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Original

Expression of HER2 and MUC1 in Advanced Colorectal Cancer : Frequency and Clinicopathological Characteristics

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Abstract : There have been many reports on the overexpression of human epidermal growth factor receptor 2 (HER2) in patients with colon cancer. However, the role and frequency of HER2 overexpression have not been clearly defined. Anti-HER2 therapy has been shown to improve the prognosis of HER2-positive patients with breast and stomach cancers. In this study, we explored HER2 expression in patients with colon cancer at stages II and III by immunohistochemistry (IHC) and dual-color *in situ* hybridization (DISH), and examined the correlation between HER2 expression and clinicopathological factors. Moreover, we examined the correlation between HER2 expression and mucin 1 (MUC1) expression. The subjects were 121 patients with colon cancer at stages II and III who underwent surgery in our hospital during the period from 2007 to 2009. Sections containing the deepest part of a lesion were subjected to immunostaining for HER2 and MUC1. HER2 expression was assessed in accordance with Ventana's Guidelines for HER2 Testing in Stomach Cancer, with sections comprising less than 10% of weakly to moderately stained tumor cells scored as 1 ＞ 2. HER2 expression scored as 2 was defined with sections comprising more than 10% of the weakly to moderately stained tumor cells. Patients with a score of 1＞2 and 2 were also subjected to DISH using a Dual ISH HER2 kit. MUC1 expression was scored according to the percentage of stained area as follows : 0, 0 to 5% ; 1, 5 to 50% ; and 2, 50% and higher. Patients with a score of 1 and 2 were defined as MUC1-positive. The analysis of HER2 by IHC yielded the following scores : 45 patients (37.2%), 0 ; 38 patients (31.4%), 1 ; 14 patients (11.6%); 1＞2 ; 24 patients (19.8%), 2 ; and 0 patients (0%), 3. For the 38 patients with a score of 1＞2 and 2, DISH returned ratios of HER2 to Chr17 expression (HER2 : Chr17 ratio) from 1.13 to 1.93 (mean = 1.46). There was no significant correlation between HER2 expression and clinicopathological factors. The numbers of MUC1-positive patients according to HER2 score were as follows : 22 patients (48.9%) in the score 0 group (45 patients); 25 patients (65.8%) in the score 1 group (38 patients); 10 patients (71.4%) in the score 1＞2 group (14 patients), and 22 patients (91.7%) in the score 2 group (24 patients). There was a positive correlation between HER2 expression and MUC1 expression. Specifically, MUC1 expression levels increased with HER2 expression level, and the percentage of MUC1-positive patients was significantly higher in

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the HER2 score 2 group than in the HER2 score 0 group ($P < 0.01$). Rates of HER2 positivity by DISH or fluorescence *in situ* hybridization (FISH) in patients who had an HER2 score of 2+ by IHC were 45% and 24% in the patients with stomach and breast cancers, respectively. However, the positivity rate was 0% in the patients with colon cancer in this study. This result indicates that patients with colon cancer who have an IHC HER2 score of 2+ are more likely to be HER2 negative by DISH than patients with breast and stomach cancers, although larger cohort studies are required before a definitive conclusion can be made. There was a positive correlation between HER2 expression and MUC1 expression in this study, although further examination is required because there were no patients who had an HER2 score of 3+ or 2+ by IHC and were HER2 positive by DISH in this study. HER2 expression in colon cancer should be cautiously assessed by both IHC and DISH.

Key words : Advanced colon cancer, HER2, MUC1

Introduction

The human epidermal growth factor receptor 2 (HER2) gene (also called *HER2/neu, c-erbB-2*) was identified as an oncogene that is similar in structure to the human epidermal growth factor receptor (EGFR) gene[1]. The HER2 gene encodes a transmembrane glycoprotein receptor that is activated by the phosphorylation of tyrosine residues and plays a role in cell proliferation via a p21/ras signaling pathway[1].

Breast cancer patients with HER2 gene amplification or HER2 protein overexpression have a poor prognosis[2,3], and anti-HER2 therapy is currently indicated for breast, stomach, and gastroesophageal junction cancers[4-6]. Although there have been many reports on the frequency of HER2 overexpression in colon cancer[7,8], the role of this oncogene in such cancers is not clearly defined.

In this study, we examined HER2 expression in patients with stage II and III colon cancer by immunohistochemistry (IHC) and dual color *in situ* hybridization (DISH). By a colorectal cancer treatment guideline (2010), a postoperative adjuvant chemotherapy is recommended for stage III, and when stage II has the high possibility of a recurrence, a postoperative adjuvant chemotherapy is considered. We also examined the correlation between HER2 expression and clinicopathological factors in these patients.

