Insulin–like Growth Factor II mRNA–Binding Protein 3 Expression in Benign and Premalignant Lesions and Carcinomas of the Stomach

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Background: Recent studies have demonstrated that insulin-like growth factor II mRNA-binding protein 3 (IMP3) is expressed in malignant tumors of various organs but not in normal tissue. We investigated IMP3 expression in various benign lesions, premalignant lesions and carcinomas of the stomach. **Methods:** IMP3 immunohistochemical staining was performed on 24 benign gastric lesions, 24 gastric adenomas, and 322 gastric carcinomas. **Results:** IMP3 was not expressed in benign gastric lesions including adenomas with low-grade dysplasia, but was expressed in 17% of adenomas with high-grade dysplasia, and in 44% of carcinomas. As the carcinomas were in the advanced stage, they expressed IMP3 more frequently and strongly. Patients with IMP3-positive tumors had poorer survival than those with negative tumors. **Conclusions:** IMP3 expression in gastric carcinoma may be related to tumor invasion and metastasis, and is an independent risk factor for poor prognosis.

Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is a member of the IMP family, which includes IMP1, IMP2 and IMP3.[1] The IMP family members play a role in RNA trafficking and stabilization, as well as in cell growth and migration during embryogenesis.[2] The *IMP3* gene is located at chromosome 7p15.[3] IMP3 is expressed in developing tissues during embryogenesis, but is found only at low or undetectable levels in adult tissues.[2] At first, IMP3 was found in pancreatic cancer cells.[4] At that time, it was called KH-domain containing protein overexpressed in cancer (KOC). KH-domains are involved in RNA binding, synthesis, and metabolism.[5] It was later demonstrated that KOC is identical to IMP3.[1]

Thereafter, several studies have demonstrated that IMP3 is expressed in malignant tumors of various organs including the bile duct,[6] colon,[7] liver,[8] lung,[9] breast,[10] and kidney[11] in addition to the pancreas.[12] Therefore, IMP3 may be regarded as an oncofetal protein. In most of these studies IMP3 was expressed more frequently and/or strongly in more aggressive and advanced carcinomas.

Although the incidence and mortality of gastric carcinoma have decreased gradually in Korea, this still remains the most common cancer in men and the third most common cancer in women.[13] Surgery is an effective treatment for gastric carcinoma, and chemotherapy is an effective adjuvant therapy following radical surgery.[14] Attempts have been made to identify biomarkers that predict patient survival or recurrence of gastric carcinoma and thus are useful for selecting adjuvant therapy for patients. E-cadherin,[15] human epidermal growth factor receptor-2,[16] p53,[17] vascular endothelial growth factor,[17] ki-67[18,19] and proliferating cell nuclear antigen[19,20] have been suggested as biomarkers. However, the ability of these candidate biomarkers to predict the outcome of gastric carcinoma is limited, and occasional conflicting results were reported among studies investigating these biomarkers.[18] Therefore, there is an urgent

need to identify reliable prognostic markers that will allow improved management and identification of potential therapeutic targets.

Gastric carcinoma is one of the most frequent cancers in Korea, but we found only one report[21] about IMP3 expression in surgical pathology specimens of gastric carcinoma, and no studies are available about IMP3 expression in gastric premalignant lesions. In this study, we explored the immunohistochemical expression of IMP3 in various benign and premalignant lesions and carcinomas of the stomach to understand at which step IMP3 begins to be expressed along the course of the development of carcinoma and to determine if IMP3 expression correlates with patient prognosis.

MATERIALS AND METHODS

We collected six cases of normal gastric mucosa, six cases of gastric mucosa with a *Helicobacter pylori* infection, 12 cases of intestinal metaplasia, 12 cases of gastric adenoma with low-grade dysplasia, and 12 cases of gastric adenoma with high-grade dysplasia for a tissue microarray. These specimens were obtained by endoscopic polypectomy or submucosal dissection. In addition, we collected 322 cases of gastric carcinoma from the surgically removed specimens. The ages of the patients with gastric carcinoma were from 24 to 85 years, with an average of 62.2 years. The male to female ratio was 211 : 111. The patients underwent surgical resection of primary gastric adenocarcinomas between 1994 and 1997 at Gyeongsang National University Hospital. The follow-up period after surgery averaged 39.4 months, with a maximum of 76 months. This study was approved by the Institutional Review Board of Gyeongsang National University Hospital (GNUHIRB-2009-054).

