Expression of IGF-1 and IGF-1R and Their Relation to Clinicopathological Factors in Colorectal Cancer

ICHITAROU SHIRATSUCHI[1], YOSHITO AKAGI[1], AKIHIKO KAWAHARA[2], TETSUSHI KINUGASA[1], KANSAKAR ROMEO[1], TAKEFUMI YOSHIDA[1], YASUHIKO RYU[1], YUKITO GOTANDA[1], MASAYOSHI KAGE[2] and KAZUO SHIROUZU[1]

[1]*Department of Surgery, and* [2]*Department of Pathology, Faculty of Medicine, Kurume University, Fukuoka 830-0011, Japan*

Abstract. *Background: Expression of insulin-like growth factor-1 (IGF-1) and IGF-1 receptor (IGF-1R) has been shown to increase in colorectal cancer. We examined the correlation between expression of IGF-1 and IGF-1R and clinicopathological factors in colorectal cancer. Patients and Methods: A prospective study was conducted of 210 colorectal cancer patients that underwent resection from January 2002 to December 2004. The clinicopathological data was correlated to expression of IGF-1 and IGF-1R obtained from immunohistochemical analysis. Statistical analysis was carried using univariate and multivariate analysis. Results: IGF1 and IGF-1R staining was positive in 169 (80%) and 139 (66%) cases, respectively. Univariate and multivariate analyses showed significant correlation between expression of IGF-1 and tumor size (p=0.0024), and depth of invasion (p=0.0147). While IGF-1R was significantly correlated to tumor size and depth of invasion in univariate analysis, only tumor size (p=0.0658) had a strong association in multivariate analysis. Conclusion: Expression of IGF-1 and IGF-1R seems to increase with tumor size in colorectal cancer.*

Colorectal cancer is a one of the most common types of cancer. Recent data suggests that an increased risk of colorectal carcinoma may result from the possible influence of dietary factors, blood insulin levels and on the bioavailability of insulin-like growth factor-1 (IGF-1) (1, 2). IGF-1 is a multifunctional peptide hormone with structures similar to insulin. It regulates cell growth, proliferation, differentiation, apoptosis and inductional activity of cell motility (3-5). Through its widespread bioactivities, IGF-1 plays an important role in the normal growth and development of the cell and maintenance of homeostasis. However, its function in promoting cell growth may have unfavorable effects in certain circumstances. Early studies of prostate, breast, colorectal and lung cancer suggested that high circulating IGF-1 concentrations were associated with increased risk of cancer (6-9).

IGF-1 exerts biological effects through activation of insulin-like growth factor type 1 receptor (IGF-1R). Overexpression of IGF-1R has been shown to play a role in colorectal, pancreatic, gastric and esophageal cancer (10-12). The expression of IGF-1 and IGF-1R in association with clinicopathological factors of colorectal cancer has not been elucidated till now. In this study we assessed the relationship between expression of IGF-1, IGF-1R and clinicopathological factors in colorectal cancer.

Patients and Methods

Patients and tissue samples. A prospective study was conducted of 210 colorectal cancer patients that underwent curative resection in Kurume University Hospital from January 2002 to December 2004. Cases with synchronous and multiple cancer were excluded from the study. Written informed consent was given by all patients and ethical approval was obtained from the Ethical Review Board, Kurume University. Tumor tissues were acquired from formalin-fixed pathological samples taken from the resected colorectal cancer specimens. Clinicopathological characteristics of patients are given in Table I.

Immunohistochemical staining method. From the tissue samples, the section of the tumor with maximal invasion was chosen and cut into sections of 4 μm which were mounted on silanized slides. The primary immunostaining was performed using IGF-1 antibody (sc-9013; Santa Cruz Biotechnology Inc., CA, USA) and IGF-1R antibody (MAB 607077; Antagene, Sunnyvale, CA, USA). Antigen retrieval was achieved by microwaving at 99˚C for 30 min in pH 6.0 citric acid buffer for IGF-1 staining and pH 9.0 Tris buffer for IGF-1R

Correspondence to: Dr. Ichitarou Shiratsuchi, Department of Surgery, Faculty of Medicine, Kurume University, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan. Tel: +81 942353311, Fax: +81 942340709, e-mail: ichitaro1217@yahoo.co.jp

Key Words: Colorectal cancer, insulin-like growth factor, IGF-1, IGF-1R.

