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Clinicopathological significance of the gene expression of *matrix metalloproteinase-7*, *insulin-like growth factor-1*, *insulin-like growth factor-2* and *insulin-like growth factor-1 receptor* in patients with colorectal cancer: *Insulin-like growth factor-1 receptor* gene expression is a useful predictor of liver metastasis from colorectal cancer

TAKASHI OSHIMA[1], MAKOTO AKAIKE[2], KAZUE YOSHIHARA[1], MANABU SHIOZAWA[2], NAOTO YAMAMOTO[1], TSUTOMU SATO[1], ROPPEI YAMADA[1], SHOICH FUJII[1], YASUSHI RINO[3], CHIKARA KUNISAKI[1], KATSUAKI TANAKA[1], MUNETAKA MASUDA[3] and TOSHIO IMADA[4]

[1]Yokohama City University Medical Center, Gastroenterological Center; [2]Department of Surgery, Kanagawa Cancer Center; [3]Department of First Surgery, Yokohama City University; [4]Yokohama City University, Yokohama, Japan

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Abstract. Matrix metalloproteinase-7 (MMP-7), secreted by cancer cells, has been implicated classically in the basement membrane destruction associated with tumor cell invasion and metastasis. Epidemiological studies have established a correlation between high levels of circulating insulin-like growth factor-1 (IGF-1) and the relative risk of colorectal cancer, which is known to produce MMP-7. We examined the clinicopathological significance of the relative expression of *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1 receptor* genes in patients with colorectal cancer, especially with regard to metastasis. We studied surgical specimens of cancer tissue and adjacent normal mucosa obtained from 205 patients with untreated colorectal carcinoma. *MMP-7*, *IGF-1*, *IGF-2*, *IGF-1R* and ß-actin mRNA in cancer tissue and adjacent normal mucosa were measured by quantitative real-time reverse-transcriptase polymerase chain reaction. *MMP-7* and *IGF-1R* gene expression levels were higher in cancer tissue than in adjacent normal mucosa. In contrast, *IGF-1* gene expression was lower in cancer tissue than in adjacent normal mucosa. As for the relationship of gene expression to clinicopathological factors, *IGF-1R* expression correlated with venous invasion and liver metastasis. *IGF-1R* gene expression is thus considered a useful predictor of liver metastasis from colorectal cancer.

Correspondence to: Dr Takashi Oshima, Yokohama City University Medical Center, Gastroenterological Center, 4-57 Urafune-cho, Minami-ku, Yokohama-shi, Kanagawa-ken 232-0024, Japan
E-mail: ohshimatakashi@yahoo.co.jp

Key words: matrix metalloproteinase-7, insulin-like growth factor-1, insulin-like growth factor-2, insulin-like growth factor-1 receptor, colorectal cancer

Introduction

Colorectal cancer, one of the most prevalent cancers worldwide (1), is the second leading cause of cancer-related mortality in developed countries (2). Tumor cell invasion and metastasis involve multiple steps, including proteolytic degradation of the basement membrane (BM) and extracellular matrix (ECM), altered cell adhesion and the physical movement of tumor cells. Among the many steps of tumor invasion and metastasis, the excessive degradation of matrix is one of the hallmarks (3).

Matrix metalloproteinases (MMPs) are a key family of proteolytic enzymes involved in extracellular matrix degradation. In colorectal cancer, several MMPs have been found to be associated with tumor stage, outcomes, or both (4). MMP-7 is a member of the MMP family and, when activated, displays broad proteolytic activity against a variety of extracellular matrix substrates, including collagens, proteoglycans, elastin, laminin, fibronectin and casein (5-7). Unlike MMPs, which are synthesized by stromal cells, MMP-7 is produced exclusively by cancer cells. Miyamoto *et al* (8) reported that MMP-7, produced by cancer cells, regulates the bioavailability of insulin-like growth factors (IGFs) in the surrounding tissue.

IGFs have been studied extensively for possible roles in cancer growth (9-12). They are expressed ubiquitously and act as endocrine, paracrine and autocrine growth factors. Insulin-like growth factor-1 (IGF-1) is associated with an increased risk of cancer (13). Functionally, IGF-1 not only stimulates cell proliferation, but also inhibits apoptosis. The combination of these mitogenic and antiapoptotic effects is

now recognized to have a profound impact on tumor growth (14). Previous studies have reported that IGF-2 is related to tumor progression and patient survival and that it has been suggested that IGF-2 acts as an autocrine growth factor in colorectal carcinoma (15). Insulin-like growth factor-1 receptor (IGF-1R) is the receptor of IGF-1 and IGF-2. IGF-1R overexpression promotes tumor growth, progression, invasion and metastasis (16).

In this study, we examined the clinicopathological significance of the relative expression of the *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1 receptor* genes in patients with colorectal cancer, especially with regard to metastasis.

Materials and methods

Patients and samples. We studied surgical specimens of cancer tissue and adjacent normal mucosa obtained from 205 patients with untreated colorectal carcinoma. The patients underwent surgery at the Yokohama City Medical Center, Gastroenterological Center and Kanagawa Cancer Center from 2002 through to 2006. Informed consent was obtained from each patient and the Yokohama City Medical Center Committee and Kanagawa Cancer Center Committee approved the study. Each tissue sample was embedded in an O.C.T. compound (Sakura Finetechnical Co., Ltd., Tokyo) and stored at -80˚C immediately before use. No patient had any other malignancy. After examining the histopathological features of specimens stained with hematoxylin and eosin, sections consisting of >80% of carcinoma cells were used to prepare total RNA.

Quantitative real-time reverse-transcriptase polymerase chain reaction (PCR). Total RNA from colorectal cancer tissue and adjacent normal mucosa was prepared with the use of Trizol (Gibco, Life Tech, Gaithersburg, MD). cDNA was synthesized from 2 μg of total RNA with the use of an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). After synthesis, the cDNA was diluted at 1:4 with water and stored at -20˚C until use. Quantitative real-time PCR was performed with iQ SYBR-Green supermix (Bio-Rad Laboratories). PCR reactions were carried out in a total volume of 15 μl, containing cDNA derived from 75 ng of RNA, 0.27 μM of each primer, 7.5 μl of iQ SYBR-Green supermix containing dATP, dCTP, dGTP and dTTP at concentrations of 400 μM each and 50 U/ml of iTag DNA polymerase. The PCR consisted of 10 min at 94˚C followed by 50 cycles of denaturation of the cDNA for 30 sec at 94˚C, annealing for 30 sec at an appropriate temperature according to Table I and a primer extension for 1 min at 72˚C, followed by 10 min at 72˚C. The PCR primer sequences of MMP-7, IGF-1, IGF-2, IGF-1R and ß-actin, used as an internal control, are shown in Table I.

Statistical analysis. Associations of the gene expression levels of colorectal cancer with those of adjacent normal mucosa were evaluated by the Wilcoxon test. The relationship of gene expression levels to potential explanatory variables, including age, gender, tumor size, histological type, depth of invasion, lymph node metastasis, tumor location, lymphatic invasion, venous invasion and liver metastasis, were assessed with the χ^2 test. Associations among variables were evaluated with the Mann-Whitney U test. Correlation coefficients between different variables were determined by a simple regression analysis. Statistical analyses were performed using Statview J 5.0 software (Abacus, CA). Two-sided P-values were calculated and P-values of <0.05 were considered to indicate a statistical significance.

Results

Comparison of MMP-7, IGF-1, IGF-2 and IGF-1R mRNA expression between colorectal cancer tissue and adjacent normal mucosa. *MMP-7* and *IGF-1R* gene expression levels were higher in cancer tissue than in adjacent normal mucosa (P<0.001, P<0.001; Fig. 1A and D). In contrast, *IGF-1* gene expression was lower in cancer tissue than in adjacent normal mucosa (P<0.001; Fig. 1B). There was no significant difference between *IGF-2* gene expression in cancer tissue and that in adjacent normal mucosa (P=0.546; Fig. 1C).