Two mm diameter core tissue biopsies were obtained from individual formalin-fixed and paraffin-embedded tissues, and arranged in new recipient paraffin blocks. In cases of gastric carcinoma, we analyzed one tissue core from the area near the invasive front.

Immunohistochemical staining was performed on a 4 μm thick section. Briefly, the tissue section was deparaffinized and rehydrated. Slides were incubated in 3% H_2O_2 for 10 minutes to reduce nonspecific background staining due to endogenous peroxidase. For epitope retrieval, specimens were heated for 20 minutes in 10 mmol/L citrate buffer (pH 6.0) in a microwave oven (700 W). After incubating with Ultra V Block (Lab Vision Corporation, Fremont, CA, USA) for 7 minutes at room

temperature to block background staining, slides were incubated with a monoclonal antibody specific to IMP3 (1 : 400, M3626, DAKO, Carpinteria, CA, USA) for 1 hour at room temperature. Antibody binding was detected by the UltraVision LP Detection System (Lab Vision Corporation), according to the manufacturer's recommendations. Color development was performed with 3,3´-diaminobenzidine and counterstained with hematoxylin.

IMP3 expression was examined with blindness to the clinical data of the patients. IMP3 was expressed in the cytoplasm. Staining intensity was assigned to a semi-quantitative, four-tired score (grade 0, < 66% of cells stained weakly; grade 1, ≥ 66% of the cells stained weakly or < 33% of cells stained strongly; grade 2, when ≥ 33% but < 66% of cells stained strongly; grade 3, ≥ 66% of cells stained strongly).

Histological type was described using the World Health Organization[22] and Lauren classification,[23] and tumor stage was classified according to the American Joint Committee on Cancer tumor, node and metastasis system.[24]

Statistical analyses were performed using SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA). The relationship between IMP3 and clinicopathological variables was evaluated with χ^2 tests (crosstabs, chi-square tests, linear-by-linear association). Survival rates were calculated with the Kaplan-Meier method, and differences in survival curves were analyzed by the log-rank test. Independent prognostic factors were identified using a Cox regression model. $p < 0.05$ was considered significant.

RESULTS

IMP3 expression in benign and premalignant lesions of the stomach

Normal surface and foveolar cells were not stained immunohistochemically by IMP3. Surface and foveolar cells in all six cases with a *H. pylori* infection did not stain either. Normal antral gland cells generally did not stain, but a few glandular cells stained weakly. Parietal and chief cells did not stain. Follicular center cells of lymphoid tissue stained weakly. Other inflammatory cells did not stain.