It has also been reported that mucin 1 (MUC1) is highly expressed in severely atypical adenomas and cancers of the colon, whereas it is scarcely expressed in the normal colon epithelium and mildly atypical adenomas[9]. MUC1 expression is related to the extent and metastasis of cancers[10], and indeed, combination therapy with a MUC1 inhibitor has been reported to reduce trastuzumab (herceptin) resistance in breast cancer cells[11]. Based on this, we finally examined the correlation between MUC1 expression and HER2 expression in our study patients with colon cancer.

Table 1 Circumferential rate is transverse diameter of the intestine ÷ maximum transverse diameter of the tumor There was no significant correlation between HER2 expression and clinicopathological factors, and HER2 expression seemed less useful as a predictive factor of prognosis.

Table 1-① HER2 expression by Tumor characteristic

		HER2 0(45)	HER2 1(38)	HER2 1＞2(14)	HER2 2(24)
Mean age		68.7(39～94)	68.8(40～88)	67.3(36～83)	69.8(40～87)
Gender	Male	28 (62.2%)	23 (60.5%)	7 (50%)	13 (54.2%)
	Female	17 (37.8%)	15 (39.5%)	7 (50%)	11 (45.8%)
Macroscopic type	type1	3 (6.7%)	0	2 (14.3%)	0
	type2	29 (64.4%)	29 (76.3%)	10 (71.4%)	20 (83.3%)
	type3	11 (26.7%)	9 (23.7%)	2 (14.3%)	4 (16.7%)
	type4	1 (2.2%)	0	0	0
	0－IIa＋IIc	1 (2.2%)	0	0	0
Histological type	tub1	15 (33.3%)	13 (34.2%)	5 (35.7%)	7 (29.2%)
	tub2	24 (53.3%)	25 (65.8%)	9 (64.3%)	14 (58.3%)
	muc	1 (4.4%)	0	0	1 (4.2%)
	pap	1 (2.2%)	0	0	1 (4.2%)
	por	4 (8.9%)	0	0	1 (4.2%)
Circumferential rate		74.6%(13～100)	72%(30～100)	63%(21～100)	67.8%(21～100)
Maximum diameter of tumor		54.1 mm(10～100)	53.3 mm(20～90)	47.9 mm(15～80)	52.4 mm(17～90)

Table 1-② HER2 expression by Tumor characteristic

		HER2 0(45)	HER2 1(38)	HER2 1＞2(14)	HER2 2(24)
ly	0	12(26.7%)	13(34.2%)	4(28.6%)	7(29.2%)
	1	19(42.2%)	19(50%)	8(57.1%)	13(54.2%)
	2	10(22.2%)	6(15.8%)	2(14.3%)	4(16.7%)
	3	4(8.9%)	0	0	0
v	0	6(13.3%)	7(18.4%)	1(7.1%)	5(20.8%)
	1	23(19%)	20(52.6%)	8(57.1%)	10(41.7%)
	2	15(33%)	10(26.3%)	3(21.4%)	9(37.5%)
	3	1(2.2%)	1(2.6%)	2(14.3%)	0
N	0	25(55.6%)	25(65.8%)	8(57.1%)	18(75%)
	1	14(31.1%)	10(26.3%)	5(35.7%)	5(20.8%)
	2	4(8.9%)	3(7.9%)	1(7.1%)	1(4.2%)
	3	1(2.2%)	0	0	0

Materials and Methods

Subjects

The subjects were 121 patients with stages II and III primary colon cancer who underwent intestinal resection and lymph node dissection in our hospital during the period from 2007 to 2009. Of these, 71 patients (58.7%) were male and 50 patients (41.3%) were female, with a mean age of 68.8 years (range : 36～94). Of the 121 patients, 76 patients were diagnosed with stage II cancer, 44 patients with stage III, and one patient underwent intestinal resection only with

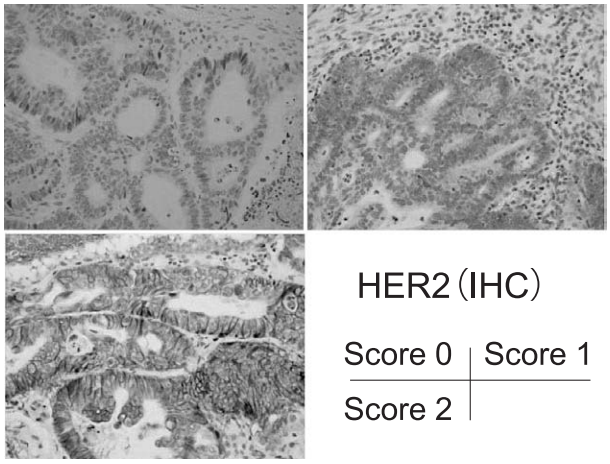

Fig. 1. Immunohistochemical staining for HER-2/neu (H & E, ×100).
Score 0: The sections containing less than 10% of weakly stained tumor cells
Score 1: The sections containing more than 10% of weakly stained tumor cells
Score 2: The sections containing more than 10% of weakly to moderately stained tumor cells

