this connection that Gum *et al.*[26] recently reported cDNA clones coding for another type of mucin core protein containing the tandem repeat of 23 amino acids, suggesting the existence of a family of mucin core proteins.

This work was financed by grants for Cancer Research from the Ministry of Education, Science and Culture, and from the Ministry of Health and Welfare, Japan; it was also supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture, and a grant from the Foundation for Promotion of Cancer Research.

(Received August 8, 1990/Accepted October 8, 1990)

REFERENCES

1) Magnani, J. L., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, Z., Koprowski, H. and Ginsburg, V. A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II. *J. Biol. Chem.*, **257**, 14365–14369 (1982).
2) Lan, M. S., Finn, O. J., Fernsten, P. D. and Metzger, R. S. Isolation and properties of a human pancreatic adenocarcinoma-associated antigen, DU-PAN-2. *Cancer Res.*, **45**, 305–310 (1985).
3) Miotti, S., Aguanno, S., Canevari, S., Diotti, A., Orlandi, R., Sonnino, S. and Colnaghi, M. I. Biochemical analsis of human ovarian cancer-associated antigens defined by murine monoclonal antibodies. *Cancer Res.*, **45**, 826–832 (1985).
4) Johnson, V. G., Schlom, J., Paterson, A. J., Bennet, J., Magnani, J. L. and Colcher, D. Analysis of a human tumor-associated glycoprotein (TAG-72) identified by monoclonal antibody B72.3. *Cancer Res.*, **46**, 850–857 (1986).
5) Hinoda, Y., Imai, K., Ban, T., Endo, T. and Yachi, A. Immunochemical characterization of adenocarcinoma-associated antigen YH206. *Int. J. Cancer*, **42**, 653–658 (1988).
6) Itzkowitz, S. H. and Kim, Y. S. New carbohydrate tumor markers. *Gastroenterology*, **90**, 491–494 (1986).
7) Rittenhouse, H. G., Manderino, G. L. and Hass, G. M. Mucin-type glycoproteins as tumor markers. *Lab. Med.*, **16**, 556–560 (1985).
8) Ban, T., Imai, K. and Yachi, A. Immunohistological and immunochemical characterization of a novel pancreatic cancer-associated antigen MUSE11. *Cancer Res.*, **49**, 7141–7146 (1989).
9) Lindholm, L., Holmgren, J., Svennerholm, L., Fredman, P., Nilsson, O., Persson, B., Myrvold, H. and Lagergard, T. Monoclonal antibodies against gastrointestinal tumor-associated antigens isolated as monosialogangliosides. *Int. Arch. Allergy Appl. Immunol.*, **71**, 178–181 (1983).
10) Kannagi, R., Fukushi, Y., Tachikawa, T., Noda, A., Shin, S., Shigeta, K., Hiraiwa, N., Fukuda, Y., Inamoto, T., Hakomori, S. and Imura, H. Quantitative and qualitative characterization of human cancer-associated serum glycoprotein antigens expressing fucosyl or sialyl-fucosyl type 2 chain polylactosamine. *Cancer Res.*, **46**, 2619–2626 (1986).
11) Chung, Y. S., Ho, J. J. L., Kim, Y. S., Tanaka, H., Nakata, B., Hiura, A., Motoyoshi, H., Satake, K. and Umeyama, K. The detection of human pancreatic cancer-associated antigen in the serum of cancer patients. *Cancer*, **60**, 1636–1643 (1987).
12) Ohyanagi, H., Saitoh, Y., Okuyama, S., Ishida, T., Uesaka, K., Ishida, H., Yamamoto, M., Uemura, Y., Kano, Y. and Suyama, T. A new monoclonal antibody-defined tumor marker (KMO1) for pancreatic carcinoma. *Mt. Sinai J. Med.*, **54**, 393–400 (1987).
13) Siddiqui, J., Abe, M., Hayes, D., Shani, E., Yunis, E. and Kufe, D. W. Isolation and sequencing of a cDNA coding for the human DF3 breast carcinoma-associated antigen. *Proc. Natl. Acad. Sci. USA*, **85**, 2320–2323 (1988).
14) Sekine, H., Ohno, T. and Kufe, D. W. Purification and sharacterization of a high molecular weight glycoprotein detectable in human milk and breast carcinomas. *J. Im-*