Table I. *Patient and tumor characteristics*.

Median follow-up, months (range)	59.5 (12-95)
Male:Female	131:79
Median age, years (range)	67 (29-87)
Location	
Right colon	52 (25%)
Left colon	81 (39%)
Rectum	77 (37%)
Median tumor size, mm (range)	45 (10-128)
CEA (mg/dl)	
<5.0	113 (61%)
≥5.0	72 (39%)
Tumor differentiation	
Well	139 (66%)
Moderate	57 (27%)
Poor	14 (7%)
Depth of invasion	
Tis	13 (6%)
T1	17 (9%)
T2	32 (15%)
T3	82 (39%)
T4	66 (31%)
LN metastasis	
Absent	138 (66%)
Present	72 (34%)
Recurrence	
Absent	189 (90%)
Present	21 (10%)

staining. Each slide was then incubated for 30 min with the antibody at room temperature; ENVISION method (Dako Cytomation, Glostrup, Denmark) was used with 3,3'-diaminobenzidine (DAB).

Evaluation of immunohistochemical staining results. The immunostaining was independently examined by two clinical pathologists who were unaware of the patient outcome. Each sample was examined in a high power field at 200 times magnification. It was determined that the samples which had less than 10% staining would be termed as being negative and those samples with 10% or more staining as being positive. The staining of IGF-1 was mainly found in the cell cytoplasm and IGF-1R in the cell membrane. The staining patterns in IGF-1 and IGF-1R are depicted in Figures 1 and 2.

Data analysis. We compared the clinicopathological factors regarding the patients with manifestation of IGF-1 and IGF-1R by immunohistochemical staining. Sex, age, preoperative carcinoembryonic antigen (CEA) level, tumor location, maximum tumor diameter, depth of invasion, lymph node metastasis, tumor differentiation, lymphatic and vascular invasion and recurrence of tumor were examined. The cut-off value for tumor size for expression of IGF-1 and IGF-1R was determined using receiver operating characteristic (ROC) curve. Univariate analysis was carried out using chi-square test, and *t*-test. The observations determined to be statistically significant in the univariate analysis were subsequently subjected to a multivariate analysis using logistic regression. A *p*-value of <0.05 was considered statistically significant. Statistical analysis was performed using SAS 9.1 JMP version 8.0 (SAS Institute, Cary, North Carolina, USA).

Table II. *Correlation between expression of IGF-1 and IGF-1R (n, %)*.

	IGF-1		*p*-Value
	Negative 41 (20%)	Positive 169 (80%)	
IGF-1R			
Negative 71 (34%)	19 (46%)	52 (31%)	0.0586
Positive 139 (66%)	22 (54%)	117 (69%)	

Results

The association of expression of IGF-1 and IGF-1R in colorectal cancer patients is depicted in Table II. IGF-1 staining was positive in 169 cases (80%) and IGF-1R staining in 139 (66%) cases. However there was no statistically significant correlation between IGF-1 and IGF-1R (p=0.0586).

Correlation between IGF-1 expression and the clinicopathological characteristics of patients with colorectal cancer are shown in Table III. There was a statistically significant correlation between IGF-1 and tumor size, tumor depth and lymphovascular invasion. There was no statistical significance in the expression of IGF-1 with regard to gender, age, location, differentiation, lymph node metastasis or recurrence of tumor. Although statistical significance was not achieved, the preoperative CEA level was strongly associated with expression of IGF-1 (p=0.0564).

Taking factors which had a significant difference in the univariate analysis, multivariate analysis using logistic regression analysis was used to search for factors independently associated with IGF-1 expression (Table IV). The tumor size (p=0.0024) and depth of invasion (p=0.0147) were statistically significantly associated with the expression of IGF-1.