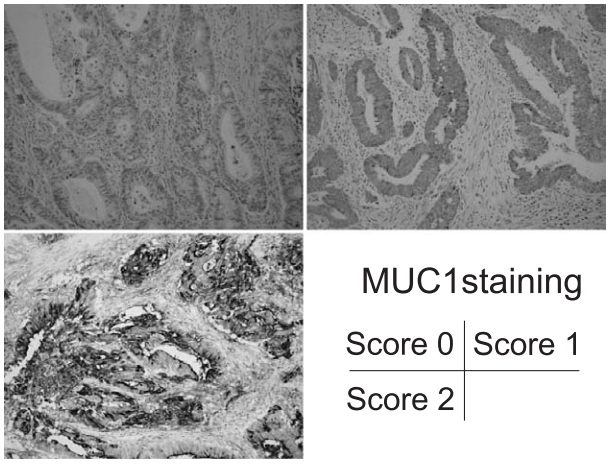

Fig. 2. Immunohistochemical staining for MUC1 (H & E, ×100).
MUC1 expression was scored according to the percentage of stained area as follows: 0, 0 to 5%; 1, 5 to 50%; and 2, 50% and higher. The patients with a score of 1 and 2 were defined as being MUC1-positive.

no need for lymph node dissection. Table 1 summarizes the patient background characteristics.

Immunostaining

For each patient sample, we selected the tissue section representing the deepest part of the lesion for HER2 and MUC1 immunostaining (Fig. 1, 2). HER2 expression was assessed in accordance with the Guidelines for HER2 Testing in Stomach Cancer[12]. The sections containing less than 10% of weakly to moderately stained tumor cells were scored $1 > 2$ in this study. Patients with a score of $1 > 2$ and 2 were also subjected to DISH using Ventana's Dual ISH HER2 kit.

MUC1 expression was scored according to the percentage of stained area as follows: 0, 0 to 5%; 1, 5 to 50%; and 2, 50% and higher, with MUC1 positivity defined by a score of 1 and 2[13].

Results

The IHC analysis of HER2 expression produced the following scores: 45 patients (37.2%) had

Table 2 Details of HER2 and MUC1 immunostaining

	Clone	Source of the primary antibody	Dilution	Antigen ritrieval	Detction
HER2	4B5	VENTANA	prediluted	CC1	DAB
MUC1	Ma695	VENTANA	1:100	CC1	DAB

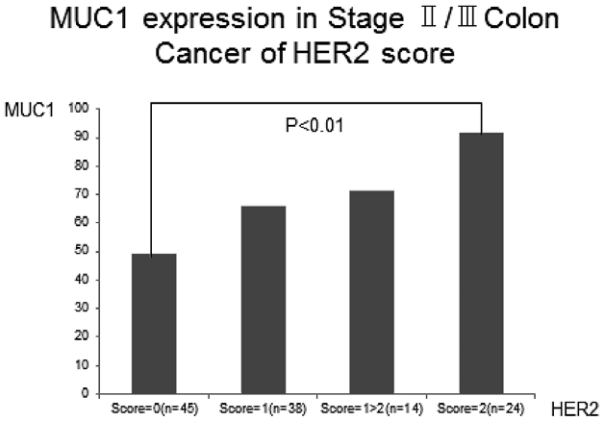

Fig. 3. The percentage of MUC1-positive patients was significantly higher in the HER2 score 2 group than in the HER2 score 0 group ($P < 0.01$). There was a positive correlation between HER2 expression and MUC1 expression, whereby the MUC1 expression level increased with HER2 expression level.

a score of 0; 38 patients (31.4%), 1; 14 patients (11.6%), 1 > 2; 24 patients (19.8%), 2; and 0 patients (0%), 3. We considered 38 patients (31.4%) including those with a score of 1 > 2 or 2 as borderline positive for HER2 expression, while the 83 patients (68.6%) with a score of 0 or 1 were considered HER2 negative.

The 38 patients with a score of 1 > 2 or 2 were subjected to DISH. The ratios of the expression level of HER2 to that of Chr17 (HER2 : Chr17 ratio) ranged from 1.13 to 1.93, with an average value of 1.46. In the cases of breast and stomach cancers, HER2 gene amplification is defined by a HER2 : Chr17 ratio of 2.2 or higher[12, 14]. According to this definition, none of the patients showed HER2 gene amplification in this study.