Relationship of clinicopathological features to MMP-7, IGF-1, IGF-2 and IGF-1R gene expression levels. After categorizing the expression levels of *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* genes as low or high according to their respective median values, we examined the relationship between the expression levels of each gene and clinicopathological features. *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* gene expression levels were unrelated to age, tumor size, histological type, lymph node metastasis, tumor location and lymphatic invasion. *IGF-1R* gene expression levels were significantly related to venous invasion (P=0.027). *IGF-1R* gene expression was significantly related to liver metastasis (P=0.033) (Table II).

Comparison of MMP-7, IGF-1, IGF-2 and IGF-1R gene expression levels between the presence and absence of venous invasion. *IGF-1R* gene expression levels differed significantly between the presence and absence of venous invasion (P=0.048) (Fig. 2).

Correlation among MMP-7, IGF-1, IGF-2 and IGF-1R expression. The results of the correlation analysis are shown in Fig. 3. No significant correlations were observed among the expression of these genes.

Discussion

Unlike other MMPs, which are produced by stromal cells, MMP-7 is produced by cancer cells and is implicated in the basement membrane destruction associated with cancer cell invasion and metastasis (17). IGF-1, IGF-2 and their receptor IGF-1R, participate in the development and progression of cancer (18-20). Previous studies have reported that MMP-7 produced by cancer cells regulates the bioavailability of IGFs in surrounding tissue (8).

In the present study, we examined *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* mRNA expression in colorectal cancer tissue and adjacent normal mucosa. We studied the relationship of these gene expression levels to clinicopathological features, as well as correlations among the expression of these genes.

Table I. PCR primers and conditions.

Gene	Primer	Temperature (°C)	Product size (bp)
MMP-7	5'-CACTGTTCCTCCACTCCATTTAG-3' 5'-CATTTATTGACATCTACCCACTGC-3'	62.6	151
IGF-1	5'-GTGGATGAGTGCTGCTTC-3' 5'-ACTTCCTTCTGGGTCTTGG-3'	58	134
IGF-2	5'-TACCGCCATCTCCCTTCTC-3' 5'-TCCCTCTGACTGCTCTGTG-3'	60	122
IGF-1R	5'-TGCCTTGGTCTCCTTGTC-3' 5'-TTTCCCTGCTTTGATGGTC-3'	58	154
β-actin	5'-AGTTGCGTTACACCCTTTCTTGAC-3' 5'-GCTCGCTCCAACCGACTGC-3'	60	171

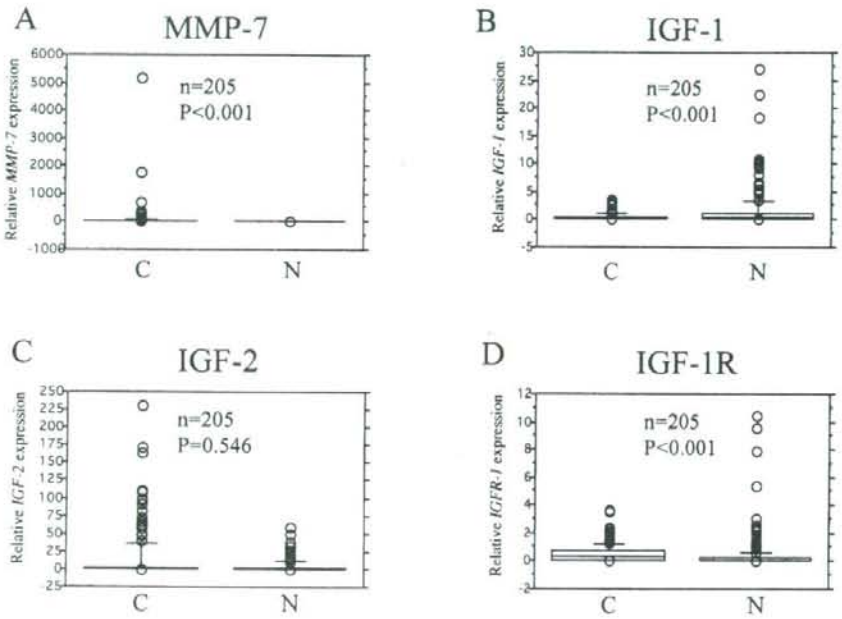

Figure 1. Comparison of *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* mRNA expression levels between colorectal cancer tissue and adjacent normal mucosa. *MMP-7* and *IGF-1* gene expression levels were higher in cancer tissue than in adjacent normal mucosa (P<0.001, P<0.001). In contrast, *IGF-1* gene expression levels were lower in cancer tissue than in adjacent normal mucosa (P<0.001). *IGF-2* gene expression did not differ significantly between cancer tissue and adjacent normal mucosa.

Several previous studies have compared *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* mRNA expression levels between colorectal cancer tissue and adjacent normal mucosa. Miyata *et al* (17) reported that the expression of MMP-7 in tumor cells was significantly higher than that in normal cells. Freier *et al* (21) found that IGF-1R gene expression was higher in colorectal cancer than in adjacent normal mucosa. Nosho *et al* (22) showed that IGF-IR mRNA expression was detected ~40% of colorectal tissues, though was undetectable in adjacent nontumor tissue. IGF-1 gene expression in colorectal cancer was reported to be higher than that in adjacent normal mucosa (21). Li *et al* (23) reported that the expression level of the IGF-2 gene was significantly increased in colorectal cancer as compared with that in adjacent normal mucosa. In our study, *MMP-7* and *IGF-1R* gene expression levels were higher in cancer tissue than in adjacent normal mucosa. Conversely, *IGF-1* gene expression was lower in cancer tissue than in adjacent normal mucosa. *IGF-2* gene expression did not differ significantly between cancer tissue and adjacent normal mucosa.

In a study of the relationship of clinicopathological features to gene expression levels, Nosho *et al* (22) found that *MMP-7* gene expression correlates with tumor size, location and histopathology in early colorectal carcinoma. Miyata *et al* (17) reported that MMP-7 expression in cancer cells correlates with an advanced pathological tumor stage. In our study, *MMP-7*

Table II. Relationship between the expression of MMP-7, IGF-1, IGF-2, or IGF-1R genes and clinicopathological features.

Variables/categories	MMP-7 expression		P-value	IGF-1 expression		P-value	IGF-2 expression		P-value	IGF-1R expression		P-value
	low (n=102)	high (n=103)		low (n=102)	high (n=103)		low (n=102)	high (n=103)		low (n=102)	high (n=103)	
Age	66.8±10.6	64.8±10.9	0.187	66.0±11.1	65.7±10.5	0.837	66.4±10.4	65.2±11.2	0.387	65.3±11.1	66.3±10.5	0.484
Gender												
Male	58	62	0.628	53	59	0.318	58	62	0.628	51	61	0.185
Female	44	41		50	42		44	41		51	42	
Size												
≤5 cm	56	59	0.731	60	55	0.434	64	51	0.056	61	54	0.287
>5 cm	46	44		42	48		38	52		41	49	
Histological type												
Well differentiated	32	29	0.700	31	30	0.926	31	30	0.864	29	32	0.457
Moderately differentiated	58	58		58	58		56	60		56	60	
Poorly differentiated	12	16		13	15		15	13		17	11	
Depth of invasion												
T1	7	9	0.888	11	8	0.837	10	9	0.178	11	8	0.559
T2	49	48		47	47		54	40		42	52	
T3	41	39		39	41		33	47		42	38	
T4	5	7		5	7		5	7		7	5	
Lymph node metastasis												
Absent	45	50	0.525	50	45	0.444	50	45	0.444	45	50	0.485
Present	57	53		52	58		52	58		58	53	
Location												
Colon	58	54	0.524	61	51	0.139	60	52	0.231	56	56	0.939
Rectum	44	49		41	52		42	51		46	47	
Lymphatic invasion												
Absent	67	67	0.924	64	70	0.824	67	67	0.924	63	71	0.281
Present	35	36		38	39		35	36		39	32	
Venous invasion												
Absent	38	39	0.928	43	34	0.176	45	32	0.054	46	31	0.027
Present	64	64		59	69		57	71		56	72	
Liver metastasis												
Absent	69	70	0.962	70	69	0.802	71	68	0.582	79	60	0.033
Present	33	33		32	34		31	35		23	43	

