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Organ-specific expression of the intestinal epithelium-related antigen A33, a cell surface target for antibody-based imaging and treatment in gastrointestinal cancer

Abstract Murine monoclonal antibody A33 (mA33) was developed by the Memorial Sloan-Kettering Cancer Center and by the New York Branch of the Ludwig Institute for Cancer Research. It is an immunoglobulin (Ig)G2a antibody that detects a protease- and neuraminidase-resistant, periodate-sensitive epitope. Serological analysis of the antigen showed that it is expressed in a few colorectal cancer cell lines and a pancreatic cancer cell line, but is basically not reactive with other types of cell line. Normal fibroblasts and normal kidney cell lines reacted negatively to mA33. Immunohistochemical study of normal tissues identified the large and small intestinal mucosa as the principal site of A33 expression. Tests in tumor samples demonstrated that only tumors of the gastrointestinal tract are consistently A33 positive. A33 is found in 95% of primary and metastatic colorectal cancers, with uniform expression throughout the tumors in most cases. A33 is also detected in 63% of gastric cancers, with uniform expression in 45% of cases. Eighty-three percent of intestinal-type gastric cancers were positive for A33, and about 50% of the diffuse-type and mucinous cancers were mA33 positive. A33 was expressed in 50% of the pancreatic cancers but with marked heterogeneity. Other epithelial cancers, sarcomas, neuroectodermal tumors, and lymphoid neoplasms were generally A33 negative. A33 is the first example of a constitutively expressed, organ-specific epithelial membrane antigen permitting highly specific tumor targeting in patients with gastrointestinal cancer. Encouraged by the success of the biodistribution and imaging characteristic studies performed at Memorial Sloan-Kettering Cancer Center by the New York Branch of the Ludwig Institute in colorectal cancers, a new clinical study of humanized monoclonal antibody huA33 against A33 antigen-positive gastric cancers has been initiated in Japan.

Key words Monoclonal antibody · A33 · Gastric cancer · Immunohistochemistry · Tumor targeting

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Introduction

The original mouse monoclonal antibody A33 (mA33) was established by the Memorial Sloan-Kettering Cancer Center by Sakamoto et al. [13]. It is an immunoglobulin (Ig)G2a antibody that detects a protease- and neuraminidase-resistant, periodate-sensitive, conformation-dependent epitope [7]. Purification and characterization of the antigen were performed by Catimel et al. demonstrating the molecular weight of the antigen using Western blotting analysis under both nonreducing (43 kD) and reducing (49 kD) conditions [3]. Detailed analysis of the structure of the antigen was performed by Heath et al. and Ritter et al., showing that the antigen is a transmembrane palmitoylated glycoprotein and a novel member of the immunoglobulin superfamily [6, 12]. Immunohistochemical analysis by Garin-Chesa et al. confirmed that the antigen is homogenously expressed by more than 95% of colon cancers and in the normal intestinal mucosa, but basically not in other epithelial cells [5]. Peripheral blood granules and mononuclear cells were also found to be nonreactive using flow cytometric techniques. Immunohistochemical staining of the normal intestinal mucosa with serially diluted samples of mA33 suggests that A33 antigen expression is greatest at the top and minimal at the base of the crypt. This characteristic distribution of A33 antigen in normal colon was of great

importance in determining the clinical use of mA33 in colorectal cancers, as replicating colonocytes are situated in the base of the crypt and would not be a crucial target for mA33-mediated damage to the normal colon.

Based on the results of these serological, biochemical, and immunohistochemical characterization studies of the A33 antigen, the biodistribution and imaging characteristics of ^{131}I-mA33 were studied at Memorial Sloan-Kettering Cancer Center by the Ludwig Institute for Cancer Research by Welt et al. in colorectal cancer patients with hepatic metastases [19]. Selective mA33 localization to the tumor tissue was demonstrated in 19 of 20 patients by both external antibody imaging and biopsy. ^{99m}Technetium-albumin blood pool studies showed that liver metastases were hypovascular, emphasizing the selective localization of mA33 despite poor tumor blood flow.

The results of the phase I localization and biodistribution studies led to a phase I/II radioimmunotherapy study at Memorial Sloan-Kettering Cancer Center with a single dose of ^{131}I-labeled mA33 [20]. Of the 23 patients entered in the study, antitumor effects were observed in five, despite the fact that only a single dose was given. Bone marrow was the dose-limiting organ toxicity, but gastrointestinal toxicity was minimal.

