Low Levels of Insulin-Like Growth Factor Type 1 Receptor Expression at Cancer Cell Membrane Predict Liver Metastasis in Dukes' C Human Colorectal Cancers

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ABSTRACT

Purpose: **The aim of this study was to evaluate the prognostic significance of insulin-like growth factor type 1 receptor (IGF-1R) expression in Dukes' C human colorectal cancers (CRCs).**

Experimental Design: **Immunohistochemical staining for IGF-1R was done on formalin-fixed, paraffin-embedded specimens from 161 patients with curatively resected Dukes' C CRC and at least 5-year follow-up periods. We investigated the association between the levels of IGF-1R expression and the clinicopathologic parameters. To evaluate the accurate prognostic value of IGF-1R expression, we investigated two patterns of recurrence-free survival (RFS) according to the mode of recurrence, the hepatic-RFS (H-RFS), and the nonhepatic-RFS (nH-RFS). The influence of the pattern of IGF-1R immunostaining (membranous or cytoplasmic) on RFS was also estimated.**

Results: **High (diffuse staining) and low (focal staining) levels of IGF-1R expression were found in 45 (28%) and 116 (72%) specimens, respectively. The recurrence rate was significantly higher in the latter group (49 of 116) than the former group (9 of 45; $P = 0.01$). H-RFS was significantly longer for the former group than the latter group ($P = 0.021$), whereas no difference was found in nH-RFS between the two groups ($P = 0.121$). In multivariate analysis, the level of IGF-1R expression was an independent factor for H-RFS ($P = 0.015$) as were the depth of invasion and lymph vessel invasion ($P = 0.006$ and 0.022, respectively). Using a combination of the level of IGF-1R expression and these two factors, the prognostic value was further increased. When IGF-1R staining patterns (membranous or cytoplasmic) were compared, membrane staining of IGF-1R possessed prognostic significance.**

Conclusions: **In Dukes' C CRC, focal membrane expression of IGF-1R in the primary tumor can predict a high risk of recurrence, especially liver metastasis. Understanding the mechanisms involved could lead to new therapeutic approaches for advanced CRC.**

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies in Western countries. Its prognosis after curative surgery depends almost completely on the appearance of metachronous metastases, especially liver metastases (1). To improve the prognosis of CRC, the most important considerations are the selection of high-risk patients and subsequently the indication of appropriate adjuvant therapy. Adjuvant chemotherapy in patients with Dukes' C CRC after curative resection is quite useful for increasing overall and disease-free survival (1–5). However, patients with Dukes' C CRC usually have a recurrence rate of 40 to 50%. Conversely, the other 50 to 60% of Dukes' C patients have no recurrence, and these patients have been forced to receive adjuvant chemotherapy unnecessarily. To increase the survival benefit of adjuvant chemotherapy, patient selection using appropriate biomarkers would be a desirable approach (4, 6).

The insulin-like growth factor (IGF) system is associated with the regulation of cell growth, transformation, and apoptosis in CRCs (7–17). Overexpression of IGF-1R has also been described in several colon cancer cell lines and in human colorectal cancer tissues (18–23). Although some studies have attempted to show the prognostic value of IGF-1R expression in CRC by immunohistochemical analysis, the importance of IGF-1R in the recurrence of CRC has not been elucidated (20–22). Hakam *et al.* (20) reported a correlation between the intensity of the IGF-1R staining and CRC tumors of high grade and high stage. Weber *et al.* (21) showed significantly stronger IGF-1R immunostaining in CRC cells compared with adjacent normal colonic epithelial cells. However, these two reports did not refer to any correlation between IGF-1R expression and patient survival rate. On the other hand, Peters *et al.* (22) reported that univariate survival analyses for IGF-1R had revealed no relevant findings because almost all cases (99.6%)

Received 3/11/04; revised 6/15/04; accepted 7/1/04.
Grant support: in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan and by a Grant-in-Aid for the 2nd Term Comprehensive 10-Year-Strategy for Cancer Control from the Ministry of Health and Welfare of Japan. M. Nakamura, S. Miyamoto, H. Maeda, S. Zhang, and T. Sangai are awardees of Research Resident Fellowships from the Foundation for Promotion of Cancer Research in Japan.
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were positive for IGF-1R expression in the primary CRC. It is noteworthy that all these reports investigated all recurrences of CRCs and did not focus on liver-specific recurrence. Recently, IGFs have also been noted to play a critical role in CRC liver metastasis as well as in primary tumor growth (7, 17, 24–28). We developed a hypothesis that the levels of IGF-1R in CRC primary tumor might predict liver metastasis.

We investigated the expression of IGF-1R immunohistochemically in 161 curatively resected Dukes' C CRC tissue samples, using an anti-IGF-1R mouse monoclonal antibody that recognizes the α subunit of IGF-1R. To estimate the prognostic value of IGF-1R, we separated the population into a recurrence-free group ($n = 103$), a recurrence to the liver group ($n = 31$), and a recurrence to other sites group ($n = 27$), and we investigated the relations between each group and IGF-1R levels. We also examined the influence of different staining patterns of IGF-1R (membrane or cytoplasmic) on the recurrence-free survival (RFS) rate.