The association of IGF-1R expression and the clinicopathological characteristic of patients with colorectal cancer in univariate analysis is shown in Table V. Only the tumor size and depth of invasion were statistically significantly associated with IGF-1R expression. The other factors had no statistically significant association. Multivariate logistic regression analysis was applied using tumor size and depth of invasion to search for factors independently associated with IGF-1R expression (Table VI). Only tumor size (p=0.0658) had strong association with the expression of IGF-1R, although this relationship was not statistically significant.

Discussion

IGF-1 is been thought to promote cell growth in colorectal cancer and overexpression of IGF-1R has been demonstrated in colorectal cancer specimens in several studies but there is no uniform consensus (13-18). Our study showed that IGF-1 staining was more positive (80%) in cases in comparison to IGF-1R (66%) in colorectal cancer.

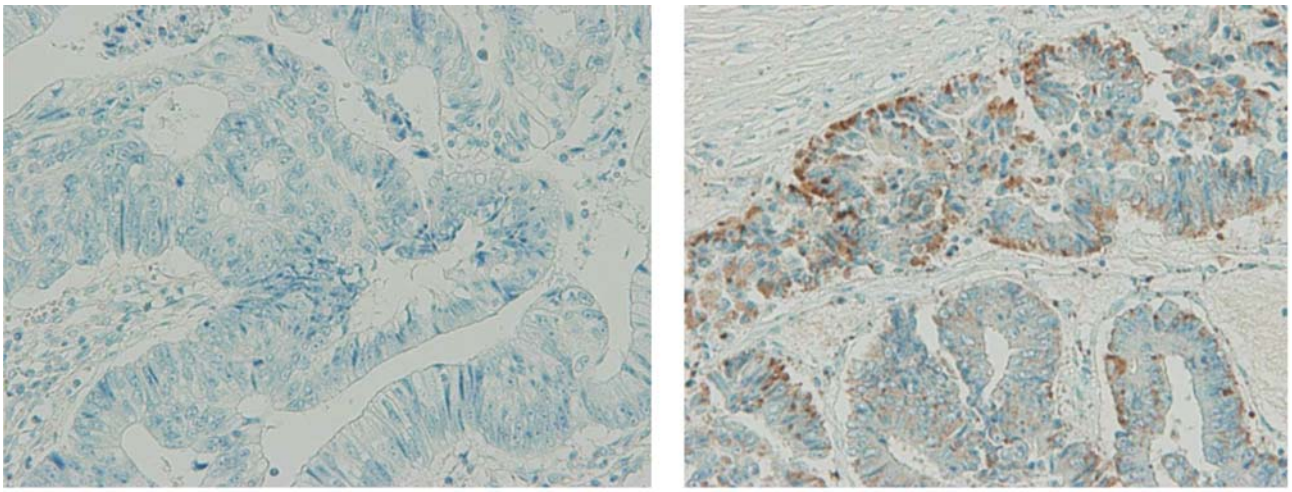

Figure 1. *Expression of IGF-1 using immunohistochemical analysis (×200).*

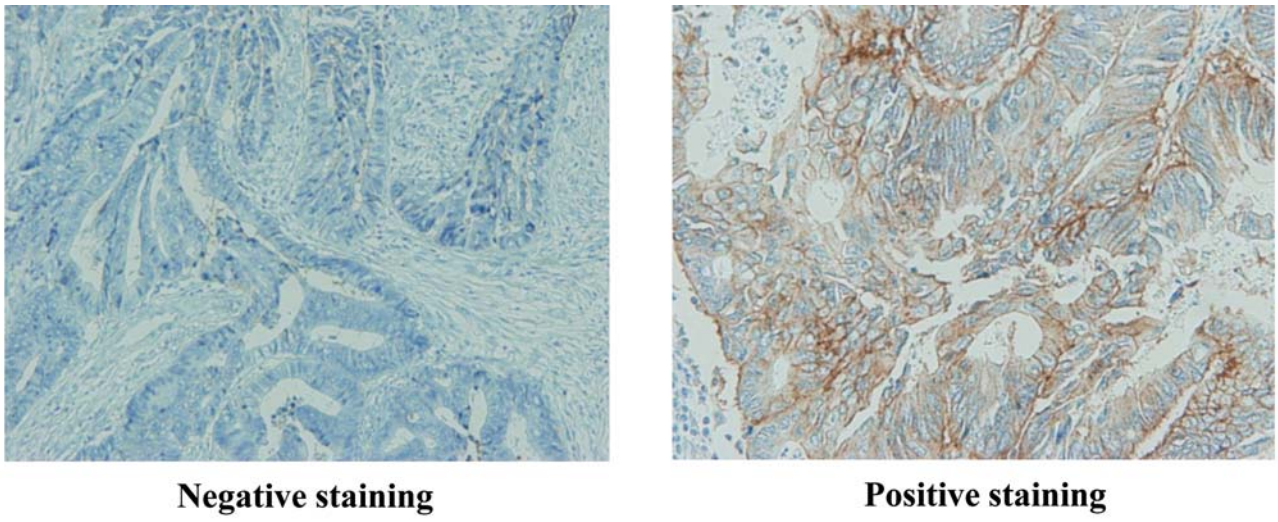

Figure 2. *Expression of IGF-1R using immunohistochemical analysis (×200).*

Several studies showed correlation of IGF-1 with clinicopathological factors such as tumor growth, and metastasis in human and animal studies. Wu *et al*. administered recombinant human IGF-1 in mice which significantly increased cecal tumor growth, weight, frequency and number of hepatic metastases (19). This showed that IGF-1 may also contribute to invasion and metastasis of colon cancer cells due to its effects on cell motility and migration. Our study showed that the tumor size, depth of invasion, and lymphatic and venous invasion seemed to increase with expression of IGF-1, which all suggest invasion and increase in tumor stage, but an increased frequency of metastasis was not seen.