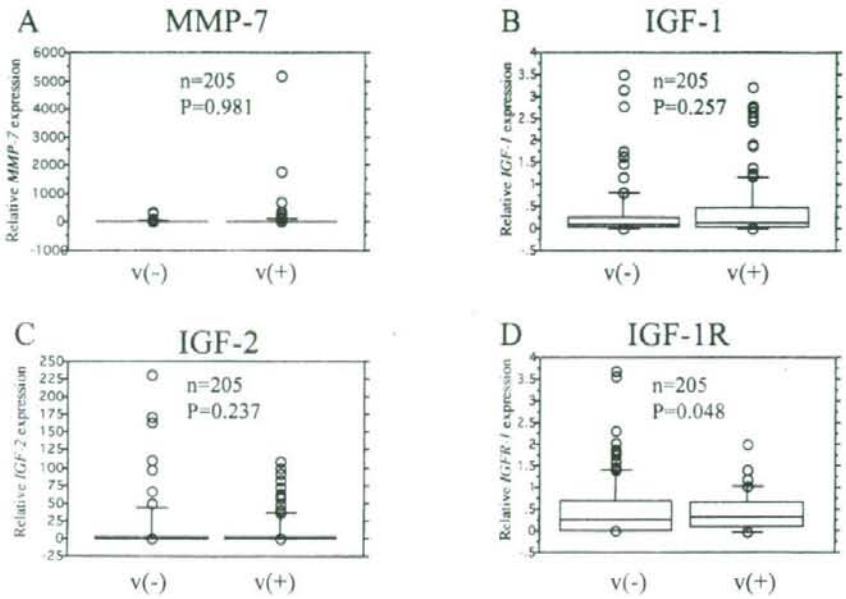

Figure 2. The association of *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* gene expression with venous invasion in 205 patients with colorectal cancer. Box boundaries, the 25th and 75th percentiles of the observed values; capped bars, the 10th and 90th percentiles; solid line, the median. P-values were assessed by the Mann-Whitney U test. The presence or absence of venous invasion was significantly related to the gene expression levels of *IGF-1R*.

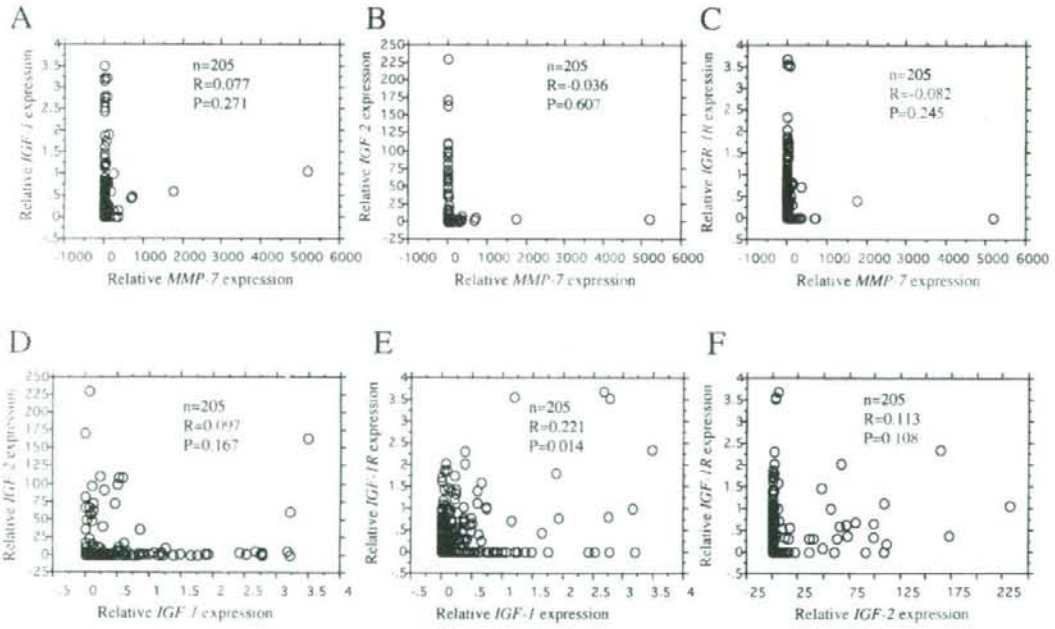

Figure 3. Correlations among gene expression levels of *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* in colorectal cancers. No significant correlations were observed among the expression levels of these genes.

gene expression levels significantly correlated with gender. As for IGFs, Peters *et al* (24) showed that *IGF-1* gene expression does not correlate with any clinicopathological characteristic. Nosho *et al* (22) reported that *IGF-2* gene expression correlates with age and tumor size, whereas *IGF-1R* gene expression does not correlate with any clinicopathological characteristic in patients with early colorectal carcinoma. Mita *et al* (25) reported that *IGF-1R* gene expression does not

correlate with any clinicopathological characteristic in prostate cancer. However, Furukawa *et al* (26) reported that increased postoperative tumor growth and the presence of liver metastasis were associated with significantly higher IGF-1R mRNA expression in gastrinomas. Our study found no significant correlation between *IGF-1* or *IGF-2* gene expression and any clinicopathological characteristic, whereas *IGF-1R* gene expression was significantly related to venous invasion and liver metastasis.

In a study examining interrelations among MMP-7, IGF-1, IGF-2 and IGF-1R, Miyamoto *et al* (8) showed that MMP-7 regulates IGF-1. Furukawa *et al* (26) reported a significant correlation ($r=0.66$, $P<0.0001$) between the expression levels of the *IGF-1* and *IGF-1R* genes. In our study, there were no significant correlations among these genes.

In conclusion, our study showed that *IGF-1R* gene expression levels were higher in adjacent normal mucosa than in cancer tissue and were significantly related to venous invasion and liver metastasis. *IGF-1R* gene expression is thus considered a useful predictor of liver metastasis from colorectal cancer.

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Overexpression of *EphA4* gene and reduced expression of *EphB2* gene correlates with liver metastasis in colorectal cancer

TAKASHI OSHIMA[1], MAKOTO AKAIKE[2], KAZUE YOSHIHARA[1], MANABU SHIOZAWA[2], NAOTO YAMAMOTO[1], TSUTOMU SATO[1], NOZAKI AKIHITO[1], YASUHIKO NAGANO[1], SHOICH FUJII[1], CHIKARA KUNISAKI[1], NOBUYUKI WADA[3], YASUSHI RINO[3], KATSUAKI TANAKA[1], MUNETAKA MASUDA[3] and TOSHIO IMADA[4]

[1]Gastroenterological Center, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama-shi, Kanagawa-ken 232-0024; [2]Department of Surgery, Kanagawa Cancer Center, 1-1-2 Nakao, Asahi-ku, Yokohama-shi, Kanagawa-ken 241-0815; [3]Department of Surgery; [4]Yokohama City University, 3-9 Fukuura, Kanazawa-ku, Yokohama-shi, Kanagawa-ken 236-0004, Japan

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Abstract. The Eph receptors, members of a large family of transmembrane receptor tyrosine kinases, play important roles in a variety of biological functions. Recent studies have suggested that EphA4 and EphB2 participate in the growth and development of various carcinomas. This study examined the relationship of *EphA4* and *EphB2* gene expression to clinicopathological factors, especially metastasis, in patients with colorectal cancer. We studied surgical specimens of cancer tissue and adjacent normal mucosa obtained from 205 patients with untreated colorectal cancer. The relative expression levels of *EphA4* and *EphB2* mRNA in the specimens were measured by quantitative real-time, reverse-transcription polymerase chain reaction. The relative expression level of *EphA4* mRNA was higher in the presence than in the absence of liver metastasis, whereas the relative expression levels of *EphB2* mRNA were similar. Analysis of the relationship between clinicopathological features and gene expression showed that high expression of the *EphA4* gene and low expression of the *EphB2* gene correlated with liver metastasis. There was no correlation between *EphA4* and *EphB2* gene expression. Our results suggest that overexpression of the *EphA4* gene and reduced expression of the *EphB2* gene might promote liver metastasis in colorectal cancer. Overexpression of the *EphA4* gene and reduced expression of the *EphB2* gene may thus be a useful predictor of liver metastasis in patients with colorectal cancer.