PATIENTS AND METHODS

Patients. The study subjects comprised 161 patients with completely resected Dukes' C CRC who underwent surgery at the National Cancer Center Hospital East between July 1992 and December 1998. Patients who received chemotherapy and radiation therapy before or after surgery were excluded from this study. The median follow-up time was 5.2 years.

Antibodies. To evaluate functional IGF-1R expression, we tested five commercially available mouse monoclonal antibodies against human IGF-1R α subunit, MAB1120, MAB1122 (clone 24–31 and 24–57, respectively; CHEMICON International, Inc., Temecula, CA), 2C8 (sc-463; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), Ab-1 (clone αIR-3; Oncogene Research Products, San Diego, CA), and Ab-4 (clone 24–60; Lab Vision Corporation, Fremont, CA). The results of preliminary experiments clearly showed that MAB1120 is the most suitable for immunohistochemical analysis in formalin-fixed paraffin-embedded sections. To confirm the reproducibility of previous reports using an antibody against human IGF-1R β subunit (20, 22), an anti-IGF-1Rβ rabbit polyclonal antibody C-20 (sc-713; Santa Cruz Biotechnology, Inc.) was selected.

Expression of IGF-1R. All tumor tissues used in this study were derived from the routine formalin-fixed pathologic samples taken from the resected colorectal specimens. One block with the most deeply invasive edge was selected from each specimen, after a review of H&E-stained slides of the surgical specimens. Sections (5 μm) were cut from the paraffin blocks and mounted on silanized slides. The sections were deparaffinized in xylene, dehydrated with graded EtOH, and then immersed in methanol with 0.3% hydrogen peroxide for 20 minutes to inhibit endogenous peroxidase activity. After being washed with distilled water, the sections were placed in a 10 mmol/L citrate buffer solution.

For antigen retrieval, the slides were heated twice at 95°C for 10 minutes in a microwave oven (H2800 Microwave Processor, Energy Beam Sciences Inc.) and then cooled for 1 hour at room temperature. The slides were washed three times in PBS, then nonspecific binding was blocked by preincubation with 2% normal swine serum in PBS (blocking buffer) for 60 minutes at room temperature. Individual slides were then incubated overnight at 4°C with anti-IGF-1R antibody, MAB1120 or C-20, at a final concentration of 2 μg/mL (dilution 1:100) in the blocking buffer. The slides were washed five times with PBS, then incubated with a peroxidase-labeled polymer conjugated to goat antimouse IgG (DAKO EnVision Peroxidase Mouse, code K4000; Dako Corp., Carpinteria, CA) and goat antirabbit IgG (DAKO EnVision Peroxidase Rabbit, code K4002; Dako Corp.) for 1 hour at room temperature. After extensive washing with PBS, the color reaction was developed in 2% 3,3′-diaminobenzidine in 50 mmol/L Tris-buffer (pH 7.6) containing 0.3% hydrogen peroxide for 5 to 10 minutes. The sections were then counterstained with Meyer's hematoxylin, dehydrated, and mounted.

In the negative controls, the primary antibody solution was substituted with the blocking buffer.

Immunoabsorption. An anti-IGF-1R antibody MAB1120 was incubated overnight at 4°C with 50-fold quantity of a recombinant human IGF-1Rα (catalogue number 391-GR; R&D Systems, Inc., Minneapolis, MN) derived from a DNA sequence encoding the extracellular domain of IGF-1R prepropeptide (MAB1120/recombinant human IGF-1Rα, 400 ng/20μg). The mixture was centrifuged at 15,000 × *g* for 30 minutes at 4°C. Supernatant (immunoabsorbed fraction) was used as a substitute for the primary antibody.

Western Blotting. Total cell lysate from human colon cancer cells (HT29) was collected as described previously (26). Samples of 40 μg of total cell lysate were electrophoresed on a 7.5% SDS-polyacrylamide mini-gel, and the separated proteins blotted electrophoretically onto a polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA). Nonspecific binding was blocked for 1 hour with 5% nonfat dry milk and 1% BSA in PBS (pH 7.4) containing 0.1% Tween 20 at room temperature. The membrane was incubated overnight in the anti-IGF-1R antibody MAB1120 (1:250) or the supernatant of immunoabsorption test (1:250) at 4°C and then for 1 hour with peroxidase-labeled rabbit antimouse antibody (1:3,000; Zymed Laboratories, Inc., San Francisco, CA). The IGF-1R bands were visualized with Lumi-Light PLUS (Roche Diagnostics Corp., Indianapolis, IN).

In the negative controls, the primary antibody solution was replaced by the blocking buffer.