Intestinal metaplasia showed grade 0 staining intensity in all 12 cases, although several metaplastic cells stained weakly. Low-grade dysplastic cells did not stain for IMP3; thus, all 12 cases of low-grade adenoma showed grade 0 staining intensity (Fig. 1A). Of the 12 cases of adenoma with high-grade dysplasia, two
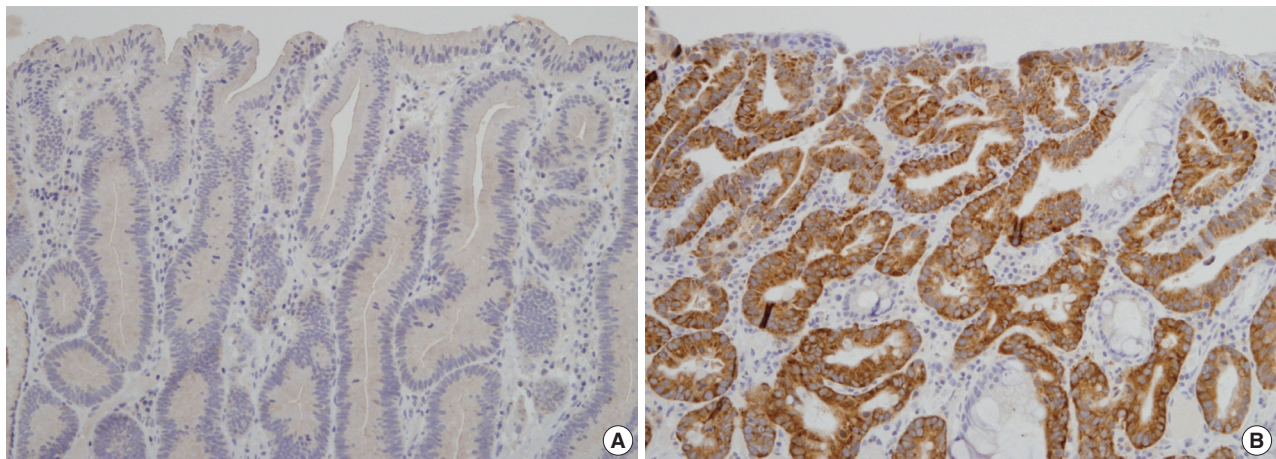
Fig. 1. (A) A gastric adenoma with low-grade dysplasia does not stain immunohistochemically for insulin-like growth factor II mRNA-binding protein 3 (IMP3). (B) A gastric adenoma with high-grade dysplasia shows a grade 3 staining intensity for IMP3. Note that intestinal metaplastic cells do not stain.
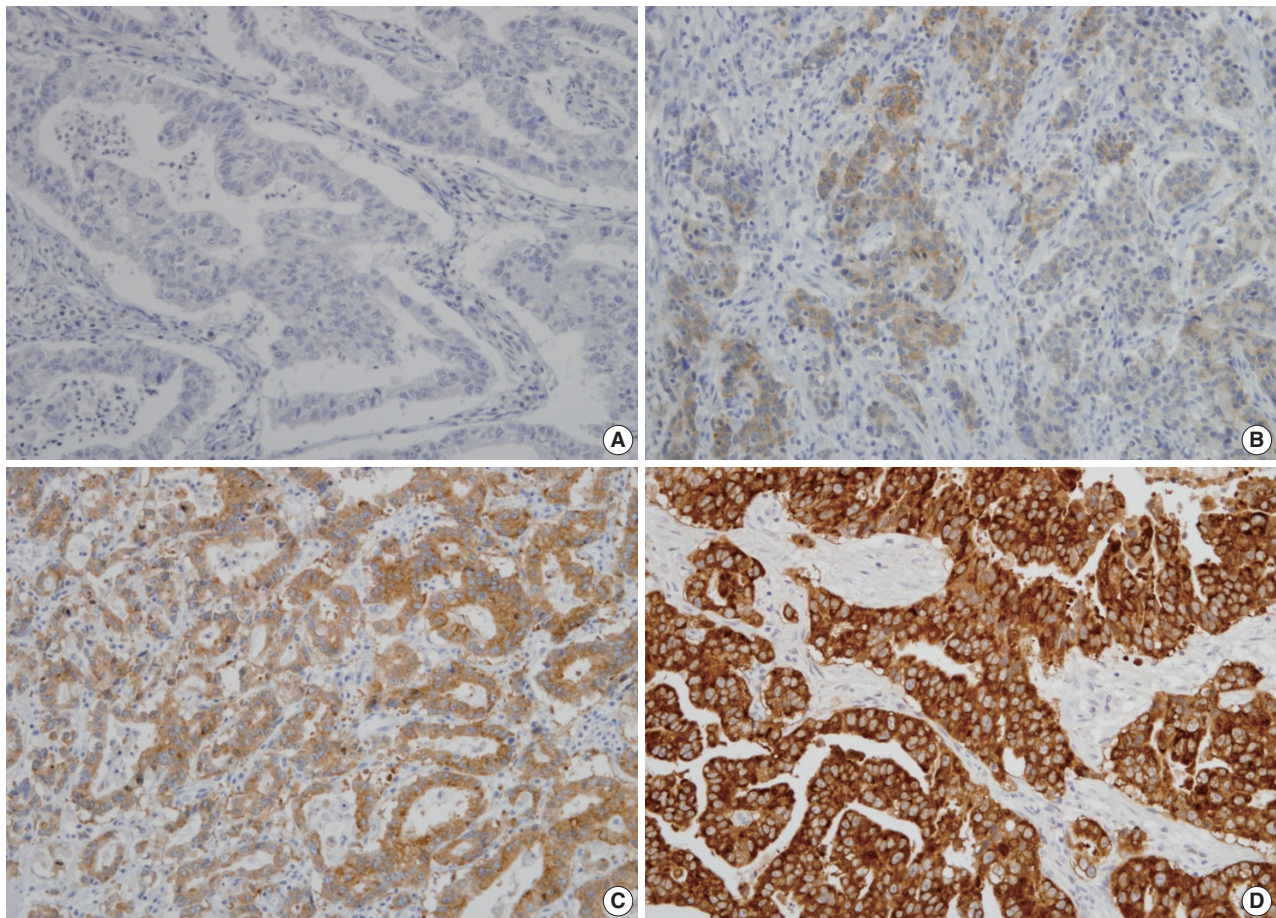