Correspondence to: Dr Takashi Oshima, Gastroenterological Center, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama-shi, Kanagawa-ken 232-0024, Japan
E-mail: ohshimatakashi@yahoo.co.jp

Key words: EphA4, EphB2, colorectal cancer

Introduction

The Eph receptor family constitutes one of the largest groups of transmembrane receptor tyrosine kinases (1). They are activated by a second family of cell surface-anchored ligands, the ephrins, which are attached to the plasma membrane via either a glycosylphosphatidylinositol (GPI) linkage (type A) or a transmembrane sequence (type B). The Eph receptors are also divided into type A or type B according to their ligand-binding specificities. In general, type A receptors bind type A ephrin ligands, and type B ephrin ligands stimulate type B receptors. One molecule that shows an exception to this rule is EphA4, which can bind and respond to type B as well as type A ephrin ligands (2). These Eph receptors and their ligands have been implicated in a variety of biological functions, including axon guidance and migration of neural crest cells in the nervous system, establishment of segmental boundaries, and formation of angiogenic capillary plexi (3-7). Among Eph receptor family members, EphA4 and EphB2 are frequently overexpressed or functionally altered in many types of cancers, suggesting a role in tumor progression or angiogenesis (8-14).

In this study, we measured expression levels of the *EphA4* and *EphB2* genes in 205 pairs of cancer tissue and adjacent normal mucosa obtained from patients with colorectal cancer. To evaluate the clinical significance of EphA4 and EphB2, we examined correlations between the relative expression of these genes and clinicopathological features.

Materials and methods

Patients and samples. We studied surgical specimens of cancer tissue and adjacent normal mucosa obtained from 205 patients with untreated colorectal cancer. The patients underwent surgery at Yokohama City Medical Center, Gastroenterological Center and at Kanagawa Cancer Center between 2002 and 2006. Informed consent was obtained from each patient and the ethics committees of Yokohama City Medical Center and Kanagawa Cancer Center approved the protocol before

Table I. PCR primers and conditions.

Gene	Primer	Temperature (°C)	Product size (bp)
EphA4	5'-AGTCCTTCTGGTCTCTGTCTC-3' 5'-CTTCATCCGCTTCTTGTTTGG-3'	60	116
EphB2	5'-GCTTTCTGCTTACTGACTTAGG-3' 5'-GGTGGGAGGAGGGAAGAG-'3	60	105
ß-actin	5'-AGTTGCGTTACACCCTTTCTTGAC-3' 5'-GCTCGCTCCAACCGACTGC-3'	60	171

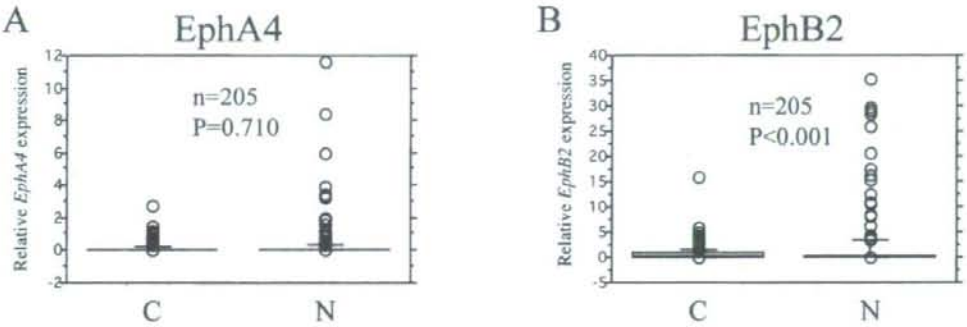

Figure 1. Comparison of *EphA4* and *EphB2* mRNA expression levels between colorectal cancer tissue and adjacent normal mucosa. P-values were calculated by the Wilcoxon test. *EphB2* gene expression levels were higher in adjacent normal mucosa than in cancer (P<0.001). *EphA4* gene expression levels did not differ significantly between cancer and adjacent normal mucosa.

initiation of the study. All tissue samples were embedded in O.C.T. compound (Sakura Finetechnical Co., Ltd., Tokyo) and immediately stored at -80°C until use. No patient had any other malignancies. The histopathological features of specimens stained with hematoxylin and eosin were examined and sections that consisted of >80% cancer cells were used to prepare total RNA.

Quantitative real-time, reverse-transcription polymerase chain reaction (PCR). Total RNA isolated from colorectal cancer and adjacent normal mucosa was prepared with the use of Trizol (Gibco, Life Tech, Gaithersburg, MD). Complemetary DNA (cDNA) was synthesized from 2 μg of total RNA with an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). After synthesis, the cDNA was diluted at 1:4 with water and stored at -20°C until use. Quantitative real-time PCR was performed with an iQ SYBR-Green Supermix (Bio-Rad Laboratories). PCR reactions were carried out in a total volume of 15 μl containing cDNA derived from 75 ng of mRNA, 0.27 μM of each primer, 7.5 μl of iQ SYBR-Green Supermix containing dATP, dCTP, dGTP, and dTTP at concentrations of 400 μM each and 50 U/ml of iTag DNA polymerase. The PCR consisted of 10 min at 94°C, followed by 50 cycles of denaturation of the cDNA for 30 sec at 94°C, annealing for 30 sec at an appropriate temperature (Table I) and a primer extension for 1 min at 72°C followed by 10 min at 72°C. The PCR primer sequences of *EphA4*, *EphB2* and ß-actin, used as internal controls, are shown in Table I.

Statistical analysis. Gene expression levels of colorectal cancer were compared with those of adjacent normal mucosa by the Wilcoxon test. The relationship between gene expression and potential explanatory variables, including age, gender, tumor size, histological type, depth of invasion, lymph node metastasis, location, lymphatic invasion, venous invasion and liver metastasis were evaluated with the χ^2 test. Associations between variables were assessed using the Mann-Whitney U test. Correlation coefficients between different variables were calculated by simple regression analysis. All statistical analyses were performed using Statview J 5.0 software (Abacus, CA). Two-sided P-values were calculated and a difference was considered significant if the P-value was <0.05.

Results

Comparison of EphA4 and EphB2 mRNA expression between colorectal cancer tissue and adjacent normal mucosa. EphB2 gene expression levels were higher in adjacent normal mucosa than in cancer (P<0.001) (Fig. 1B). *EphA4* gene expression levels were similar in cancer and adjacent normal mucosa (P=0.710) (Fig. 1A).

Relationship of EphA4 and EphB2 gene expression levels to clinicopathological features. Expression levels of the *EphA4* and *EphB2* genes were categorized as low or high according to their median values. The relationship between the expression of these genes and clinicopathological features were then examined. Expression levels of the *EphA4* and *EphB2* genes

Table II. Relationship between expression of the *EphA4*, *EphB2* genes and clinicopathological features.

Variables/categories	*EphA4* expression		P-value	*EphB2* expression		P-value
	low (n=103)	high (n=102)		low (n=103)	high (n=102)	
Age	65.4±10.5	66.3±11.1	0.534	66.0±10.9	65.6±10.7	0.912
Gender						
Male	60	52	0.296	55	57	0.721
Female	43	50		48	45	
Size						
≤5 cm	52	63	0.104	62	53	0.235
>5 cm	51	39		41	49	
Histological type						
Well differentiated	26	35	0.258	30	31	0.864
Moderately differentiated	64	52		60	56	
Poorly differentiated	13	15		13	15	
Depth of invasion						
T1	10	9	0.071	6	13	0.059
T2	38	56		52	42	
T3	48	32		36	44	
T4	7	5		9	3	
Lymph node metastasis						
Absent	49	46	0.722	47	48	0.838
Present	54	56		56	54	
Location						
Colon	54	58	0.524	63	49	0.059
Rectum	49	44		40	53	
Lymphatic invasion						
Absent	71	63	0.281	70	64	0.376
Present	32	39		32	38	
Venous invasion						
Absent	34	43	0.176	37	40	0.626
Present	69	59		66	62	
Liver metastasis						
Absent	78	63	0.031	64	77	0.039
Present	25	39		39	25	

were unrelated to age, gender, tumor size, lymph node metastasis, lymphatic invasion and venous invasion. High expression of the *EphA4* gene and low expression of the *EphB2* gene correlated with liver metastasis (P=0.031, 0.039) (Table II).