Evaluation of Immunostaining. Immunohistochemical staining was evaluated independently by three investigators (M. N, S. M, and A. O) who were blinded to the clinical outcomes. To evaluate functional IGF-1R expression, we used an anti-IGF-1R antibody MAB1120 and evaluated focusing on only the cell membrane expression and ignoring cytoplasmic staining (defined as CRITERIA-1). The immunohistochemical results for IGF-1R were classified under the "diffuse-positive pattern" if the tumor cells showed an unequivocally strong membranous reaction in >50% of the carcinoma cells (Fig. 2*C*). Cases with faint staining or with a positive reaction in <50% of tumor cells were classified under the "focal-positive pattern" (Fig. 2*D*). Two different criteria were applied for evaluation: CRITERIA-1, in which positive staining was evaluated in membranous staining alone, and CRITERIA-2, in which evaluation of either membranous or cytoplasmic staining was done. CRITERIA-1 was

used because of the importance of evaluating membrane staining alone. CRITERIA-2, which had been used in previous studies (20–22), was used to enable comparison with the earlier immunohistochemical staining studies. When evaluating the staining pattern of anti-IGF-1Rβ antibody C-20, we targeted cytoplasmic staining alone.

Detection and Mode of Recurrence. Abdominal computed tomography, ultrasound sonography (US), and chest X-ray were done every 3 months during the 1st year after the resection and every 6 months thereafter. The mode of recurrence was defined as that initially detected. The hepatic-recurrence free survival (H-RFS) and nonhepatic-recurrence free survival (nH-RFS) were calculated from the day of surgical resection to the day of first detection of liver metastasis and other sites of metastasis, respectively.

Statistical Analysis. In the analysis of H-RFS or nH-RFS, patients who died of a disease other than colon cancer recurrence were censored at the date of death. Statistical analysis of the association between the levels of IGF-1R expression and clinicopathologic parameters was based on Fisher's exact test. The association between the clinicopathologic parameters and the risk of recurrence was evaluated with the Cox proportional hazard model. We plotted H-RFS and nH-RFS curves as graphs using calculations based on the Kaplan-Meier method. Survival differences between the two groups were assessed with the log-rank test. The observations determined to be statistically significant in the univariate analysis were subsequently subjected to a multivariate analysis. In all analyses, a P value <0.05 was considered statistically significant.

Table 1 Correlation between IGF-1R staining and clinicopathologic factors

Variable	Cases (%) (N = 161)	IGF-1R staining: Focal positive (%)	IGF-1R staining: Diffuse positive (%)	P value
Age				
≤65 years	109 (68)	76 (70)	33 (30)	
>65 years	52 (32)	40 (77)	12 (23)	NS
Gender				
Male	97 (60)	71 (73)	26 (27)	
Female	64 (40)	45 (70)	19 (30)	NS
Location				
Colon	87 (54)	62 (71)	25 (29)	
Rectum	74 (46)	54 (73)	20 (27)	NS
Tumor size				
≤5 cm	88 (55)	64 (73)	24 (27)	
>5 cm	73 (45)	52 (71)	21 (29)	NS
Histologic type				
W/D & M/D	137 (85)	96 (70)	41 (30)	
Others	24 (15)	20 (83)	4 (17)	NS
Depth				
Serosa negative	68 (42)	51 (75)	17 (25)	
Serosa positive	93 (58)	65 (70)	28 (30)	NS
Lymph vessel invasion				
Absent	70 (43)	52 (74)	18 (26)	
Present	91 (57)	64 (70)	27 (30)	NS
Venous invasion				
Absent	34 (21)	22 (65)	12 (35)	
Present	127 (79)	94 (74)	33 (26)	NS
Recurrence				
Absent	103 (64)	67 (65)	36 (35)	
Present	58 (36)	49 (84)	9 (16)	0.010
[Liver	31 (19)	27 (87)	4 (13)	0.024] *

Abbreviations: W/D, well differentiated; M/D, moderately differentiated; NS, not significant.

* Hepatic recurrence cases compared with free cases.

RESULTS

Clinicopathologic Characteristics of the Subjects. The patients included 97 men and 64 women, ranging from 26 to 92 years (mean, 61 years; Table 1).

The primary tumors analyzed here were distributed as follows: 26 tumors in the cecum and ascending segment, 7 in the transverse segment, 12 in the descending segment, 42 in the sigmoid segment, and 74 in the rectum. The median tumor size was 5.0 cm with a range of 1 to 13.5 cm. Histologic types of the primary tumors were determined as well differentiated in 22 cases, moderately differentiated in 115 cases, and others (including 15 poorly differentiated adenocarcinomas, 7 mucinous adenocarcinomas, 1 signet ring cell carcinoma, and 1 adenosquamous cell carcinoma) in 9 cases. The depth of invasion of primary tumors was serosa-positive in 93 cases and serosa-negative in 68 cases. Lymph vessel and venous invasion of tumor cells was found in 91 cases and 127 cases, respectively.

For at least 5 years after curative surgery, all patients were carefully followed to determine postoperative survival. During this period, tumors recurred in 58 patients (36%). Sites of recurrence included the liver in 31 patients, lung in 8 patients, lymph nodes in 5 patients, the peritoneum in 4 patients, bone in 1 patient, and the recurrence was local in 9 patients.