One of the attractive features of the A33 antigenic system for immunotherapy is the in vitro evidence for rapid internalization of A33 antigen/antibody complexes into colon cancer cells and the ability of mA33 conjugates with intracellular sites of action to kill colon cancer cells in vitro and in the nu/nu mice xenograft model [4]. ^{125}I-labeled mA33 was suggested to be useful in patients because this radionuclide exerts its cytotoxic effects primarily through short-range Augar electrons, which are most effective when generated in close proximity (1–4 μm) to the cell nucleus. Accordingly, one of the benefits of ^{125}I-mA33, compared with ^{131}I-mA33, is the reduced bone marrow toxicity, and this expectation was confirmed in the nu/nu mouse tumor model. In a second phase I/II clinical trial at Memorial Sloan-Kettering Cancer Center by the Ludwig Institute for Cancer Research, a total of 22 advanced colorectal cancer patients who had failed conventional chemotherapy were treated with ^{125}I-mA33 at an initial dose of 50 μCi/m^2 to 350 μCi/m^2 [21]. Tumor imaging by external ^{125}I scanning with a collimator was possible in all patients, and tumor images have been followed for up to 35 days after antibody infusion. Four patients showed evidence of an antitumor effect of ^{125}I-mA33. With the exception of one patient with transient grade 3 thrombocytopenia, there were no significant toxicities and no significant gastrointestinal symptoms.

Because of the frequent development of an anti-mouse IgG immune response, patients in mA33 therapy studies could be treated with only a single course of the antibody. To overcome this limitation, a genetically engineered, fully humanized, CDR-grafted A33 IgGl monoclonal antibody (huA33) was developed by the Ludwig Institute for Cancer Research in conjunction with Celltech Therapeutics (Slough, UK), and clinical grade huA33 was purified, enabling repeated antibody administration and treatment [10].

Three clinical trials with huA33 have been conducted to date. An initial phase I multiple-infusion protocol in metastatic colorectal cancer has been conducted by Welt in the New York Branch of the Ludwig Institute for Cancer Research [22]. In this trial, some antitumor responses were observed, and immunogenicity represented by the generation of a human anti-human antibody was noted in some patients after multiple injections. A phase II trial of huA33, also conducted by Welt, is currently active at Memorial Sloan-Kettering Cancer Center. A phase I, single-infusion, biopsy-based study of ^{131}I-huA33 in patients with colorectal carcinoma has also been conducted by Scott at the Melbourne, Australia, Branch of the Ludwig Institute [16]. This study confirmed that the biodistribution of huA33 was identical to that in prior studies of mA33.

In Japan, a clinical phase I study with huA33 in advanced gastric cancer is planned in Aichi Prefectural Hospital and Gunma University in conjunction with the Ludwig Institute for Cancer Research. The present immunohistochemical study was conducted to evaluate the appropriate target for this phase I huA33 trial by examining the expression of A33 antigen in gastric cancer in relation to histopathological type.

Materials and methods

Tissues

Primary gastric carcinoma tissue was obtained from 38 patients undergoing surgical resection at Aichi Prefectural Hospital. Fifteen were diffuse, 21 were intestinal, and two were mucinous carcinomas. In all cases normal mucosa distant from the tumor lesion was obtained from the same patient. Intestinal metaplasia was detected in three and obvious signs of gastritis were noted in two of the 38 patients. All diagnoses were confirmed using hematoxylin and eosin-stained sections. Samples were embedded in OCT compound (Tissue-Tek, Sakura Fine Chemical Co., Tokyo, Japan), frozen in isopentane pooled in liquid nitrogen, and stored at −70 °C.

Antibody

The mA33 hybridoma line was derived from a fusion between spleen cells from a BALB/C mouse immunized with the AsPc-1 pancreas cancer cell line and SP2/0 myeloma cells. The specificity of mA33 was examined by the mixed hemagglutination test using a panel of cancer and normal cell lines (Fig. 1). As a source of mA33 (IgG2a subclass) for immunohistochemistry, either hybridoma culture supernatant or purified Ig was used [5]. Unrelated mouse monoclonal antibodies of the same immunoglobulin subclass were used as negative and positive controls throughout the study.