Relationship of EphA4 and EphB2 gene expression levels to liver metastasis. The highest rate of liver metastasis was associated with high expression of the *EphA4* gene and low expression of the *EphB2* gene (Fig. 2).

Associations of EphA4 and EphB2 gene expression with lymph node metastasis in patients with colorectal cancer. There was no significant association between the expression level of either gene and the presence or absence of lymph node metastasis (Fig. 3).

Associations of EphA4 and EphB2 gene expression with liver metastasis in patients with colorectal cancer. EphA4

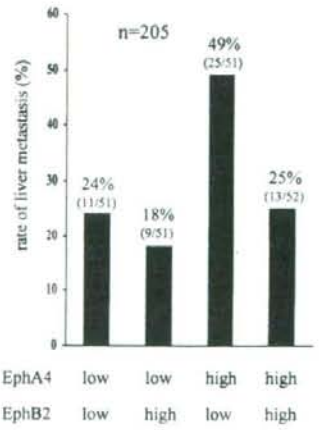

Figure 2. Relationship of *EphA4* and *EphB2* gene expression levels to liver metastasis. The highest rate of liver metastasis was associated with high expression of the *EphA4* gene and low expression of the *EphB2* gene.

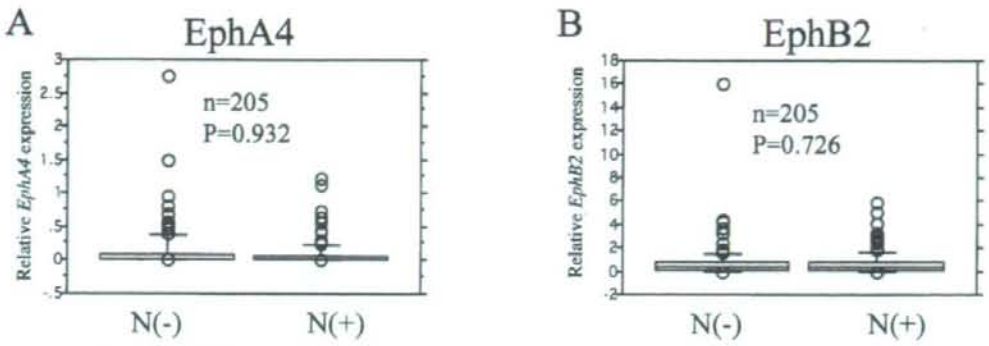

Figure 3. Associations of *EphA4* and *EphB2* gene expression with lymph node metastasis in 205 patients with colorectal cancer. Box boundaries, the 25th and 75th percentiles of the observed values; capped bars, the 10th and 90th percentiles; solid line, median. P-values were calculated by the Mann-Whitney U test. There was no correlation between the expression level of either gene and the presence or absence of lymph node metastasis.

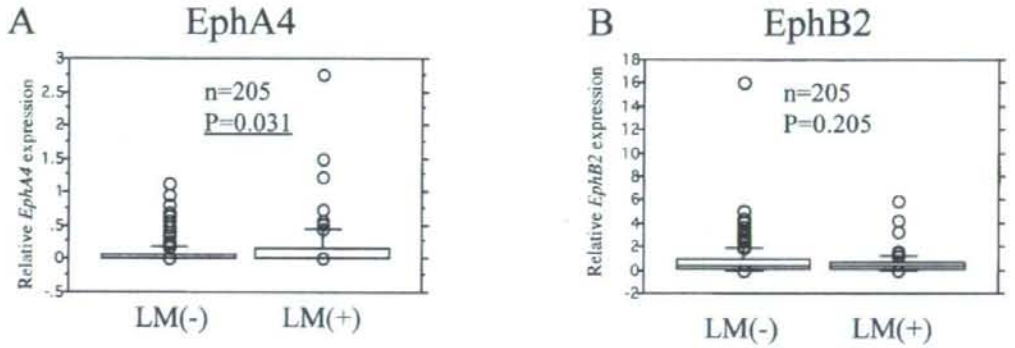

Figure 4. Associations of *EphA4* and *EphB2* gene expression levels with liver metastasis in 205 patients with colorectal cancer. Box boundaries, the 25th and 75 th percentiles of the observed values; capped bars, the 10th and 90th percentiles; solid line, median. P-values were calculated by the Mann-Whitney U test. *EphA4* gene expression levels were higher in the presence than in the absence of liver metastasis (P=0.031).

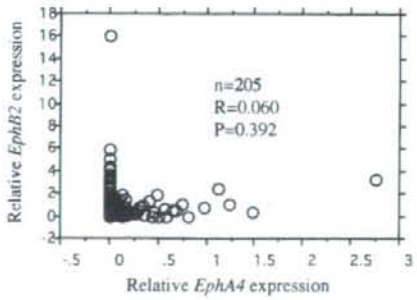

Figure 5. Correlation between *EphA4* and *EphB2* gene expression levels in colorectal cancers. Each gene expression level is relative to that of the ß-actin gene. There was no correlation between *EphA4* and *EphB2* expression levels.

gene expression levels were higher in the absence than in the presence of liver metastasis (P=0.031) (Fig. 4A).

Correlation between EphA4 and EphB2 gene expression. The correlation between *EphA4* and *EphB2* gene expression levels is shown in Fig. 5. There was no correlation between *EphA4* expression and *EphB2* expression.

Discussion

Receptor tyrosine kinases and their ligands play critical roles in the regulation of a variety of cell activities, including cellular survival, proliferation, differentiation and tissue organization (15). Eph receptors and their ligands, ephrins, are indeed involved in several cell processes during embryonic development, such as pattern formation, cell aggregation and migration, segmentation, neural development, angiogenesis, and vascular hierarchical remodeling (3-7). The overexpression of some Eph receptor family members has an important role in the development and progression of various cancers. In particular, EphA4 and EphB2 overexpression is frequently associated with human invasive cancers (10-12,16,17).

In this study, we examined expression levels of the *EphA4* and *EphB2* genes in colorectal cancer and in adjacent normal mucosa. We also studied the relationship of these gene expression levels to clinicopathological features, as well as correlations among the expression of these genes.

Several previous studies have compared *EphA4* and *EphB2* mRNA expression levels between colorectal cancer tissue and adjacent normal mucosa. Ashida *et al* (17) found that the expression of *EphA4* is significantly higher in human prostatic cancer than in adjacent normal prostatic epithelium. Liu *et al*

(18) reported that the expression of EphB2 mRNA is lower in nonmalignant cell lines than in the colon cancer cell lines *in vitro*. Mao *et al* (19) showed that the expression of *EphB2* is higher in colorectal cancer tissue than in normal colorectal tissue (n=11). However, Guo *et al* (20) found that the expression of EphB2 protein is significantly higher in normal colorectal mucosa than in colorectal cancer. Our study (n=205) demonstrated that *EphB2* gene expression levels were higher in adjacent normal mucosa than in colorectal cancer tissue. This finding is consistent with the results of a previous study showing that EphB2 suppresses carcinogenesis, including the transition from colorectal adenoma to carcinoma (20). In contrast, *EphA4* gene expression levels did not differ significantly between cancer and adjacent normal mucosa.

A previous study examining the relationship between clinicopathological features and gene expression levels, found no significant correlation between EphA4 expression and the histological type of pancreatic ductal adenocarcinoma (16). This result was unexpected because the expression of exogenous EphA4 promotes the growth of pancreatic ductal adenocarcinoma cells (16). In our study, there was no significant correlation of EphA4 expression with tumor size, histological type, invasion, or lymph node metastasis in colorectal cancer. However, high *EphA4* gene expression correlated with liver metastasis.