Specificity of an Anti-IGF-1R Antibody MAB1120. We did an immunoabsorption test to confirm the specificity of the anti-IGF-1R antibody, MAB1120. The immunoreactivity of IGF-1R by MAB1120 (Fig. 1*A*) was diminished after immunoabsorption by recombinant human IGF-1Rα (Fig. 1*B*) to the level of the negative control (Fig. 1*C*). Furthermore, the specificity was also tested by Western blot analysis against the lysates of HT-29 cells expressing IGF-1R. A band corresponding to the α subunit of the IGF-1R (M_r 130,000) was detected by MAB1120, and this band disappeared after the immunoabsorption procedure (Fig. 1*D*).

Immunoreactivity of IGF-1R. For evaluating IGF-1R expression of Dukes' C CRC tissues, we confirmed that two antibodies, MAB1120 and C-20, were suitable for immunohistochemical analysis (Fig. 2*A* and *B*, respectively). In cases that used MAB1120, IGF-1R expression was distributed in both the membrane and cytoplasm of the tumor cells (Fig. 2*A*). On the other hand, in cases that used C-20, IGF-1R expression was distributed diffusely in the cytoplasm of the tumor cells, and positive staining was observed in 158 of 161 specimens (98.1%; Fig. 2*B*). There was no correlation between IGF-1R expression and the prognosis. This result was compatible with a previous report in which the same antibody used (20). Therefore, we used the anti-IGF-1Rα antibody, MAB1120, for the further evaluation of IGF-1R expression in this study.

Relations between the Levels of IGF-1R Expression and Clinicopathologic Factors. Using MAB1120, we observed two typical immunostaining patterns for IGF-1R according to CRITERIA-1 (Fig. 2*C*, diffuse-positive case; 2*D*, focal-positive

case). Of the 161 colorectal cancers analyzed, 45 (28%) were classified as diffuse-positive cases, and 116 (72%) were focal-positive cases. Expression levels of IGF-1R in CRCs were first compared with the clinicopathologic factors. In the various clinicopathologic factors, the recurrence rate was significantly higher in focal-positive cases than in diffuse-positive cases [42% (49 of 116) *versus* 20% (9 of 45), respectively; $P = 0.01$]. The association between the IGF-1R expression level and recurrence was more evident in the cases with metastases to the liver than to other sites (Table 1).

Influence of Clinicopathologic Factors, Including IGF-1R Expression, on Recurrence-Free Survival. To elucidate the prognostic factors for Dukes' C CRC patients, we investigated IGF-1R expression and several clinicopathologic factors for liver metastasis using CRITERIA-1 evaluated by membranous staining alone. A univariate analysis showed that the depth of invasion, lymph vessel invasion, venous invasion, and IGF-1R expression pattern was associated with H-RFS ($P = 0.003$, 0.023, 0.02, and 0.021, respectively; Table 2). The specimens with focal-positive IGF-1R expression had a significantly shorter H-RFS than those with diffuse-positive expression ($P = 0.021$, Fig. 3*A*), whereas IGF-1R expression was not associated with nH-RFS (Fig. 3*B*).

Subsequently, a multivariate analysis of these four variables indicated that IGF-1R expression, the depth of invasion, and lymph vessel invasion could independently affect H-RFS ($P = 0.015$, 0.006, and 0.022, respectively; Table 3). Furthermore, double stratification for IGF-1R expression and lymph vessel invasion showed that patients with focal-positive IGF-1R expression and positive lymph vessel invasion had significantly shorter H-RFS than those with diffuse-positive IGF-1R expression and negative lymph vessel invasion (log-rank test, $P = 0.005$; Fig. 4*A*). On the other hand, double stratification for IGF-1R expression and the depth of tumor invasion showed that patients with focal-positive IGF-1R expression and serosa positivity had significantly shorter H-RFS than those with diffuse-positive IGF-1R expression and serosa negativity, those with diffuse-positive expression and serosa positivity, and those with focal-positive expression and serosa negativity (log rank, $P = 0.013$, 0.014, and 0.002, respectively; Fig. 4*B*).

Influence of Two Different Criteria of IGF-1R on Recurrence-Free Survival. We also investigated the influence of the two different criteria, CRITERIA-1 and CRITERIA-2, on RFS. As shown in Table 4, in the case of using CRITERIA-1 on H-RFS, we classified 40 (30%) and 94 (70%)