Immunohistochemistry

The avidin-biotin complex immunoperoxidase method was used as described previously [5, 17]. Briefly, 4–5 μm-thick frozen sections were cut, mounted on poly-L-lysine-coated slides, air-dried, and fixed in acetone at 4 °C for 10 min. Sections were treated with 0.3% H_2O_2 for 3 min to block endogenous peroxidase, followed by blocking with normal horse serum for 30 min at room temperature.

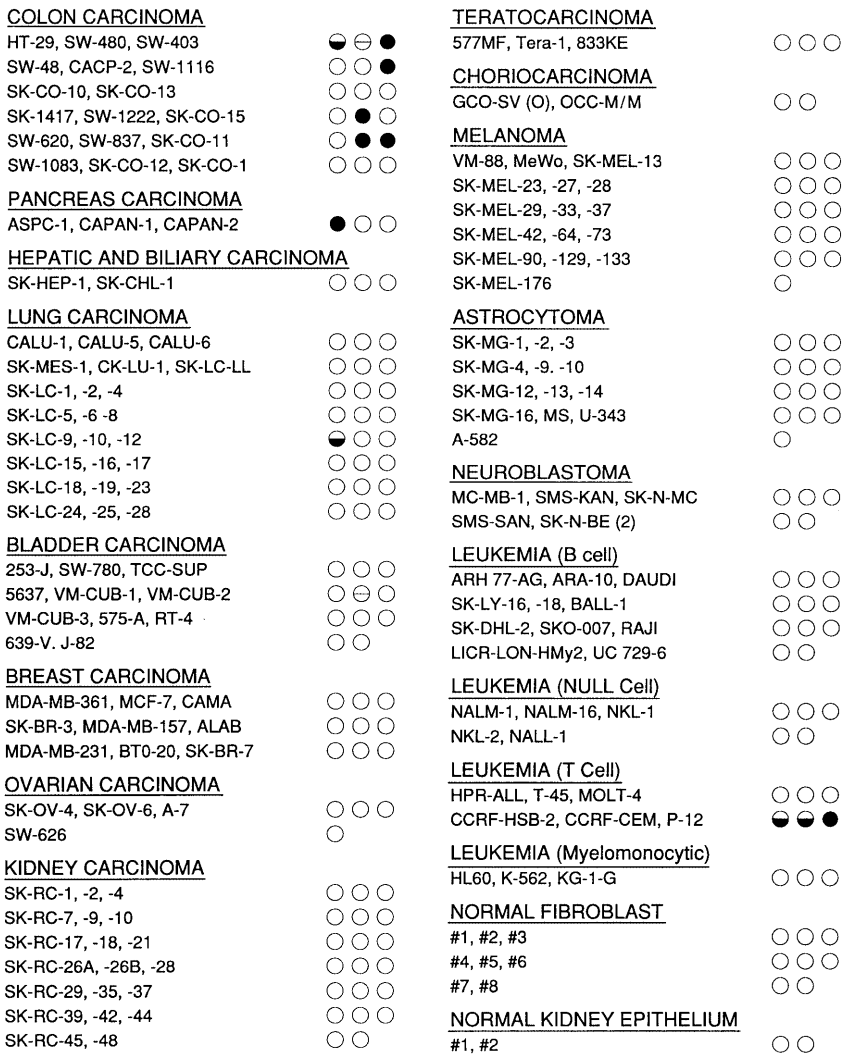

Fig. 1 Reactivity of mouse monoclonal antibody A33 with a panel of tumor and normal cell lines

Slides were incubated overnight at 4 °C with mA33 or isotype-matched positive and negative control monoclonal antibodies. Sections were washed and incubated with biotinylated horse anti-mouse serum (1:100; Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature, followed by avidin-biotin-horseradish peroxidase complex (1:100 dilution at a 1:1 ratio). The final reaction product was visualized with chromogen 3,3′-diaminobenzidine. Sections were counterstained with Harris hematoxylin.

Results

A33 expression in gastric cancers

A total of 38 gastric carcinomas were analyzed. As shown in Table 1 and Fig. 2, 24 (63%) expressed A33.