The relationship between IGF-1R expression and aggressiveness of tumor remains unclear. Hakam *et al*. reported strong correlation between IGF-1R positivity in higher grade and higher stage tumors ($p<0.01$) (15). On the other hand, Nakamura *et al*. reported low IGF-1R expression correlated with increased risk of liver metastasis in Dukes' C colorectal cancer (20). Our study did not show any correlation with the grade (tumor differentiation) of the tumor or metastasis with IGF-1R expression. However, in our study, tumor depth and size had significant correlation in univariate analysis and tumor size had strong correlation in multivariate analysis, although it was not statistically significant. Tumor

Table III. *Univariate analysis of correlation between IGF-1 staining and clinicopathological factors.*

	IGF-1 staining		
	Negative (n=41)	Positive (n=169)	*p*-Value
Gender			
Male	28 (68%)	103 (61%)	0.3837
Female	13 (32%)	66 (39%)	
Age (years)	64.51±1.92	64.78±0.94	0.8979
CEA			
Normal	25 (76%)	88 (58%)	0.0564
Increased	8 (24%)	64 (42%)	
Location			
Colon	21 (51%)	112 (66%)	0.0728
Rectum	20 (49%)	57 (34%)	
Tumor size (mm)			
<35	23 (56%)	34 (20%)	<0.0001
≥35	18 (44%)	135 (80%)	
LN metastasis			
Absent	32 (78%)	106 (63%)	0.0636
Present	9 (22%)	63 (37%)	
Recurrence			
Absent	36 (88%)	153 (91%)	0.6015
Present	5 (12%)	16 (9%)	
Tumor differentiation			
Well	32 (78%)	107 (63%)	0.0736
Other	9 (22%)	62 (37%)	
Depth of invasion			
Tis-T1	18 (44%)	12 (7%)	<0.0001
T2-4	23 (56%)	157 (93%)	
Lymphatic invasion			
Absent	30 (73%)	92 (54%)	0.0292
Present	11 (27%)	77 (46%)	
Venous invasion			
Absent	22 (54%)	38 (22%)	<0.0001
Present	19 (46%)	131 (78%)	

Table V. *Univariate analysis of correlation between IGF-1R staining and clinicopathological factors.*