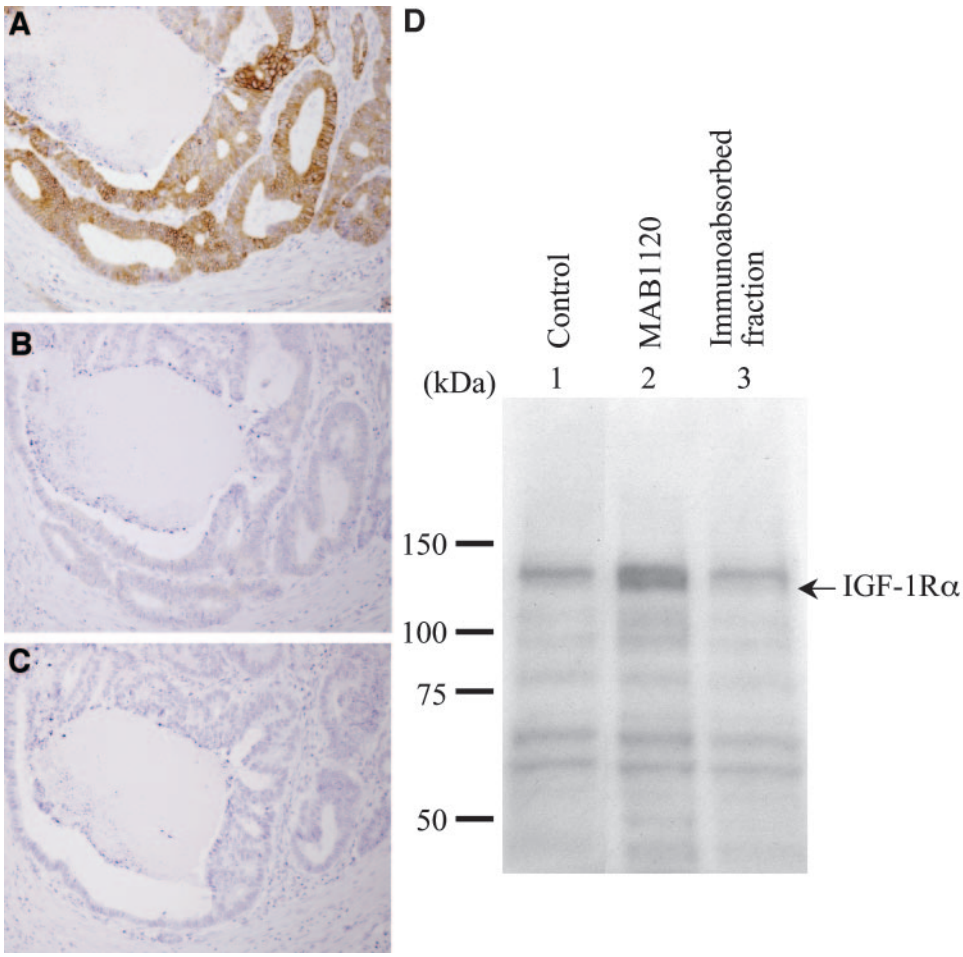

Fig. 1 Specificity of the anti-IGF-1Rα antibody, MAB1120. Positive staining for IGF-1Rα by MAB1120 is observed in the colorectal cancer cells (*A*). Immunoreactivity of IGF-1R was diminished after immunoabsorption (*B*) as complete as the negative control (*C*; original magnification, ×200). The specificity of MAB1120 was also analyzed by Western blotting: control (*Lane 1*), MAB1120 (*Lane 2*), and immunoabsorbed fraction (*Lane 3*). A band corresponding to the α subunit of the IGF-1R (M_r 130,000) is markedly diminished after immunoabsorption procedure (*arrow*, *D*).

