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A clinicopathological evaluation of anti-fucosylated antigens antibody (YB-2) in colorectal carcinoma

Hiroshi Naitoh, Takashi Nakajima*, Yukio Nagamachi and Shin Yazawa†

*First Department of Surgery, *Department of Pathology and †Department of Legal Medicine, Gunma University, Gunma, Japan*

A newly generated monoclonal antibody, YB-2, reacts simultaneously with Y (Fucα1 → 2Galβ1 → 4[Fucα1 → 3]GlcNAcβ), Le^b (Fucα1 → 2Galβ1 → 3[Fucα1 → 4]GlcNAcβ) and H type 2 (Fucα1 → 2Galβ1 → 4GlcNAcβ) antigens (*Jpn J Cancer Res* 1993: 84; 641–8). Since these antigens have been reported to be expressed strongly in malignant colorectal tissues, we investigated the usefulness of this antibody as an immunochemical tool for diagnosis of colorectal cancer. The rate of positive staining with YB-2 antibody in colorectal carcinoma ($n = 101$), adenoma ($n = 26$) and normal tissues ($n = 25$) was 95.0, 50.0 and 12.0%, respectively. The specimens with negative staining were restricted in Dukes' A patients but 75% of Dukes' C patients were strongly positive. The intensity of positive staining with YB-2 antibody was also significantly related to the clinico-pathological features such as the depth of invasion, metastasis, histological types and tumor location. Moreover, the 5-year survival in patients whose tumors were positive with YB-2 antibody was found to be significantly low. Therefore, YB-2 antibody could be useful for immunodiagnosis and, possibly, immunotherapy of colorectal carcinoma.

Keywords: anti-fucosylated antigen antibody, colorectal cancer, immunohistochemical diagnosis, tumor-associated antigen

Introduction

A number of glycoconjugates with different fucosylated structures have been reported in human epithelial tissues [1]. Some of the fucosylated antigens are involved in blood group substances and some of those on cell surfaces are now considered to have important roles in biological functions including cell-to-cell interactions [2–4]. The presence of aberrant fucosylated antigens, which are absent or present at only low levels in normal tissues, has been widely observed in various tumor tissues and, moreover, the expression of such antigens has been demonstrated to be closely related to the invasion and the metastasis of tumors [5–10]. The inappropriate expression of the antigens in cancer tissues has also been reported and some antigens have been demonstrated to be cancer-associated [1, 5–10]. The accumulation of either Y, Le^b or H antigens which are commonly present in other normal tissues, but hardly present in normal colorectal tissues, has been reported in colorectal carcinomas [11–15]. In our previous study [15], a novel monoclonal antibody was developed against fucosylated antigens and was found to react with not only Y (Fucα1 → 2Galβ1 → 4[Fucα1 → 3]GlcNAcβ) but also Le^b (Fucα1 → 2Galβ1 → 3[Fucα1 → 4]GlcNAcβ) and H type 2 (Fucα1 → 2Galβ1 → 4GlcNAcβ) antigens to the same extent. Moreover, immunohistochemical studies on colorectal carcinoma showed that YB-2 monoclonal antibody could be useful for diagnosis

Address correspondence to: Shin Yazawa, Department of Legal Medicine, School of Medicine, Gunma University, 3-39-22 Showa-machi, Maebashi 371, Gunma, Japan. Tel: (+81) 272 31 7221; Fax: (+81) 272 32 3379.

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and evaluation of the progression of colorectal cancer.

The structural changes of fucosylated glycoconjugates have led to aberrant fucosylations and must be caused by the action of some fucosyltransferases [1, 16–17]. Recently, we demonstrated that the presence of aberrant $\alpha 1 \rightarrow 2$fucosyltransferase in the colorectal cancer tissues, which is responsible for new biosynthetic pathways synthesizing Y and Le^b antigens from X (Gal$\beta 1 \rightarrow 4$[Fuc$\alpha 1 \rightarrow 3$] GlcNAcβ) and Le^a (Gal$\beta 1 \rightarrow 3$[Fuc$\alpha 1 \rightarrow 4$] GlcNAcβ) antigens, respectively, and, further, for the accumulation of these antigens in colorectal tumors.

In this study, specimens from patients with colorectal carcinomas and adenomas as well as from normal tissues were examined immunohistochemically with YB-2 antibody, and the usefulness of this antibody was evaluated for the diagnosis of colorectal cancer.

Materials and methods

Clinical materials

Colorectal carcinomas ($n = 101$), adenomas ($n = 26$) and normal colorectal tissues ($n = 25$) were selected from the clinical files of the First Department of Surgery, Gunma University. Tumors were classified according to Dukes' stages and the histological typing was performed as shown in Table 2 [18]. All tissues were fixed in 10% (v/v) formalin and embedded in paraffin.