As for EphB2, Wu *et al* (21) reported that *EphB2* gene expression does not correlate with clinical stage or histological grade in breast cancers. Guo *et al* (20) found that low expression of EphB2 correlates with invasion and metastasis in colorectal cancers. In our study, reduced *EphB2* gene expression correlated with liver metastasis in colorectal cancer. This result is considered reasonable, because EphB2 receptor activity suppresses colorectal cancer progression and metastasis (22,23). Thus, overexpression of the *EphA4* gene and reduced expression of the *EphB2* gene is associated with liver metastasis in colorectal cancer.

When expression levels of the *EphA4* and *EphB2* genes were contrasted with the presence or absence of lymph node metastasis, no correlation was noted for either gene. We also examined potential correlations of gene expression levels with the presence or absence of liver metastasis. Iiizumi *et al* (16) reported that EphA4 contributes to properties such as invasiveness or metastasis in a wide range of malignancies. Thorstensen *et al* (22) found that loss of heterozygosity at the *EphB2* locus was frequently associated with liver metastasis. In our study, *EphA4* gene expression levels were higher in the presence than in the absence of liver metastasis. This finding suggested that overexpression of *EphA4* mRNA might contribute to liver metastasis in colorectal cancer.

We then examined correlations between EphA4 and EphB2 gene expression in colorectal cancers. There was no significant correlation between the expression levels of these genes.

In conclusion, our results show that overexpression of the *EphA4* gene and reduced expression of the *EphB2* gene correlates with liver metastasis in colorectal cancer. Overexpression of the *EphA4* gene and reduced expression of the *EphB2* gene may thus be a novel marker or predictor of liver metastasis.

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大腸癌の術中腹腔洗浄細胞診の有用性

横浜医学
別　刷

横浜医学，59，33-39（2008）

原　著

大腸癌の術中腹腔洗浄細胞診の有用性

藤井正一[1]，池　秀之[1]，大田貢由[1]，山岸　茂[4]，
長田俊一[4]，市川靖史[5]，國崎主税[1]，大木繁男[2]，
野沢昭典[3]，嶋田　紘[4]

[1] 横浜市立大学附属市民総合医療センター 消化器病センター，[2] 同手術部，[3] 同病理部，
[4] 横浜市立大学大学院医学研究科 消化器病態・腫瘍外科学，[5] 横浜市立大学大学院医学研究科 臨床腫瘍科学

要　旨：目的：大腸癌術後腹膜再発の予知因子として原発巣切除時の腹腔洗浄細胞診（Cy）の有用性を知る．方法：大腸癌298例を対象に切除直前にCyを施行し，臨床病理学的諸因子別の陽性率，腹膜再発率，生存率の比較，Cy(+)例の臨床経過を検討した．結果：全例のCy陽性率は6.0%（18／298例）で腹膜転移（P）陽性例は46.7%（7／15）でP陰性例の3.9%（11／283）に比べ有意に高かった．P陰性例283例で病理学的諸因子を検討するとリンパ節転移，深達度，脈管侵襲陽性が高度であるほど，組織型ではpor/mucにCy(+)率が高い傾向はあるが有意でなかった．肝転移（H）を認めたものでは有意にCy陽性率が高かった．腹膜再発率はCy陽性では9.1%（1／11），Cy陰性では1.1%（3／272）で陽性群に高い傾向はあるが有意差はなかった．大腸癌取り扱い規約に拠る治癒切除であるとされる根治度A，Bが得られた264例では，Cyによる生存率に差はなく，また病理学的諸因子の多変量解析でも生存に寄与する独立因子としてCy陽性は選択されなかった．結論：Cyは腹膜再発の予知因子として有用ではない．

Key words: 大腸癌（Colorectal cancer），腹腔洗浄細胞診（Intraperitoneal lavage cytology），
腹膜再発（Peritoneal recurrence）

はじめに

大腸癌の再発形式としては肝臓，肺転移，次いで腹膜再発が多い．前二者に対しては近年では外科療法により良好な成績が得られるようになってきた[1)~7)]．しかし腹膜再発に対しては，早期の診断方法とともに有効な治療法も少ないのが現状である[8)9)]．腹膜再発の予知として腹腔洗浄細胞診が行われるようになったが，その有用性については一定していない[10)11)]．第7版の大腸癌取扱い規約[12)]では腹水を認めた場合に細胞診を行い，癌細胞を認めた場合はCy1と記載することを明記しているが，洗浄細胞診については臨床的意義は不明としている．また，その手技，方法は十分に確立されていない．本研究では当院で施行された大腸癌切除術前の腹腔洗浄細胞診と，臨床病理学的諸因子や腹膜再発などの術後経過，生存率の相関について検討し，その意義を考察した．

対象と方法

1995年9月から2001年6月に当院で切除術を施行した原発性大腸癌の手術中切除前の腹水あるいは腹腔洗浄細胞診のうち，原発巣の病理学的所見が明らかな298例を対象とした．開腹直後，病巣の切除前にヘパリン加生食200mlで腹腔内を洗浄，吸引にて回収し，それを1500 rpm，5minで遠沈して塗沫標本とした．Papanicolaou染色，May Giemsa染色標本を作製，鏡検，ClassⅢb以上を陽性とした．本来ClassⅢbは擬陽性であるが，ClassⅣ以上を陽性として今回の分析を行ったところ，根治度AあるいはBが得られた症例のうちClassⅣ以上は5例（1.9%）のみで全例に腹膜再発を認めなかったため，統計学的な検討が成り立たず，ClassⅢb以上を陽性として分析した．

臨床病理学的事項は大腸癌取扱い規約に従い記載し

藤井正一，横浜市南区浦舟町4－57（〒232-0024）横浜市立大学附属市民総合医療センター 消化器病センター
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た．これに基づいて以下の検討を行った．

1）性差，年齢，部位別の細胞診陽性率
2）手術時の腹膜転移有無での細胞診陽性率
3）腹膜転移のなかった283例での各臨床病理学的諸因子別の細胞診陽性率
4）腹膜再発率
5）細胞診陽性症例の臨床経過
6）根治度 A，B264例の生存率

術後の腹膜再発の診断は臨床所見と Computed tomography scan（CT）あるいは Magnetic Resonance Image（MRI）による画像診断で病変の描出があったものとした．

統計学的には χ^2 検定，Mann-Whitney の U 検定で危険率5％未満を有意差ありとした．生存率は Kaplan-Meier 法にて算出し logrank test で検定し危険率5％未満を有意差ありとした．生存率に対する病理学的諸因子の多変量解析は Cox 比例ハザードモデルを用いて行った．

成　績

1）性差，年齢，部位別の細胞診陽性率

全大腸癌の洗浄細胞診陽性率は6.0％（18／298例）であった．性差は男3.6％（6／168例），女9.2％（12／130例），年齢は陽性群66.8±8.1歳，陰性群64.5±10.2歳でともに有意差はなかった．部位は盲腸9.1％（1／11例），上行結腸6.3％（2／32例），横行結腸7.7％（2／26例），下行結腸16.7％（2／12例），S 状結腸癌5.2％（5／97例），上部直腸6.1％（5／82例），下部直腸2.6％（1／32例）で有意差を認めなかった（Table 1）．

2）腹膜転移有無での細胞診陽性率

開腹時に腹膜転移ありでは46.7％（7／15例），なしでは3.9％（11／283例）で有意差を認めた．P1では0％（0／3例），P2では42.9％（3／7例），P3では80％（4／5例）で腹膜転移の程度が高いほど陽性率は高かった（Table 2）．

3）腹膜転移のなかった283例での各臨床病理学的諸因子別の細胞診陽性率

i）組織型

高・中分化腺癌では3.7％（10／270例），低分化腺癌・粘液癌では8.3％（1／12例）で差を認めなかった．

ii）組織学的深達度

mp 以浅で1.5％（1／65例），ss/a1 で2.8％（4／

Table 1 Patients characteristics and peritoneal lavage cytology

	Cy (+)	Cy (-)	
Number of patients	18(6.0%)	280	
	Class3: 5 (1.7%)	Class1: 92 (30.9%)	
	Class4: 1 (0.3%)	Class2: 189 (63.4%)	
	Class5: 12 (4.0%)		
Age (y.o, mean±SD)	66.8±8.1	64.5±10.2	N.S.
Gender; men	6(3.6%)	162	N.S.
women	12(9.2%)	118	
Site of lesion			
C	1 (9.1%)	10	N.S.
A	2 (6.3%)	30	
T	2 (7.7%)	24	
D	2 (16.7%)	10	
S	5 (5.2%)	92	
Rs/Ra	5 (6.1%)	77	
Rb/P	1 (2.6%)	37	
curability			
A	4 (1.6%)	247	P<0.05
B	3 (15.8%)	16	
C	11 (39.3%)	17	

