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

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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
ß-actin	5'-AGTTGCGTTACACCCTTTCTTGAC-3' 5'-GCTCGCTCCAACCGACTGC-3'	60	171

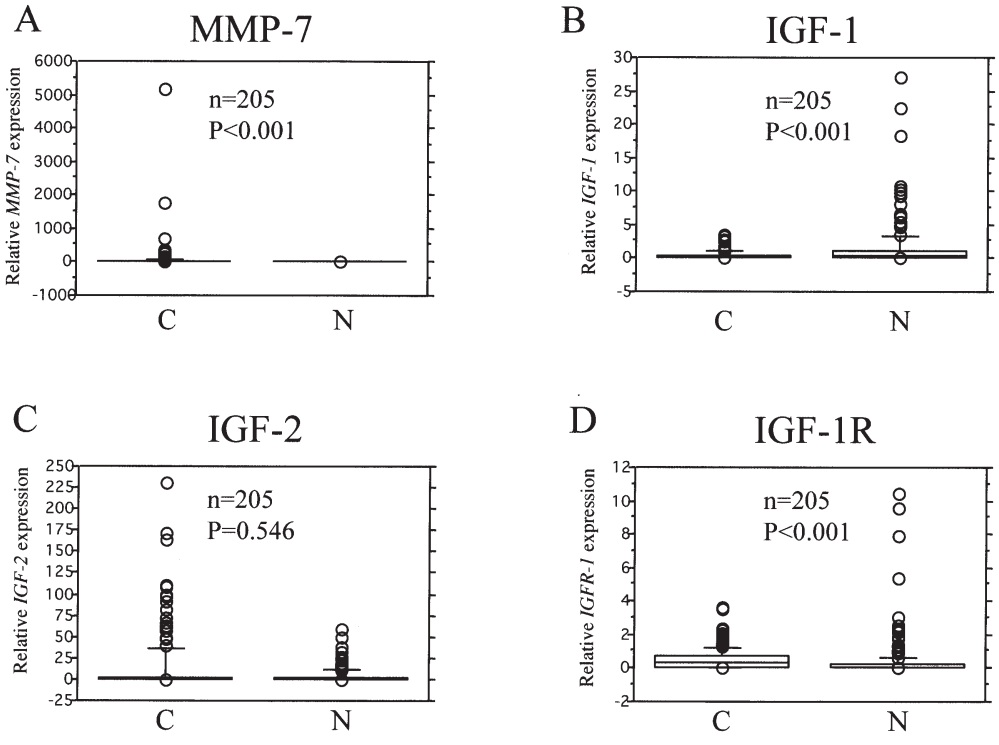

Figure 1. Comparison of *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* mRNA expression levels between colorectal cancer tissue and adjacent normal mucosa. *MMP-7* and *IGF-1* gene expression levels were higher in cancer tissue than in adjacent normal mucosa (P<0.001, P<0.001). In contrast, *IGF-1* gene expression 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												
Tl	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	