Antibody

YB-2 antibody was obtained as described previously [15]. The specificity of this antibody has been determined: it reacts with Y as well as Le^b and H type 2 antigens.

Immunostaining procedure

The avidin–biotin–peroxidase method [19] was used. The sections were placed on slides, deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol, and then rinsed in 0.01 M phosphate buffered saline, pH 7.4 (PBS). Endogenous peroxidase activity in each section was destroyed by treatment with 100% (v/v) methanol containing 0.3% (v/v) hydrogen peroxide for 30 min. The sections were preincubated in 5% (v/v) of normal goat serum (DAKO, Kyoto, Japan) for 30 min, and then they were incubated overnight with YB-2 monoclonal antibody at 4°C. After washing with PBS the sections were treated by biotinylated goat anti-mouse IgM (Vector Laboratories, Burlingame, CA) for 30 min. After washing with PBS, the sections were incubated with avidin–biotin–complex (Vecor Laboratories) for 15 min. Then they were developed for color with a solution containing diaminobendizine and 0.03% (v/v) hydrogen peroxide in 0.05 M Tris–HCl buffer, pH 7.6, and counterstained with hematoxylin and mounted. Slides were observed without knowledge of clinico-pathological information and classified into three groups based upon the intensity of staining with YB-2 antibody as follows: grade 1, negative; grade 2, positive with intratumor heterogeneity; grade 3, uniformly positive.

Statistical analysis

Survival curves were obtained according to the method of Kaplan–Meier and the differences in survival rates of each group were calculated by the Cox–Mantel test. Other data were analyzed by the chi-square test.

Results

The incidence of positive staining with YB-2 antibody in colorectal carcinoma was 95.0% (96/101), and that in adenoma and in normal tissues was 50.0% (13/26) and 12.0% (3/25), respectively ($P < 0.01$) (Table 1). Three normal specimens whose tissues stained positively were obtained from ascending colon. Five cancer specimens (two from distal colon and three from rectum) with Dukes' A staging showed negative staining. There was no correlation between the immunohistochemical grades of staining with YB-2 antibody and their ages and sexes (data not shown). On the other hand, significant correlations were found in specimens from grades 2 and 3 between the grades of staining and the clinico-pathological features, such as the tumor location, depth of tumor invasion, histological types, Dukes' stages, metastasis either to lymph node or liver (Table 2).

The overall 5-year survival rates were investigated according to the grade of immunostaining with YB-2 antibody, and estimated to be 100% in grade 1, 82% in grade 2 and 41% in grade 3 (Figure 1). The difference between grades 2 and 3 was significant ($P < 0.01$); in particular, in patients with Dukes' B staging ($P < 0.05$), but not in patients with Dukes' stage C (Figure 2). Survival rates of grade 3 were significantly lower than those of grade 2 in both colon ($P < 0.01$) and rectum

Table 1. The incidence of staining with YB-2 antibody in neoplastic and normal colorectal tissues

YB-2 antigen expression	No. of cases (%)		*P* value
	Positive	Negative	
Normal ($n = 25$)	3 (12.0)	22 (88.0)	*
Adenoma ($n = 26$)	13 (50.0)	13 (50.0)	*
Cancer ($n = 101$)	96 (95.0)	5 (5.0)	*

*$P < 0.01$.

Table 2. Relationship between grades of staining with YB-2 antibody and clinico-pathological features

	No.	No. of cases (%)			*P* value[a]
		Grade 1	Grade 2	Grade 3	
Tumor location					
colon	48	2 (4.2)	21 (43.8)	25 (52.0)	**
rectum	53	3 (5.7)	10 (18.9)	40 (75.4)	**
Depth of invasion					
to sm	26	5 (19.2)	14 (53.9)	7 (26.9)	*
deeper than pm	75	0 (0.0)	17 (22.7)	58 (77.3)	*
Histological type					
well[b]	46	3 (6.5)	20 (43.5)	23 (50.0)	**
moderately[b]	48	2 (4.2)	10 (20.8)	36 (75.0)	**
others	7	0 (0.0)	1 (14.3)	6 (85.7)	
Dukes' stage					
A	26	5 (19.2)	14 (53.8)	7 (27.0)	*
B	31	0 (0.0)	9 (29.0)	22 (71.0)	*
C	44	0 (0.0)	8 (18.2)	36 (81.8)	*
Lymph node metastasis					
−	57	5 (8.7)	23 (40.4)	29 (50.9)	*
+	44	0 (0.0)	8 (18.2)	36 (81.8)	*
Liver metastasis					
−	83	5 (6.0)	30 (36.2)	48 (57.8)	*
+	18	0 (0.0)	1 (5.6)	17 (94.4)	*

*$P < 0.01$, **$P < 0.02$.
[a] *P* values were calculated between grade 2 and 3.
[b] Differentiated adenocarcinoma.