Table 2 Peritoneal metastases and peritoneal lavage cytology

	Cy (+)	Cy (-)	
P (-)	11(3.9%)	272	P<0.05
P (+)	7 (46.7%)	8	
P1	0 (0.0%)	3	
P2	3 (42.9%)	4	
P3	4 (80.0%)	1	

142例)，se/a2 で6.5%（4／62例)，si/ai で14.2%（4／14例）で深達度が深いほど陽性率は高かったが有意ではなかった．

iii）リンパ節転移

n0 で1.9%（3／159例)，n1 で5.3%（4／76例)，n2 で7.4%（2／27例)，n3 で9.1%（1／11例)，n4 で14.3%（1／7例）とリンパ節転移の程度が高いほど陽性率は高かったが有意ではなかった．

iv）脈管侵襲

ly(+) で5.1%（10／196例)，ly(−) で1.2%（1／84例）で ly(+) に陽性率が高い傾向がみられたが，有意ではなかった．ly1 で4.0%（4／101例)，ly2 で5.9%（4／68例)，ly3 で8.0%（2／25例）で侵襲の程度が高いほど陽性率は高い傾向がみられたが，有意ではなかった．

v(+) で5.9%（8／135例)，v(−) で2.1%（3／145例）で v(+) に陽性率が高い傾向がみられたが，有意ではなかった．v1 で9.5%（4／79例)，v2 で8.1%（3／37例)，v3 で5.6%（1／18例）で侵襲の程度に陽性率は差がなかった

v）肝転移

肝転移ありでは15.0%（3／20例)，なしでは3.0%（8／263例）で有意差を認めた．H1，H2 では0%（0／8例，0／1例）であったが，H3 では27.3%（3／11例）であった（Table 3)．

4）腹膜再発

腹膜転移のなかった283例で腹膜再発率を検討した．全283例の腹膜再発率は1.4%（4／283例）で細胞診陽性群では9.1%（1／11例)，細胞診陰性群では1.1%（3／272例）で陽性群に腹膜再発率が高い傾向はみられたが有意差はなかった（Table 4)．

5）細胞診陽性症例の臨床経過

腹膜転移がなく細胞診陽性であった11例の臨床経過を検討した．4例は癌死で全例肝転移を認めた．そのうち

Table 3 Clinicopathological factors and peritoneal lavage cytology in patients excluding peritoneal metastases

	Cy (+)	Cy (-)	
Histology			
well/mod	10(3.7%)	270	N.S.
por/muc	1 (8.3%)	11	
Depth			
m/sm/mp	1 (1.5%)	64	N.S.
ss/a1	4 (8.0%)	138	
se/a2	4 (6.5%)	58	
si/ai	4 (14.2%)	10	
Lymph node metastasis			
n0	3 (1.9%)	156	N.S.
n1	4 (5.3%)	72	
n2	2 (7.4%)	25	
n3	1 (9.1%)	10	
n4	1 (14.3%)	6	
Lymphovascular invasion			
ly(-)	1 (1.2%)	83	N.S.
ly(+)	10 (5.1%)	186	
ly1	4 (4.0%)	97	
ly2	4 (5.9%)	64	
ly3	2 (8.0%)	23	
v(-)	3 (2.1%)	142	N.S.
v(+)	8 (5.9%)	127	
v1	4 (9.5%)	75	
v2	3 (8.1%)	34	
v3	1 (5.6%)	17	
Hepatic metastasis			
H (-)	8 (3.0%)	255	P<0.05
H (+)	3 (15.0%)	17	
H1	0 (0.0%)	8	
H2	0 (0.0%)	1	
H3	3 (27.3%)	8	

Table 4 Peritoneal recurrence and peritoneal lavage cytology

	Peritoneal recurrence(+)	Peritoneal recurrence(-)	
Cy (+)	1(9.1%)	10	N.S.
Cy (-)	3(1.1%)	269	

3例は手術時にcurCであった．腹膜再発を認めたのは1例で，手術時既にH3で術後2ヶ月に癌性腹膜炎と肝転移による死亡であった（Table 5）．

6）生存率

curCを除いたcurA，B264例での生存率を検討した．細胞診陽性7例の5年生存率は83.3%に対し，細胞診陰性257例では89.9%で差を認めなかった（Fig. 1）．生存率に対する細胞診を含めた病理学的諸因子を多変量解析により分析すると，生存に寄与する独立因子は組織型と深達度であり，細胞診は選択されなかった（Table 6）．

考　察

大腸癌の腹膜転移に対しては有効な治療法は少なく，診断時には終末期の対症療法となることも多い．しかし胃癌や膵癌と比し，癌性腹膜炎の状態となってからも予想外に生存期間が長く，時にはイレウス状態となりその解除術も行われることある．腹膜再発を早期に予見し，進行を妨げる方策を講じる必要があることはいうまでもない．

Table 5 clinical courses of patients with positive peritoneal lavage cytology

Age	Gender	Curability	Cy	H	Site	Histology	Depth	n	P-stage	ly	v	Prognosis	P rec.	Cause of death
67	F	A	4	0	RaRb	w	ss	0	2	0	1	55mo,A	(-)	
62	F	A	3	0	RbPE	w	a1	1	3a	2	0	42mo,A	(-)	
64	F	B	5	0	Rs	w	ss	1	3a	1	0	31mo,A	(-)	
55	M	A	3	0	S	w	mp	0	1	1	0	24mo,A	(-)	
63	M	B	5	3	Rb,S	w	se	1	4	1	2	11mo,A	(-)	
74	F	C	3	0	Rbas	m	a2se	4	4	3	1	10mo,A	(-)	
61	F	A	5	0	A	m	si	2	3b	2	1	9mo,A	(-)	
68	F	C	3	3	S	m	ss	1	4	1	3	26mo,D	(-)	Hepatic metastasis
86	F	C	5	0	Rs	w	se	2	3b	3	2	12mo,D	(-)	Hepatic metastasis
70	F	B	5	0	RSRa	muc	si	0	3a	2	2	9mo,D	(-)	Hepatic and lung metastasis
65	M	C	5	3	D	w	se	3	4	2	1	2mo,D	(+)	Hepatic and peritoneal metastasis

w: well differentiated adenocarcinoma
m: moderately differentiated adenocarcinoma
muc: mucinous carcinoma
P rec.: peritoneal recurrence

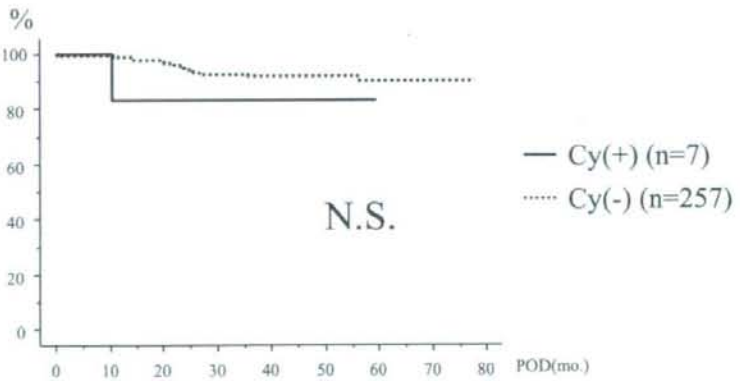

Fig.1 Cumulative survival rates in patients underwent curative operation

Table 6 Multivariate analysis using the Cox's proportional Hazards model between survival and risk of clinicopathological factors