munol., **135**, 3610–3615 (1985).
15) Hayes, D. F., Sekine, H., Ohno, T., Abe, M., Keefe, K. and Kufe, W. D. Use of a murine monoclonal antibody for detection of circulating plasma DF3 antigen levels in breast cancer patients. *J. Clin. Invest.*, **75**, 1671–1678 (1985).
16) Hinoda, Y., Imai, K., Ban, T., Endo, T. and Yachi, A. A sandwich enzyme immunoassay of an adenocarcinoma-associated antigen, YH206, in cancer sera. *Jpn. J. Cancer Res.*, **78**, 607–613 (1987).
17) Boucher, D., Cournoyer, D., Stanners, C. P. and Fuks, A. Studies on the control of gene expression of the carcino-embryonic antigen family in human tissue. *Cancer Res.*, **49**, 847–852 (1989).
18) Gendler, S., Taylor-Papadimitriou, J., Duhig, T., Rothbard, J. and Burchell, J. A highly immunogenic region of a human polymorphic epithelial mucin expressed by carcinomas is made up of tandem repeat. *J. Biol. Chem.*, **263**, 12820–12823 (1988).
19) Imai, K., Tsujisaki, M., Hishikawa, N., Tokuchi, S., Higashide, T., Itoh, J., Sato, Y., Ono, A., Sugiyama, T. and Yachi, A. Preparation of monoclonal antibodies to synthetic CEA peptide. *In* "The Carcinoembryonic Antigen Gene Family," ed. A. Yachi and J. E. Shively, pp. 1–11 (1989). Elsevier, Amsterdam.
20) Lan, M. S., Bast, R. C., Colnaghi, M. I., Knapp, R. C., Colcher, D., Schlom, J. and Metzger, R. S. Co-expression of human cancer-associated epitopes on mucin molecules. *Int. J. Cancer*, **39**, 68–72 (1987).
21) Merlo, G. R., Siddiqui, J., Cropp, C. S., Liscia, D. S., Lidereau, R., Callahan, R. and Kufe, D. W. Frequent alteration of the DF3 tumor-associated antigen gene in primary human breast carcinomas. *Cancer Res.*, **49**, 6966–6971 (1989).
22) Xing, P. X., Tjandra, J. J., Reynolds, K., Mclaughlin, P. J., Purcell, D. F. J. and Mckenzie, F. C. Reactivity of anti-human milk fat globule antibodies with synthetic peptides. *J. Immunol.*, **142**, 3503–3509 (1989).
23) Burchell, J., Taylor-Papadimitriou, J., Boshell, M., Gendler, S. and Duhig, T. A short sequence, within the amino acid tandem repeat of a cancer-associated mucin, contains immunodominant epitopes. *Int. J. Cancer*, **44**, 691–696 (1989).
24) Abe, M. and Kufe, D. W. Identification of a family of high molecular weight tumor-associated glycoproteins. *J. Immunol.*, **139**, 257–261 (1987).
25) Croghan, G. A., Pepsidero, L. D., Valenzuela, L. A., Nemoto, T., Penetrante, R. and Chu, T. M. Tissue distribution of an epithelial and tumor-associated antigen recognized by monoclonal antibody F36/22. *Cancer Res.*, **43**, 4980–4987 (1983).
26) Gum, J. R., Byrd, J. C., Hicker, J. W., Toribata, N. W., Lamport, D. T. A. and Kim, Y. S. Molecular cloning of human intestinal mucin cDNAs. *J. Biol. Chem.*, **264**, 6480–6487 (1989).