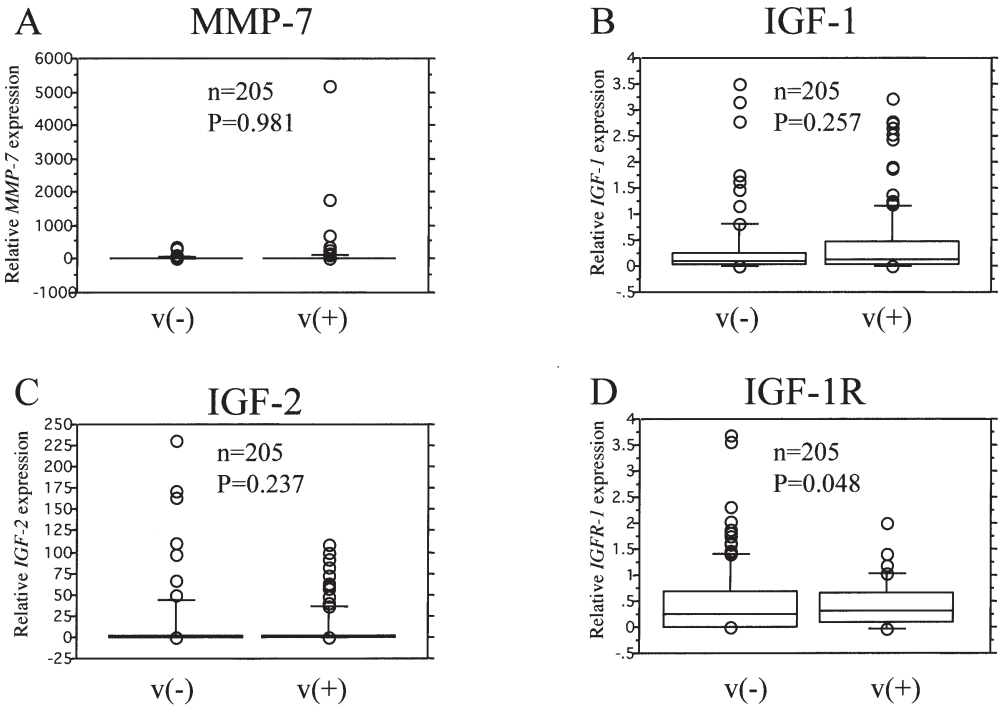

Figure 2. The association of *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* gene expression with venous invasion in 205 patients with colorectal cancer. Box boundaries, the 25th and 75th percentiles of the observed values; capped bars, the 10th and 90th percentiles; solid line, the median. P-values were assessed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

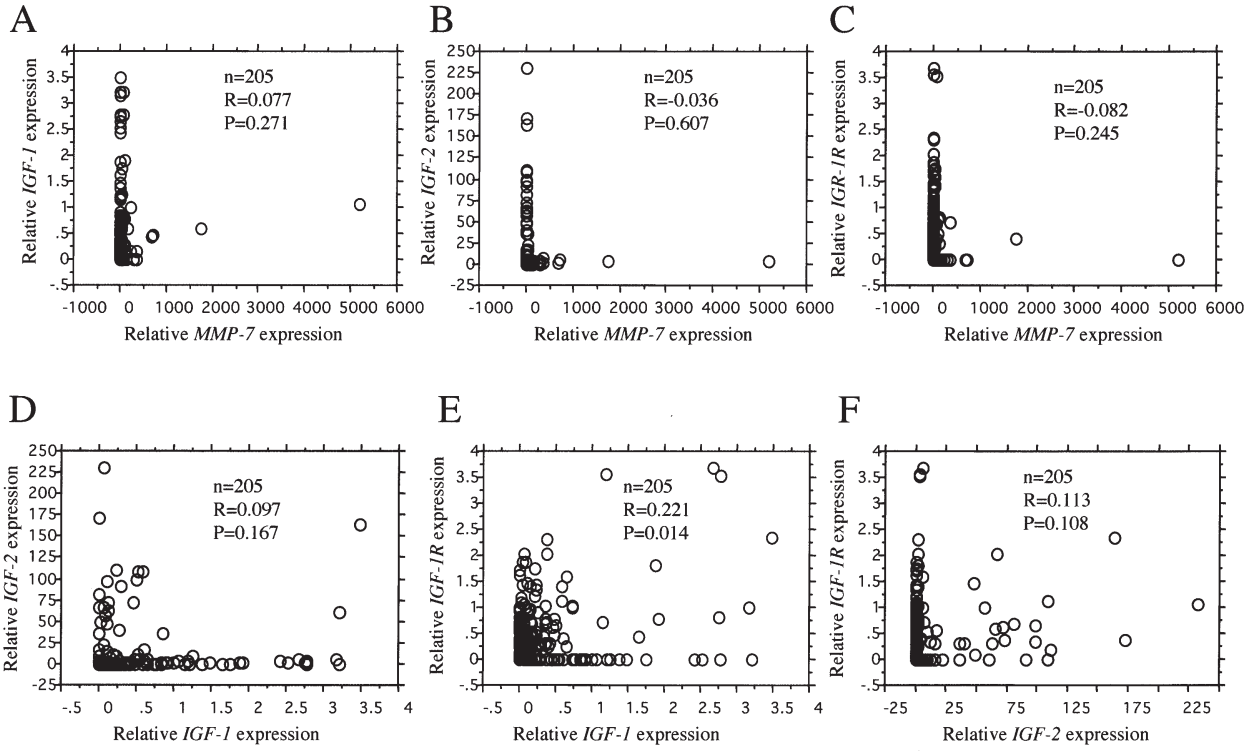

Figure 3. Correlations among gene expression levels of *MMP-7*, *IGF-1*, *IGF-2* and *IGF-1R* in colorectal cancers. No significant correlations were observed among the expression levels of these genes.

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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