Fig. 2. Gastric carcinomas show grade 0 (A), 1 (B), 2 (C) and 3 (D) staining intensity on immunohistochemistry for insulin-like growth factor II mRNA-binding protein 3.

(17%) showed grade 3 staining intensity (Fig. 1B) and one (8%) showed grade 1 staining intensity.

IMP3 expression in gastric carcinomas

Gastric carcinomas showed grade 0 staining intensity in 133 (41.3%) cases, grade 1 in 47 (14.6%) cases, grade 2 in 79 (24.5%) cases, and grade 3 in 63 (19.6%) cases (Fig. 2). The relationship between IMP3 expression and the clinicopathological variables of gastric carcinoma is shown in Table 1. Tubular adenocarcino-

mas expressed IMP3 more frequently and strongly than mucinous or signet ring cell carcinomas (p < 0.001). Intestinal or mixed type adenocarcinomas expressed IMP3 more frequently and strongly than diffuse type adenocarcinomas (p = 0.001). Gastric adenocarcinomas expressed IMP3 more frequently and strongly as the tumors invaded more deeply (p = 0.024), when they metastasized to lymph nodes (p = 0.015), and as the tumors advanced in stage (p = 0.029). In contrast, IMP3 expression in gastric adenocarcinomas had no or little relationship to patient age, gender, or tumor size, and no significant correlation was ob-

Table 1. The relationship between insulin-like growth factor II mRNA-binding protein 3 (IMP3) expression and gastric carcinoma clinicopathological variables