Table 1 Expression of A33 antigen in gastric cancers and in normal gastric epithelium. Figures in parentheses indicate number of cases with uniform antigen expression

	+	−	Total
Cancer			
Diffuse	11 (8)	10	21
Intestinal	12 (8)	3	15
Mucinous	1 (1)	1	2
Intestinal metaplasia	3 (3)	0	3
Gastritis	0	2	2
Normal			
Foveolar epithelium	0	38	38
Gastric gland	0	38	38

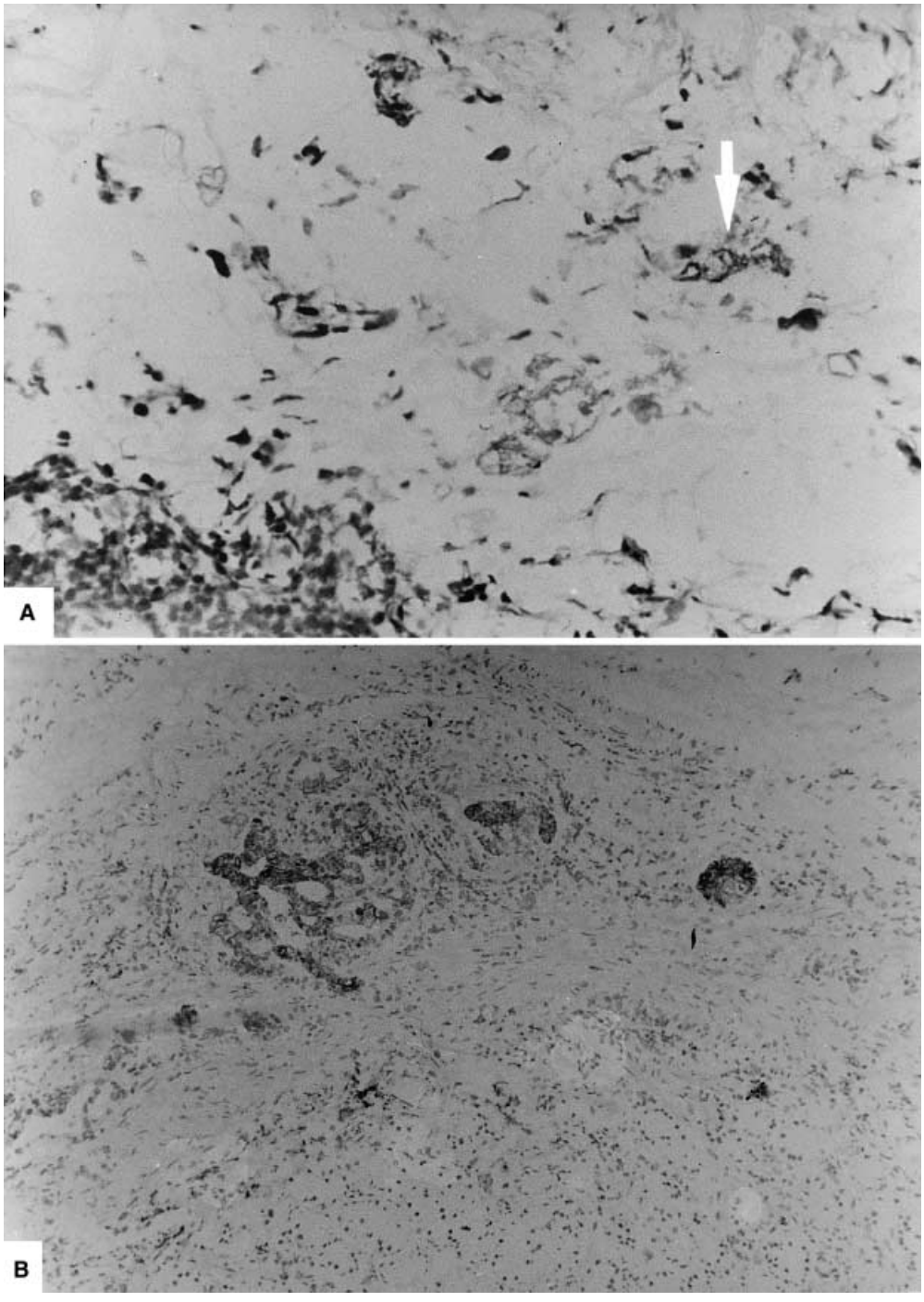

Fig. 2 Immunohistochemical detection of A33 antigen expression in diffuse (A) and in intestinal-type(B) gastric cancers