Variable	Hazard ratio	95% Confidential interval	χ^2	p-value
Peritoneal lavage cytology	2.663	0.289-24.500	0.748	0.3870
Histology	10.378	2.805-38.392	12.290	0.0005
Depth of invasion	6.813	2.345-19.392	12.438	0.0004
Lymph node metastasis	0.750	0.275-2.050	0.314	0.5753
Lymphatic invasion	2.432	0.476-12.432	1.141	0.2855
Vascular invasion	1.392	0.467-4.148	0.353	0.5525

Table 7 Clinical utility of intraperitoneal lavage cytology as prognostic factor for peritoneal recurrence in colorectal cancer

Author	Clinical utility
Jiro Nasu et al. [10]: Examination of trap coeliac washing cytology in large intestine cancer. Nihonsyoukakigekagakkaizasshi 28:1991-1994,1995	It is.
Wind P et al. [18]: Long-term prognostic value of positive peritoneal washing in colon cancer. Scand J Gastroenterol 34:606-610, 1999	None
Vogel P, et al. [11]: Prognostic value of microscopic peritoneal dissemination: comparison between colon and gastoric cancer. Dis Colon Rectum 43: 92-100, 2000	None

腹膜転移の予知因子として，腹腔細胞診は胃癌ではその有用性は広く認められ，多変量解析でも独立した予後規定因子であるとする報告も多い[3)~10)]．多くの施設で細胞診の結果如何によっては術式の変更や，腹腔内化学療法の施行[11]など治療方針の決定に大きな役割を担っている．ところが大腸癌では胃癌と異なり，腹腔洗浄細胞診が腹膜転移の予知因子となりうるとする報告[10]は少ない．反論も多く現在もまだその有用性については一定していない[11,18]（Table 7）．これは癌細胞の腹膜転移形成能のような胃癌と大腸癌の生物学的特性の差であると考えられる．細胞診陽性率をみても明らかで，胃癌では se 以深では40%前後である[12)~17)]のに比べ，大腸癌では20%前後[10,18]と低く，自験例でも10.5%であった．胃癌では露出した腫瘍から容易に癌細胞が脱落しやすくさらに仮に小さな癌細胞巣でも比較的容易に生着し転移を形成することが想像される．これに対し大腸癌ではたとえ腹腔内に遊離した癌細胞があっても容易には生着しにくく，それを規定するものは血行転移成立のモデルに考えられている細胞の遊離，血管内浸入，標的臓器の血管内皮への接着，血管外脱出，標的臓器での増殖といった各過程に強く関わる転移関連形質などの異常ではないかと考えられる[19]．シアリルルイス x 抗原の高発現群で肝転移などの再発が多いことなどが知られるように[20]，腹膜転移でも一部の糖鎖の高発現が有意であるとの報告[21]も散見され，今後分子生物学的手法による大腸癌腹膜播種転移の解明が待たれる．

細胞診の結果を大きく左右する要因として，判定の精度の問題がある．最も擬陽性となりやすいのは腹膜の中皮細胞が癌細胞と誤認されやすいことがある[10]．自験例では腹腔転移がある場合の陽性率が46.7%，多くの報告での50~60%と大差はなかった[10,18]．細胞診陽性例の背景を検討すると，深達度が深いほど陽性率が高い傾向であったことからも概ね信頼できるものと判断した．しかし偽陽性の疑いとして mp1 例，ss3 例，Rb1 例の陽性例があった．これらの詳細をみると，mp 症例と Rb の a1 症例は共に高分化腺癌であるが，ly(+) であった．ss の3症例のうち1例は H3，1例は ly(+) であるが，もう1例は ly(−) で n(−) の高分化腺癌であった．前4者については浸襲陽性のリンパ管あるいは肝転移巣からの癌細胞の遊出の可能性は考えられるが，漿膜下より遊出する可能性については不明であるとしても，前出最後の症例は偽陽性であった可能性がある．

他の背景因子では，リンパ節転移が高度であるほど陽性率が高かった．これは胃癌でも同様の報告があり[3]，腹腔内へ癌細胞が遊離する経路として単に原発巣からの脱落のみでなく転移リンパ節や腹腔に開放するリンパ管が存在しそこから遊出する可能性もあり得ることを示唆する．組織型では粘液癌に陽性率が高いという報告があるが[22]，粘液癌 低分化腺癌と高 中分化腺癌との間に差がなかった．これは粘液癌8例のうち5例は RbP であったためであることと，症例数が少なかったこともあると考えられる．肝転移例では有意に高かった．しかし H1，2 では陽性例は0であるが，H3 に陽性を認めたことから転移巣から脱落の可能性も否定できない．

腹膜再発は細胞診陽性例に高い傾向はみられたが，有意ではなく腹腔洗浄細胞診が腹膜再発の有用な予知因子であるとはいえない結果となった．このことは第1に細胞診の検体採取方法の問題を提起する．Hase ら[23]は切除前後で細胞診を施行し，前後ともに陽性ならば50%の高い腹膜再発率であるが切除前のみ陽性では16.7%であったと報告した．切除後の細胞診の意義としては切断されたリンパ管からの癌細胞の遊出の可能性[24]など単に癌の進行度のみならず手術操作による播種の影響もあり得る．

またこのことは第2として従来の鏡検による細胞診の限界の可能性をも示唆する．本研究での多変量解析では curA，B 例では細胞診陽性例は生存に寄与する独立因子ではなかった．このことは腹膜転移のみならず，肝，肺など他の全身再発の危険因子ではないことを意味する．同様の報告はこれまでもみられ[11,18]，また山村ら[25]は腹膜再発に寄与する臨床病理学的諸因子は多変量解析によると何もなかったと報告している．腹腔内の遊離癌細胞の検出としては免疫組織学的手法が細胞診よりも鋭敏かつ有用であるという多くの報告がある[26)~29)]．近年では RT-PCR 法によりさらに鋭敏かつ迅速に細胞診の補助診断ができるという報告もみられるようになった[30]．これらの遺伝子診断は急速な進歩がみられ，近い将来に実用化を迎えることになると思われる．

また腹膜転移の診断上の問題として，微小な転移はCTやMRIで画像上描出するのは事実上困難なことがある[1]．近年FDG-PETの応用など新しい診断技術により感度や正診率が上がるという報告がある[10]．今後これらの画像診断精度の上昇をみた上で，大腸癌取扱い規約に記載がある以上，細胞診が微小な腹膜転移，再発の予知となり得るかを，その手技を含め判定方法や意義を再度検討し直す必要があるものと思われた．

結　語

大腸癌の術中腹腔洗浄細胞診は腹膜再発の予知因子として有用ではない．

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Abstract

EVALUATION OF INTRAPERITONEAL LAVAGE CYTOLOGY BEFORE RESECTION OF COLORECTAL CANCER

Shoichi FUJII[1], Hideyuki IKE[1], Mitsuyoshi OTA[1], Shigeru YAMAGISHI[1], Shunnichi OSADA[4], Yasushi ICHIKAWA[5], Chikara KUNISAKI[1], Shigeo OHKI[2], Akinori NOZAWA[3], Hiroshi SHIMADA[4]

[1] *Department of Surgery, Gastroenterological Center, Yokohama City University Medical Center*

[2] *Operation unit, Yokohama City University Medical Center*

[3] *Division of Pathology, Yokohama City University Medical Center*

[4] *Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine*

[5] *Division of Clinical Oncology, Yokohama City University Graduate School of Medicine*

The aim of this study conducted in 298 cases of colorectal cancer was to assess the usefulness of intraperitoneal lavage cytology (Cy) before resection as a predictive factor for peritoneal recurrence. The Cy(+) rate, peritoneal recurrence rate and 5-year survival rate were examined in relation to various clinicopathological factors. The overall Cy(+) rate was 6.0%. The Cy(+) rate in the group with peritoneal and hepatic metastases was significantly higher than that in the group without metastasis (46.7 vs. 3.9% and 26.9 vs. 4.0%, respectively). There were no significant differences in the Cy(+) ratio in relation to other clinicopathological factors. The peritoneal recurrence rate was higher in the Cy(+) group than in the Cy(−) group, but the difference was not significant. There was no survival difference, regardless of the Cy status, in the 263 curatively resected patients. Thus, the Cy status before resection was not a useful predictive factor for peritoneal recurrence in cases of colorectal cancer.