Variables	Total No. of case	Grade of IMP3 expression				
		Grade 0	Grade 1	Grade 2	Grade 3	p-value
Age (yr)						
<65	160	67 (41.9)	24 (15.0)	39 (24.4)	30 (18.7)	0.722
≥65	162	66 (40.7)	23 (14.2)	40 (24.7)	33 (20.4)	
Sex						
Male	211	86 (40.8)	31 (14.7)	54 (25.6)	40 (18.9)	0.931
Female	111	47 (42.4)	16 (14.4)	25 (22.5)	23 (20.7)	
Tumor size (cm)						
<3	116	49 (42.3)	21 (18.1)	26 (22.4)	20 (17.2)	0.380
≥3	206	84 (40.8)	26 (12.6)	53 (25.7)	43 (20.9)	
Histologic type						
Tubular	283	107 (37.8)	41 (14.5)	74 (26.1)	61 (21.6)	< 0.001
Mucinous	8	5 (62.5)	2 (25.0)	0	1 (12.5)	
Signet ring cell	31	21 (67.7)	4 (12.9)	5 (16.1)	1 (0.03)	
Tubular adenocarcinoma						
W/D	73	27 (37.0)	13 (17.8)	15 (20.5)	18 (24.7)	0.081
M/D	112	34 (30.3)	15 (13.4)	32 (28.6)	31 (27.7)	
P/D	98	46 (46.9)	13 (13.3)	27 (27.6)	12 (12.2)	
Lauren classification						
Intestinal	238	88 (37.0)	35 (14.7)	61 (25.6)	54 (22.7)	0.001
Mixed	8	2 (25.0)	2 (25.0)	3 (37.5)	1 (12.5)	
Diffuse	76	43 (56.6)	10 (13.2)	15 (19.7)	8 (10.5)	
Depth of invasion						
Mucosa	80	37 (46.2)	18 (22.5)	15 (18.8)	10 (12.5)	0.024
Submucosa	88	39 (44.3)	13 (14.7)	18 (20.5)	18 (20.5)	
Muscle	38	15 (39.5)	3 (7.9)	13 (34.2)	7 (18.4)	
Subserosa and serosa	116	42 (36.2)	13 (11.2)	33 (28.5)	28 (24.1)	
Lymph node metastasis						
Absent	212	97 (45.8)	34 (16.0)	42 (19.8)	39 (18.4)	0.015
Present	110	36 (32.7)	13 (11.8)	37 (33.7)	24 (21.8)	
TNM stage						
I	222	103 (46.4)	35 (15.8)	44 (19.8)	40 (18.0)	0.029
II	43	13 (30.2)	3 (7.0)	14 (32.5)	13 (28.3)	
III	44	11 (25.0)	8 (18.2)	16 (36.4)	9 (20.4)	
IV	13	6 (46.2)	1 (7.7)	5 (38.4)	1 (7.7)	
Recurrence						
No recurrence	266	114 (42.8)	46 (17.3)	55 (20.7)	51 (19.2)	0.040
Recurrence	56	19 (33.9)	1 (1.8)	24 (42.9)	12 (21.4)	

Numbers in parentheses are percentages. p-values are calculated by linear-by-linear test using R-by-C crosstabs analysis. With histologic types, the p-value is the result of comparison between tubular adenocarcinoma and combined category of mucinous and signet ring cell carcinoma, and with Lauren classification, between diffuse type and combined category of intestinal and mixed type.
W/D, well-differentiated; M/D, moderately-differentiated; P/D, poorly-differentiated; TNM, tumor, node and metastasis.
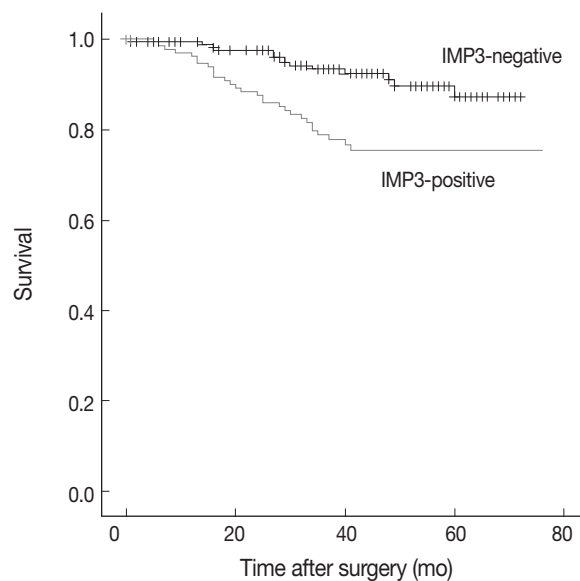
Fig. 3. Patients with insulin-like growth factor II mRNA-binding protein 3 (IMP3)-positive gastric carcinoma have poorer survival than those with IMP3-negative gastric carcinoma. Log rank test p < 0.001.

served between IMP3 expression and the differentiation of tubular adenocarcinoma.