($P < 0.01$) (Figure 3), and in both well ($P < 0.01$) and moderately differentiated adenocarcinoma ($P < 0.01$) (Figure 4).

Discussion

Many well-defined monoclonal antibodies raised against glycoconjugates are now available and have been determined to show restricted specificities with glycoconjugates in glycosylated antigens [1]. Changes of the expression of glycoconjugates have been described in a variety of cancers [20–21] and the use of highly specific monoclonal antibodies, which detect such changes, has an advantage in demonstrating cancer-associated antigens for immunodiagnosis or immunotherapy. Accumulation and inappropriate expression of fucosylated antigens in a variety of human cancers have been regarded as a cancer-associated phenomenon, and levels of some fucosylated antigens have been reported to be elevated in sera and tissues from cancer patients [2–4]. In colorectal carcinoma tissues, the expression of blood group substances and related antigens has been reported to be different from that in normal colon, *i.e.* the expression of

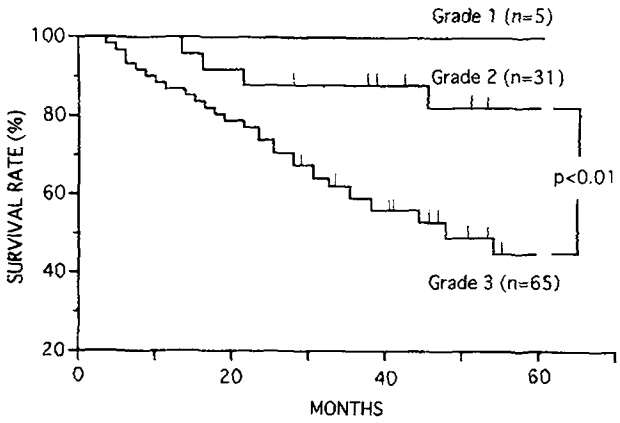
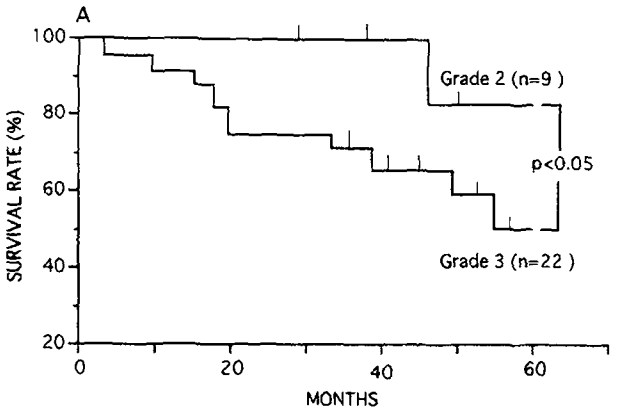


Figure 1. Survival curves of patients with colorectal cancer according to the grades of immunohistochemical staining with YB-2 antibody.



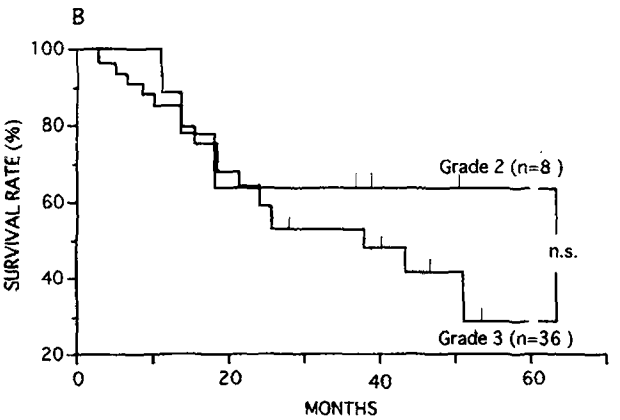
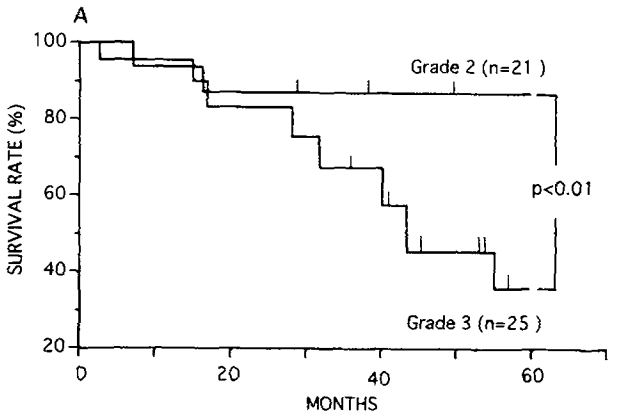


Figure 2. Survival curves of patients with colorectal cancer according to the grades and Dukes' stage. A, Dukes' stage B; B, Dukes' stage C.