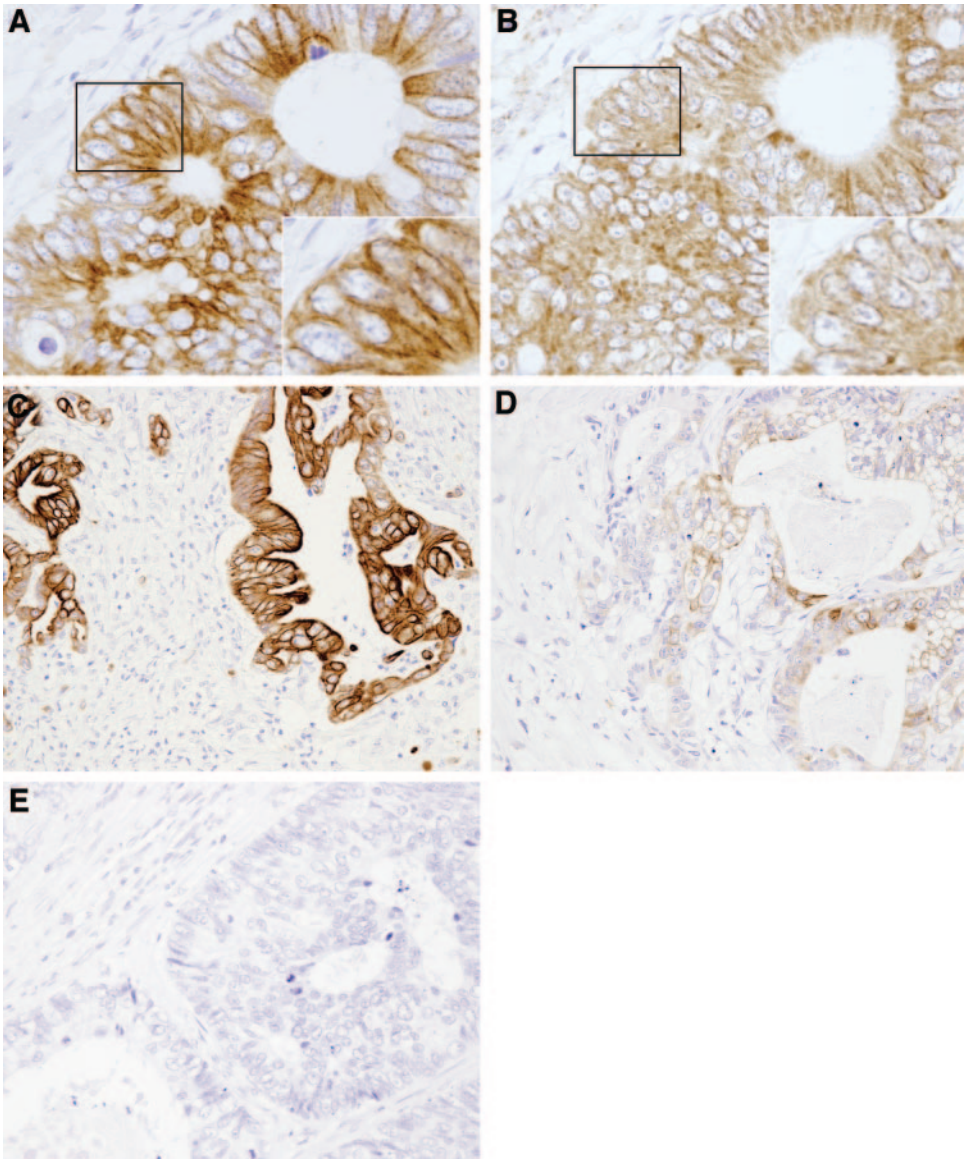

Fig. 2 Representative examples of immunohistochemistry in Dukes' C CRC. Anti-IGF-1Rα antibody MAB1120 and anti-IGF-1Rβ antibody C-20 (*A* and *B*, respectively; shown at higher magnification in the *inset images*). *C*, typical pattern of IGF-1R diffuse-positive staining by MAB1120. *D*, typical pattern of IGF-1R focal-positive staining by MAB1120. *E*, negative control. Original magnification, ×200 for *C*, *D*, and *E*; ×400 for *A* and *B*.

specimens as diffuse positive and focal positive, respectively. On the other hand, in the case of using CRITERIA-2 on H-RFS, 63 (47%) and 71 (53%) specimens were classified as diffuse positive and focal positive, respectively. That is, 23 specimens defined as diffuse positive in CRITERIA-2 were transvalued as focal positive in CRITERIA-1. Compared with Fig. 3 using CRITERIA-1 (evaluated by membranous staining alone), we found no difference between the focal-positive group and diffuse-positive group in RFS using CRITERIA-2 (evaluated by either membrane or cytoplasmic staining; Fig. 5*A* and *B*). In neither CRITERIA-1 nor CRITERIA-2 did the levels of IGF-1R expression affect overall survival ($P = 0.098$ and 0.741, respectively).

DISCUSSION

We showed that low membranous expression of IGF-1R was a significant and independent unfavorable prognostic factor in patients with Dukes' C CRCs. Our results raise the possibility that IGF-1R expression alone or in combination with other conventional pathologic factors could be a surrogate marker for liver metastasis after curative resection of Dukes' C CRCs and might provide a guide as to which patients should undergo adjuvant chemotherapy. In particular, it was noteworthy that the combination of IGF-1R expression and a simple depth of tumor measurement by pathologic assessment could predict a better outcome.

Previous reports that evaluated the prognostic value of IGF-1R expression using immunohistochemical techniques did not take account of the mode of recurrence (20–22). IGFs are noted to play a critical role in not only the primary growth but also in liver metastasis from CRCs (7, 17, 24–29). One reason why we could show the prognostic significance of IGF-1R may be attributable to the focus of the study on the mode of recurrence, especially liver metastases.

Another reason is the difference in our definition of IGF-1R expression. In all previous studies evaluating the prognostic value of IGF-1R expression in CRCs, the levels of IGF-1R expression were defined both by cell membrane staining and by cytoplasmic staining (CRITERIA-2). Because IGF-1R is a transmembrane tyrosine kinase receptor, like the members of the human epidermal growth receptor family, it is reasonable that only membranous staining of IGF-1R, which implies a functional receptor, had prognostic significance in H-RFS. Conversely, cytoplasmic staining of IGF-1R may imply a nonfunctional receptor, including precursor molecules of the receptor and degraded and recycled receptors (30). Our results correspond to several immunohistochemical studies in breast cancer that regarded membranous staining as the only evidence for the assessment of human epidermal growth receptor-2 expression (31). Moreover, it might be more difficult to evaluate the membrane staining precisely with an anti-IGF-1R antibody toward the β subunit (intracellular) portion of the receptor, which could not discriminate between cell membrane staining and cytoplasmic staining of the receptor. However, we used an antibody that recognized the extracellular α subunit of the receptor, showed no cross-reaction with the insulin receptor, and could simplify the evaluation of IGF-1R expression.