When grade 0 or 1 staining intensity was considered negative and grade 3 or 4 staining intensity was considered positive for IMP3, IMP3-positive tumors had a significantly higher recurrence rate than IMP3-negative tumors (p = 0.040), and patients with IMP3-positive tumors had poorer survival than those with IMP3-negative tumors (p < 0.001) (Fig. 3). In a multivariate analysis based on the Cox regression model, tumor stage and IMP3 expression were independent prognostic factors (p = 0.001) (Table 2).

DISCUSSION

We reconfirmed the results of a previous study by Jeng *et al.*[21] that IMP3 represents an independent prognostic factor for survival in patients with gastric carcinoma. The differences between the previous study and ours included the size of tumors and the Lauren type.

In the study by Jeng *et al.*,[21] large tumors expressed IMP3 more frequently than small tumors. But in this study, IMP3 expression had little relationship with tumor size. The reason may be related to the difference in the average tumor stage between the two studies. In the study by Jeng *et al.*,[21] the carcinomas were somewhat evenly distributed among the four stages from I to IV. In contrast, in the present study, the carcinomas

Table 2. Multivariable Cox regression analysis of patient survival with gastric carcinoma

Variables	n	Hazard ratio (95% confidence interval)	p-value
TNM stage			
I	222	1	0.010
II	43	20.4 (1.78-235)	
III-IV	57	74.8 (4.01-1,395.3)	
IMP3 expression			
Negative	180	1	0.010
Positive	142	2.5 (1.1-5.2)	

TNM, tumor, node and metastasis; IMP3, insulin-like growth factor II mRNA-binding protein 3.

were stage I in about 70% of the patients, and 52% of the carcinomas were limited to the mucosa or submucosa. As the tumors invade more deeply, the tumors probably increase in size and in IMP3 expression. However, within the same stage or status of invasion, the tumors may show similar degrees of IMP3 expression regardless of tumor size.

Tubular adenocarcinomas expressed IMP3 more frequently than signet ring cell carcinomas, and all of the signet ring carcinomas were classified as diffuse type by the Lauren classification. This may be the reason why intestinal type adenocarcinomas expressed IMP3 more frequently than those of the diffuse type. However, in the study by Jeng *et al.*[21] little difference was found for IMP3 expression between the intestinal and diffuse types. The reason for the difference between the two studies is unknown.

In addition to carcinomas, we investigated IMP3 expression in gastric dysplastic lesions. As a result, IMP3 was expressed in some high-grade dysplastic lesions but not in low-grade dysplastic lesions. IMP3 may be partially involved in the process of progression from low-grade to high-grade dysplasia, or the dysplastic lesions that strongly express IMP3 might have progressed rapidly if they had once invaded through the basement membrane.

Our results indicate that IMP3 may play a role promoting tumor invasion and metastasis. However, IMP3 does not seem to be an essential factor for tumor progression. Many advanced gastric carcinomas were negative (grade 0 or 1 staining intensity) for IMP3. Indeed, 42% of the carcinomas with stage II or higher were negative for IMP3.

Evidence is accumulating regarding the role of IMP3 in cell movement and tumor invasion. The Xenopus homologue of IMP3 has been localized on the leading edge of migrating neural crest cells.[25] The intracellular location of these proteins may facilitate cell migration or directional outgrowth. Vikesaa *et al.*[26]

reported that IMP3 modulates the expression of specific extracellular matrix and cell adhesion proteins (e.g., collagen V a1 and ALCAM) and stabilizes CD44 mRNA, thereby promoting invadopodia formation in cervical cancer cells.

If IMP3 plays a role promoting tumor invasion and metastasis, it may become a target of cancer therapy. Indeed, a phase I clinical trial of immunotherapy targeting IMP3 has been performed in non-small cell lung cancer.[27] The results showed a high level of safety but evidence of immune activation was limited; therefore, further clinical testing is necessary.

In conclusion, IMP3 was expressed in some high-grade dysplastic lesions and carcinomas of the stomach, but not in normal, metaplastic, or low-grade dysplastic epithelium. IMP3 expression in gastric carcinoma may be related to tumor invasion and metastasis and is an independent risk factor for a poor prognosis.

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