ABH and Le^b antigens is almost lost from normal distal colonic tissue, but is preserved in proximal tissue, while Le^a antigen appears to be more uniformly distributed throughout the proximal and



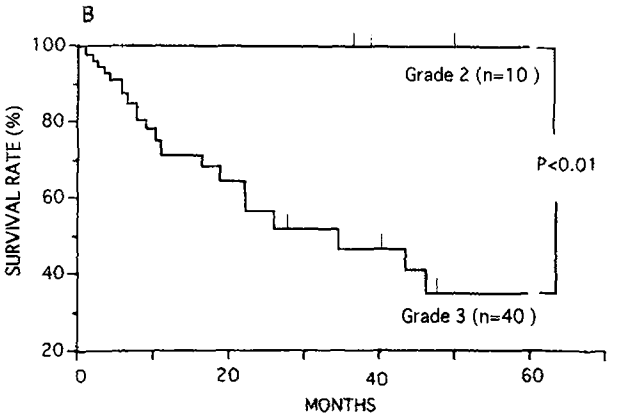


Figure 3. Survival curves of patients with colorectal cancer according to the grades and tumor location. Colon (A) and rectum (B) cancer patients.

distal colon. The ABH and Le^b antigens are re-expressed in colorectal tumors, and, in particular, accumulation of Y, Le^b and H antigens has been shown in colon cancer [11–15]. In our previous study [15], it was demonstrated that YB-2 monoclonal antibody, reacting simultaneously with Y, Le^b and H type 2 antigens, could be used for the diagnosis of colorectal cancer. More recently [17], we also found the presence of aberrant $\alpha 1 \rightarrow$ 2fucosyltransferase, responsible for the synthesis of the aforementioned fucosylated antigens, in colorectal tumors. In this study, we have demonstrated from the immunohistochemical results of a large number of sections that YB-2 monoclonal antibody reacts very sensitively (95%) and specifically (88%) toward colorectal cancer, and, moreover, that the incidence of positive staining with YB-2 antibody was related to the histological types, tumor location and the occurrence of metastasis either to lymph node or to liver. It was of particular interest that overall 5-year survival rates were clearly associated with the grade of immunostain-

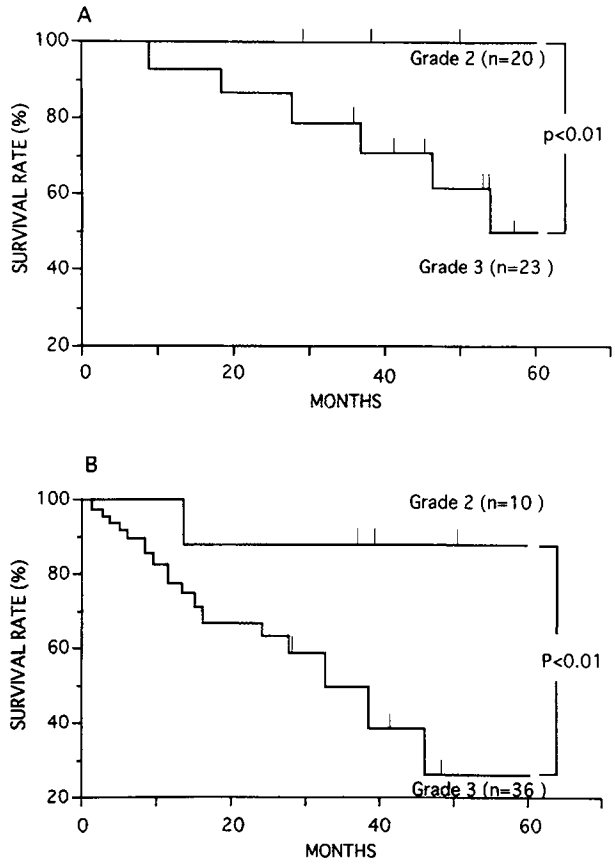


Figure 4. Survival curves of patients with colorectal cancer according to the grades and histological types. Well (A) and moderately (B) differentiated adenocarcinoma.

ing with YB-2 antibody. Therefore, it is clear that immunohistochemical studies with YB-2 antibody are potentially useful in predicting the prognosis of colorectal cancer and are an independent factor of clinicopathological value in estimating malignant potential.

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