Table 2 shows the clinicopathological factors of the patients classified into HER2 score groups. There was no significant correlation between HER2 expression and patient age or gender; the macroscopic type, histological type, circumferential rate, and maximum diameter of the tumor; or the presence of lymphatic invasion, venous invasion, and lymph node metastasis.

MUC1-positive patients were then classified into HER2 score groups as follows: 22 patients (48.9%) in the score 0 group (45 patients); 25 patients (65.8%) in the score 1 group (38 patients); 10 patients (71.4%) in the score 1 > 2 group (14 patients); and 22 patients (91.7%) in the score 2 group (24 patients). There was a positive correlation between HER2 expression

and MUC1 expression, indicating that MUC1 expression level increased with HER2 expression level, and the percentage of MUC1-positive patients was significantly higher in the HER2 score 2 group than in the HER2 score 0 group ($P < 0.01$).

Discussion

HER2 protein overexpression detected by IHC was reported in 15～25% of patients with breast cancer[2,3] and in 8～31% of patients with stomach cancer[15], while HER2 gene amplification and HER2 protein overexpression have been linked to a poor prognosis. Moreover, a positive correlation was found between FISH results and IHC scores in patients with breast and stomach cancers, whereby the HER2 positivity rate by FISH increases with IHC score. In patients with colon cancer, the levels of HER2 protein overexpression by IHC in previous reports range from 0 to 83%[7,8,16–19]; however, studies published during the last five years, from 2008 onwards, report a HER2 protein expression level of approximately 10%[20,21].

In this study, no patient was found to be HER2 positive by both DISH and IHC. Because the number of HER2-positive patients with colon cancer was smaller than those with breast and stomach cancers, we were unable to correlate the HER2 expressions by IHC and DISH in this study. The results on HER2 expression in colon cancer show inconsistencies among the previous reports, and this could reflect the varied mechanisms regulating HER2 gene expression. Moreover, the interpretation of immunostaining results for colon cancer specimens may vary because there are no standardized criteria such as those prescribed for stomach and breast cancers[22,23].

There was no significant difference in clinicopathological factors among the patients in each HER2 score group. These findings indicate that patients with colon cancer are more likely to express HER2 than those with breast or stomach cancers and that it is difficult to correlate HER2 expression with clinicopathological factors or to predict the clinicopathological prognosis on the basis of HER2 expression.

HER2-positivity rates using FISH for patients with a borderline HER2 score of 2+ by IHC have been reported to be approximately 24%[24] and 45%[4] in the patients with breast and stomach cancers, respectively. Based on the present results, the patients with breast and stomach cancers who had a HER2 score of 2+ by IHC should be re-examined by FISH. In this study, none of the 24 patients with colon cancer who had an HER2 score of 2+ by IHC were HER2 positive by DISH, indicating that, unlike for patients with breast and stomach cancers, patients with colon cancer and with a HER2 score of 2+ by IHC are likely to be HER2 negative by DISH. Studies involving larger patient numbers are now required to validate this hypothesis.

MUC1 is normally expressed on the luminal surface of many epithelial cells and also in some hematopoietic cells and activated T cells. MUC1 is also called polymorphic epithelial mucin (PEM), episialin, CD227 antigen, DF3 antigen, and epithelial membrane antigen (EMA), and the expression of this protein has been related to cancer extent and metastatic potential[10]. In this study, 79 patients (65.3%) were MUC1-positive, which agreed with previous reports[9,10], and the percentage of MUC1-positive patients was significantly higher in the HER2 score 2 group than in the HER2 score 0 group ($P < 0.01$). Thus, there was a positive correlation between

HER2 expression and MUC1 expression. This is a very interesting finding, although further examination of a larger number of patients is required in the future because there was no patient who had a HER2 score of 3+ or 2+ by IHC and was HER2 positive by DISH in this study.

We examined HER2 expression in 121 patients with stage II or III colon cancer by IHC and DISH. We found that HER2 expression in patients with colon cancer was weak and infrequent, and there was no significant correlation between HER2 expression and clinicopathological factors. Thus, HER2 expression seems less useful as a predictive factor of prognosis in such cancer patients.

The patients with colon cancer in this study who had an HER2 score of 2+ by IHC were likely to be HER2 negative by DISH, although the accumulation of more cases is required to draw definitive conclusions. Nevertheless, the results suggest that HER2 expression should be assessed and determined carefully by both IHC and DISH.

There was also a positive correlation between HER2 expression and MUC1 expression, although further examination will be required because there was no patient who had a HER2 score of 3+ or 2+ in IHC and were HER2-positive in DISH in this study.

Conflict of interest

The authors have declared no conflict of interest.

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