Our results, which indicated an association between low levels of IGF-1R and a high risk of metastasis, may be paradoxical. However, similar results have been reported in several studies that used clinical samples of breast and prostate cancers (32–35). In animal models, Pennisi *et al.* (36) described that a decrease in the expression of IGF-1R in MCF-7 cells led to increased motility and decreased ability to form aggregates in culture, resulting in characteristics consistent with a more metastatic phenotype. Mauro *et al.* (37) showed that overexpression of IGF-1R significantly decreased the invasiveness of MCF-7/6

Table 2 Influences of several cofactors on H-RFS: univariate analysis

Variable	Cases (N = 134)	Metastasis rate (%)	HR (95% CI)	P value (Log-rank test)
Location				
Colon	70	14 (20)	Referent	
Rectum	64	17 (27)	1.40 (0.69–2.83)	NS
Tumor size				
≤5 cm	73	16 (22)	Referent	
>5 cm	61	15 (25)	1.13 (0.56–2.29)	NS
Histologic type				
W/D & M/D	120	27 (23)	Referent	
Others	14	4 (29)	1.40 (0.45–4.89)	NS
Depth				
Serosa negative	60	7 (12)	Referent	
Serosa positive	74	24 (32)	3.27 (1.42–5.80)	0.003
Lymph vessel invasion				
Absent	63	9 (14)	Referent	
Present	71	22 (31)	2.39 (1.12–4.58)	0.023
Venous invasion				
Absent	29	2 (7)	Referent	
Present	105	29 (28)	4.69 (1.17–6.03)	0.020
IGF-1R expression				
Diffuse positive	40	4 (10)	Referent	
Focal positive	94	27 (28)	3.21 (1.14–5.16)	0.021

Abbreviations: W/D, well differentiated; M/D, moderately differentiated; HR, hazard ratio; CI, confidence interval; NS, not significant.

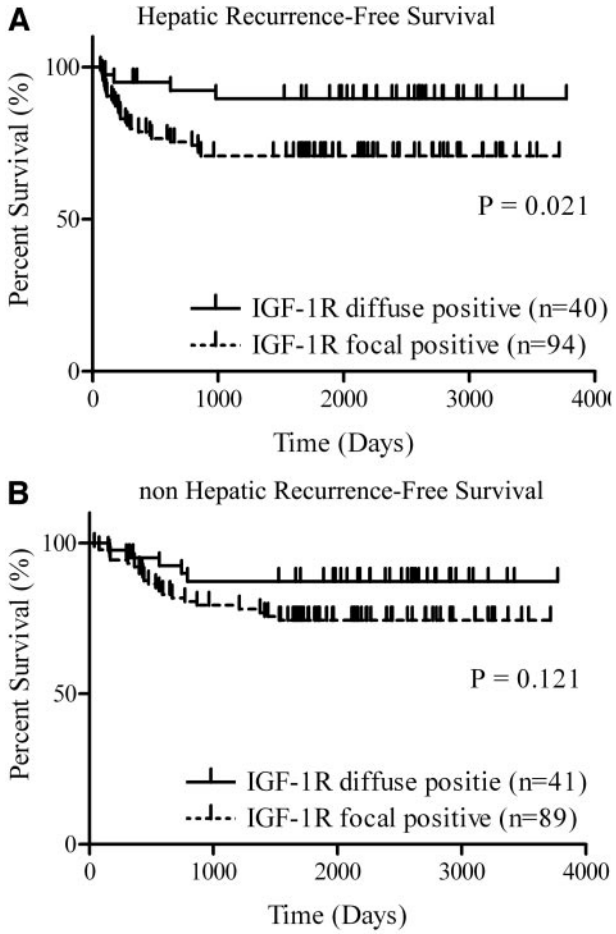

Fig. 3 Kaplan-Meier survival curves showing the levels of IGF-1R expression evaluated by membrane staining on H-RFS (*A*) and on nH-RFS (*B*).

Table 3 Influences of several cofactors on H-RFS: multivariate analysis

Variable	Cases (N = 134)	HR (95% CI)	P value
IGF-1R expression			
Diffuse positive	40	Referent	
Focal positive	94	3.70 (1.29–10.63)	0.015
Depth			
Serosa negative	60	Referent	
Serosa positive	74	3.32 (1.42–7.75)	0.006
Lymph vessel invasion			
Absent	63	Referent	
Present	71	2.48 (1.14–5.41)	0.022
Venous invasion			
Absent	29	Referent	
Present	105	3.54 (0.84–14.98)	NS

Abbreviations: W/D, well differentiated; M/D, moderately differentiated; HR, hazard ratio; CI, confidence interval; NS, not significant.

cells and suggested that overexpression of IGF-1R might reduce metastatic potential in E-cadherin–positive cells. Lee *et al.* (38) suggested two possible explanations in their review of the IGF system in breast cancer. First, a high expression of IGF-1R may reflect an absence of ligands and low receptor signaling, whereas a low IGF-1R content may indicate that the receptor is fully active, thus stimulating tumor growth and leading to a worse prognosis. Second, IGF-1R expression could also identify a relatively well-differentiated tumor that still requires IGF for proliferation. In addition, on the basis of a report indicating that tumor microenvironmental stress, like hypoxia, low pH, and low glucose, could increase the IGF-1R expression (39), we speculated that IGF-1R expression might indicate the requirement of the IGF system for adapting to tumor microenvironmental stress. Conversely, the cancer cells with low levels of IGF-1R may represent a subpopulation that was able to adapt to the stress and thus develop a more metastatic phenotype.