Seventeen of the 38 tumors showed uniform A33 immunostaining. Among the 21 diffuse-type gastric carcinomas (poorly differentiated carcinomas and signet ring cell carcinomas), A33 was expressed in 11 (52%). Uniform staining was observed in eight (38%) of the diffuse-type gastric cancers. Among the 15 intestinal-type gastric carcinomas (well- and moderately well-differentiated adenocarcinomas), A33 was expressed in 12 (80%), and uniform expression was noted in eight (53%). Uniform expression was also observed in one of two mucinous cell carcinomas.

Expression in intestinal metaplasia and gastritis

In five patients with gastric cancers, areas of either intestinal metaplasia or gastritis were present in the gastric mucosa in the resected specimens. In three patients, these areas of intestinal metaplasia showed homogenous A33 expression. However, in two patients with gastritis, A33 was not expressed.

Expression in normal gastric epithelium

The normal gastric epithelium at least 3 cm from the tumor margin in the resected specimens from 38 patients was examined. Neither foveolar epithelium nor gastric gland [14] reacted positively to mA33. In contrast to normal colonic, small intestinal, or duodenal epithelium, normal gastric epithelium does not express A33 antigen.

Discussion

Numerous clinical trials of monoclonal antibody-targeted cancer therapies have been performed in a broad range of human tumors. Although considerable progress has been reported in the treatment of hematopoietic tumors like lymphoma [9] or leukemia [8], few studies on solid tumors have shown remarkable results.

Among gastrointestinal cancers, most specific immunotherapy trials have been performed in colorectal cancers. Several antigens, such as carcinoembryonic antigen, blood group-related antigens, 17-lA antigen, and A33 antigen have been used for diagnostic and therapeutic purposes, and in some trials promising results have been achieved [11, 21]. A33 could be an ideal target for specific immunotherapy. Unlike the mucin-type, high molecular-weight glycoconjugate antigens, A33 is not secreted or shed at detectable levels into extracellular tissue spaces or the serum of cancer patients. The findings of the successive immunolocalization and immunotherapy studies using monoclonal antibody against A33 antigen by Welt et al. [20–22], and production of humanized A33 monoclonal antibody huA33 [10], encouraged us to attempt multidisciplinary use of the antibody [1, 18] and in other gastrointestinal tract malignancies.

In the present study, A33 antigen was expressed in $>50\%$ of the gastric cancers, both diffuse and intestinal, examined. Although it is taken for granted that variability of expression within a tumor mass may curtail the diagnostic and therapeutic utility of monoclonal antibodies, $>50\%$ of intestinal-type and 38% of diffuse-type gastric cancers with uniform antigen expression could be candidates for a huA33 trial in gastric cancer.

There are several advantages in A33-positive gastric cancer with uniform expression of the antigen in the tumor tissue compared with colorectal cancer. One possibility is for its use as a preoperative and intraoperative diagnostic tool. Since normal stomach and adjacent tissues are A33 antigen negative, preoperative administration of the antibody could be an accurate and useful tool to detect qualitative lymph node metastases. Since extended lymph node dissection for gastric cancer is associated with a higher risk of postoperative complications [2, 15], the antibody could indicate the appropriate operative procedure, which would be a meaningful advance particularly in gastric cancers.

Another possibility for the use of huA33 in gastric cancer is in the prevention and treatment of peritoneal metastases. With the exception of colorectal cancer, about half of advanced or recurrent gastric cancer patients die of peritoneal metastases, especially those with tumors of the diffuse type. Unlike liver or lung metastases that can be treated by surgery and/or irradiation, no effective therapy for peritoneal metastases has yet been established. Since A33 antigen is expressed even in diffuse-type cancers, it is possible that huA33 could be exploited against this incurable peritoneal dissemination in the case of both an advanced tumor mass and minimal residual disease.

A new phase I radioimmunolocalization and dosimetry study in advanced gastric cancer has recently been initiated in Aichi Prefectural Hospital and in Gunma University in conjunction with the Ludwig Institute for Cancer Research.

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