	IGF-1R expression		
	Negative (n=71)	Positive (n=139)	*p*-Value
Gender			
Male	48 (68%)	83 (60%)	
Female	23 (32%)	56 (40%)	0.240
Age (years)	66.5±1.45	63.8±1.04	0.1341
CEA			
Normal	39 (63%)	74 (60%)	
Increased	23 (37%)	49 (40%)	0.7182
Location			
Colon	46 (65%)	87 (63%)	
Rectum	25 (35%)	52 (37)	0.7544
Tumor size (mm)			
<40	33 (46.5%)	41 (29.5%)	
≥40	38 (53.5%)	98 (70.5%)	0.0148
LN metastasis			
Absent	46 (65%)	92 (66%)	
Present	25 (35%)	47 (34%)	0.8400
Recurrence			
Negative	63 (89%)	126 (91%)	
Positive	8 (11%)	13 (9%)	0.6617
Tumor differentiation			
Well	50 (70%)	89 (64%)	
Others	21 (30%)	50 (36%)	0.3542
Depth of invasion			
Tis-T1	15 (21%)	15 (11%)	
T2-T4	56 (79%)	124 (89%)	0.0429
Lymphatic invasion			
Absent	43 (61%)	79 (57%)	
Present	28 (39%)	60 (43%)	0.6044
Venous invasion			
Absent	24 (34%)	36 (26%)	
Present	47 (66%)	103 (74%)	0.2304

Table IV. *Correlation of expression of IGF-1 and clinicopathological factors using multivariate logistic regression analysis.*

	Odds ratio	95% CI	*p*-Value
CEA Increased vs. normal	1.229	0.467-3.402	0.6792
Depth of invasion T2-4 *vs*. Tis-T1	4.378	1.349-14.830	0.0147
Tumor size 35 mm *vs*. <35 mm	4.058	1.642-10.156	0.0024
Lymphatic invasion Present *vs*. absent	1.513	0.595-3.981	0.3876
Venous invasion Present *vs*. absent	1.953	0.670-5.421	0.2039

Table VI. *Multivariate logistic regression analysis of correlation between expression of IGF-1R and clinicopathological factors.*

	Odds ratio	95% CI	*p*-Value
Depth of invasion T2-4 *vs*. Tis-T1	1.678	0.720-3.894	0.228
Tumor size (mm) ≥40 *vs*. <40	1.816	0.962-3.421	0.0658

metastasis probably depends on multiple factors, whereas IGF may only contribute to a part of this complex process.

As IGF-1 is one of the growth factors associated with colorectal cancer, this might have been the reason for positive correlation between expression of IGF-1, IGF-1R and tumor size. In the circulation, IGF-1 binds mainly to the major IGF-binding protein, IGFBP-3, which is believed to inhibit the IGF-1-mediated effects (4). Because the biological effects of IGF-1 are determined by the balance of IGF-1 and IGFBP-3, it may be necessary to consider the expression of IGFBP-3 together with IGF. IGFBP-3 was not assessed here, which might be considered as a drawback of this study.

The expression of staining of IGF-1 seems to be more frequent than that of IGF-1R in colorectal cancer specimens. The clinicopathological factors in colorectal cancer that have significant association with IGF-1 and IGF-1R expression are tumor size and depth of invasion, but the independence of significance of these associations was only confirmed for IGF-1.

References

1 Giovannucci E: Insulin and colon cancer. Cancer Causes Control *6*: 164-179, 1995.

2 Kaaks R, Toniolo P, Akhmedkhanov A, Lukanova A, Biessy C, Dechaud H, Rinaldi S, Zeleniuch-Jacquotte A, Shore RE and Riboli E: Serum C-peptide, IGF-I, IGFBPs, and colorectal cancer risk in women. J Natl Cancer Inst *92*: 1592-1600, 2000.

3 Khandwala HM, McCutcheon IE: The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr Rev *21*: 215-244, 2000.

4 Jones JI and Clemmons DR: Insulin-like growth factor and their binding proteins: Biological action. Endocr Rev *16*: 3-34, 1995.