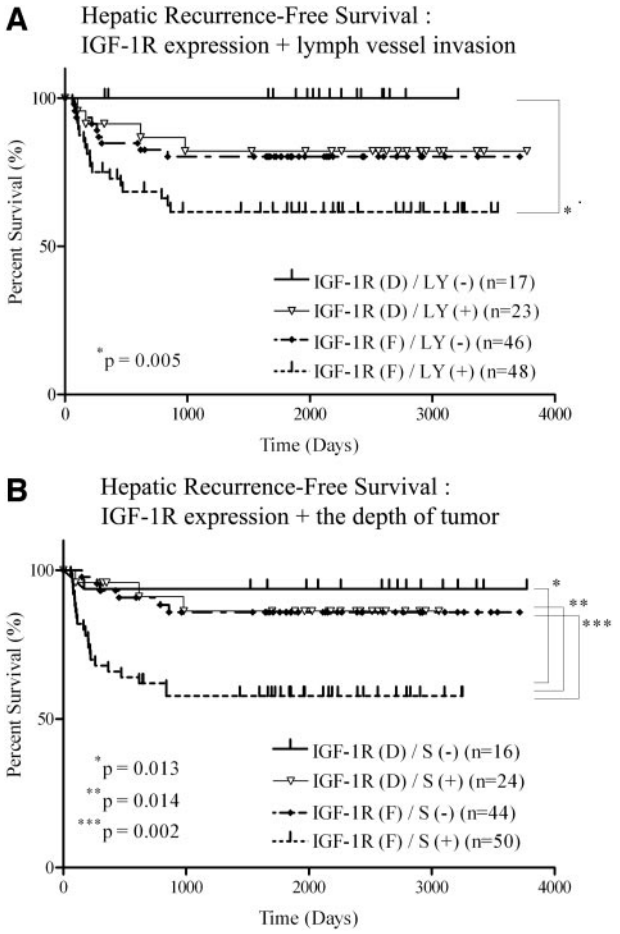

Fig. 4 Kaplan-Meier survival curves showing the levels of IGF-1R expression combined with the presence or absence of lymph vessel invasion (*A*) and the depth of tumor (*B*) evaluated by membrane staining alone (CRITERIA-1) on H-RFS. *D*, diffuse-positive cases of IGF-1R expression; *F*, focal-positive cases of IGF-1R expression; *LY*, lymph vessel invasion; *S*, Serosa.

Table 4 Influences of two different criteria of IGF-1R on H-RFS

IGF-1R expression	CRITERIA-1 (membranous staining alone)	CRITERIA-2 (membranous and cytoplasmic staining)
Diffuse positive	30% (40/134)	47% (63/134)
Focal positive	70% (94/134)	53% (71/134)

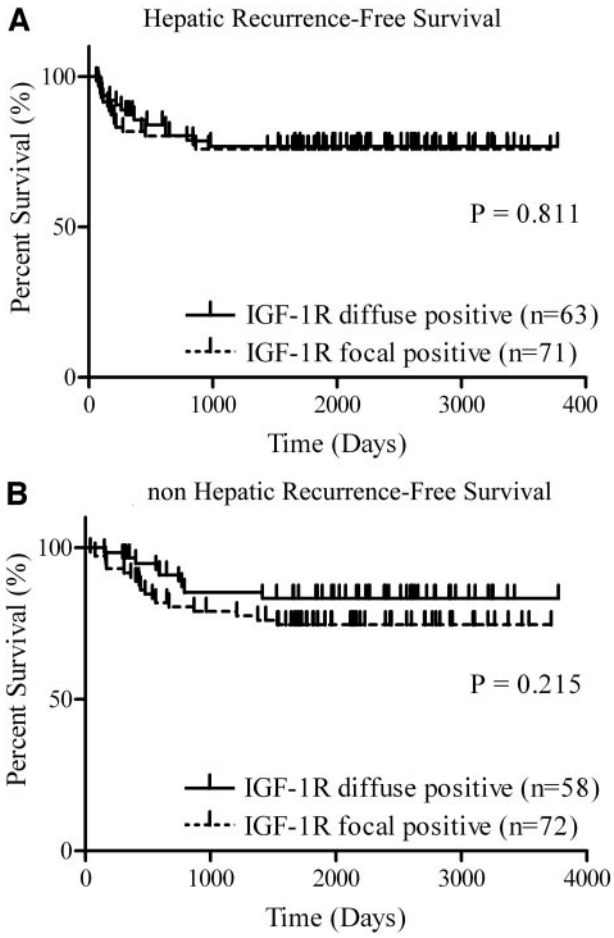

Fig. 5 Kaplan-Meier survival curves showing the levels of IGF-1R expression evaluated by both membrane and cytoplasmic staining (CRITERIA-2) on H-RFS (*A*) and on nH-RFS (*B*).

In conclusion, IGF-1R expression is useful for predicting liver metastasis from Dukes' C CRCs. However, additional studies are needed to clarify the role of the IGF system in liver metastasis of CRCs.

ACKNOWLEDGMENTS

The technical assistance of C. Okumura and Y. Okuhara as well as the secretarial help of M. Suzaki are gratefully acknowledged.

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Clin Cancer Res 2004;10:8434-8441.