5 Clemmons DR: Role of insulin-like growth factor binding proteins in controlling IGF actions. Mol Cell Endocrinol *140*: 19-24, 1998.

6 Cham JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH and Pollak M: Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science *279*: 563-566, 1998.

7 Hankinson SE, Willet WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE and Pollack M: Circulating concentration of insulin-like growth factor-I and risk of breast cancer. Lancet *351*: 1393-1396, 1998.

8 Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH and Stampfer MJ: Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst *91*: 620-625, 1999.

9 Yu H, Spitz MR, Mistry J, Gu J, Hong WK and Wu X: Plasma level of insulin-like growth factor-I and lung cancer risk: a case–control analysis. J Natl Cancer Inst *91*: 151-156, 1999.

10 Min Y, Adachi, Yamamoto H, Ito H, Itoh F, Lee CT, Nadaf S, Carbone DP and Imai K: Genetic blockade of the insulin-like growth factor-I receptor: a promising strategy for human pancreatic cancer. Cancer Res *63*: 6432-6441, 2003.

11 Min Y, Adachi Y, Yamamoto H, Imsumran A, Arimura Y, Endo T, Hinoda Y, Lee CT, Nadaf S, Carbone DP and Imai K: Insulin-like growth factor I receptor blockade enhances chemotherapy and radiation responses and inhibits tumor growth in human gastric cancer xenografts. Gut *54*: 591-600, 2005.

12 Imsumran A, Adachi Y, Yamamoto H Li R, Wang Y, Min Y, Piao W, Nosho K, Arimura Y, Shinomura Y, Hosokawa M, Lee CT, Carbone DP and Imai K: Insulin-like growth factor-I receptor as a maker for prognosis and a therapeutic target in human esophageal squamous cell carcinoma. Carcinogenesis *28*: 947-956, 2007.

13 Guo YS, Narayan S, Yallampalli C and Singh P: Characterization of insulin-like growth factor I receptors in human colon cancer. Gastroenterology *102*: 1101-1108, 1992.

14 Guo YS, Jin GF, Townsend CM Jr., Zhang T, Sheng HM, Beauchamp RD and Thompson JC: Insulin-like growth factor-II expression in carcinoma in colon cell lines: implications for autocrine actions. J Am Coll Surg *181*: 145-154, 1995.

15 Hakam A, Yeatman TJ, Lu L, Mora L, Marcet G, Nicosia SV, Karl RC and Coppola D: Expression of insulin-like growth factor-1 receptor in human colorectal cancer. Hum Pathol *30*: 1128-1133, 1999.

16 Weber MM, Fottner C, Liu SB, Jung MC, Engelhardt D and Baretton GB: Overexpression of the insulin-like growth factor I receptor in human colon carcinomas. Cancer *95*: 2086-2095, 2002.

17 Peters G, Gongoll S, Langner C, Mengel M, Piso P, Klempnauer J, Ruschoff J, Kreipe H and Wasielewski R: IGF-1R, IGF-1 and IGF-2 expression as potential prognostic and predictive markers in colorectal cancer. Virchows Arch *443*: 139-145, 2003.

18 Freier S, Weiss O, Eran M, Flyvbjerg A, Dahan R, Nephesh I, Safra T, Shiloni E and Raz I: Expression of the insulin-like growth factors and their receptors in adenocarcinoma of the colon. Gut *44*: 704-708, 1999.

19 Wu Y, Yakar S, Zhao L, Hennighausen L and LeRoith D: Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis. Cancer Res *62*: 1030-1035, 2002.

20 M Nakamura, Miyamoto S, Maeda H, Zhang SC, Sangai T, Ishii G, Hasebe T, Endoh Y, Saito N, Asaka M and Ochiai A: Low levels of insulin-like growth factor type 1 receptor expression at cancer cell membrane predict liver metastasis in Dukes' C human colorectal cancers. Clin Cancer Res *10*: 8434-8441, 2004.

Received April 7, 2011
Revised June 10, 2011
Accepted June 13, 2011