Aberrant expression of cortactin and fascin are effective markers for pathogenesis, invasion, metastasis and prognosis of gastric carcinomas

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Abstract. Tumor metastasis depends on cell adhesiveness, motility and deformability, resulting from quantitative alterations and rearrangement of various actin-binding cytoskeletal components, such as cortactin and fascin. To clarify the involvement of cortactin and fascin expression in tumorigenesis and progression of gastric carcinoma, we performed immunohistochemistry (IHC) on tissue microarray containing gastric carcinomas, adenomas and adjacent non-neoplastic mucosa (ANNM) using the antibodies against cortactin (Ab-466, -421) and fascin as well as a comparison of their expression with clinicopathological parameters of the tumors. Gastric carcinoma cell lines MKN28, AGS, MKN45, KATO-III and HGC-27 were studied for both proteins by IHC. Cortactin-466 was found to be highly expressed in adenoma, compared with ANNMs and carcinoma ($p<0.05$), and more frequently in ANNMs than in carcinoma ($p<0.05$). Cortactin-421 expression was higher in gastric carcinomas than in adenoma and ANNMs ($p<0.05$). There was increased fascin expression in gastric carcinoma and adenoma than in ANNMs ($p<0.05$). Most of the gastric carcinoma cell lines showed expression of cortactin and fascin at different levels. Cortactin-466 expression was inversely correlated with tumor size, depth of invasion, lymphatic and venous invasion, lymph node metastasis and UICC staging ($p<0.05$). The converse was observed for cortactin-421 and fascin ($p<0.05$). There was stronger positivity of both cortactins in intestinal- versus diffuse-type carcinomas ($p<0.05$). Univariate analysis indicated the cumulative survival rate of patients with positive cortactin-466 expression to be higher than without its expression even stratified according to the depth of invasion ($p<0.05$). However, it was the converse for fascin ($p<0.05$). Multivariate analysis showed that age, depth of invasion, lymphatic invasion, lymph node metastasis, UICC staging and Lauren's classification were independent prognostic factors for carcinomas ($p<0.05$). It was suggested that aberrant expression of cortactin and fascin possibly contributes to the pathogenesis, growth, invasion and metastasis of gastric carcinomas. Thus, they may be objective and effective markers to indicate the pathobiological behaviors and prognosis of gastric carcinomas.

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Introduction

Metastasis is one of the major obstacles to the treatment of malignancies and remains the cause of 90% of deaths from solid tumors (1). The aggressive phenotype depends on cell adhesiveness, motility and deformability, which are thought to result from quantitative alterations and rearrangement of various cytoskeletal components. According to a popular definition, cytoskeleton is composed of actin filaments (microfilaments), intermediate filaments and microtubules. In particular, such actin-binding proteins as cortactin and fascin also play an essential role in cytoskeleton formation, cell migration and signaling pathways (2,3).

Cortactin (also designated as Ems-1) is a filamentous actin (F-actin)-binding monomer and consists of an amino-terminal acidic (NTA) region, 37-residue-long segments, a proline-rich region and an SH3 domain (2). This basic structure is highly conserved among all species which express cortactin. The NTA region harbors a short motif called DDW, which is necessary for binding to the Arp2/3 complex (4). Cortactin was originally identified as a substrate for the protein kinase src and is activated via phosphorylation in response to extracellular signals such as growth factors, adhesion sites, or pathogenic

invasion of the epithelial layer. Much of its function is closely linked to the regulation of actin cytoskeleton, including endocytosis, vesicular transport, organization of transmembrane receptors and neuronal polarization, cell invasion and metastasis. A large body of evidence indicates that cortactin is implicated in human neoplasia, and its up-regulation was observed in several epithelial carcinomas, including esophageal cancer, head and neck cancer, hepatocellular carcinoma and salivary gland adenoid cystic carcinoma (4-7).

Fascin is a monomeric actin filament bundling protein originally isolated from sea urchin egg, but is also found in mature dendritic cells, mesenchymal cells, endothelial cells and neurons of the human (3,8,9). Fascin binds to ß-catenin, a molecule that is not only part of cell-cell adhesion, but also a part of the wnt signaling pathway. It has been proposed that fascin reduces the cytoplasmic pool of soluble ß-catenin and so would be expected to act as an anti-oncogene (10,11). Fascin, an actin-crosslinking protein, participates in cell motility. *In vitro* experimental evidence indicated that fascin overexpression correlated with increased proliferation, altered ß1 integrin distribution, enhanced invasive capacity and an altered differentiation status in colonic adenocarcinoma (12). Up-regulated fascin induces a high potential for invasion and metastasis in various malignancies, including carcinomas of the pancreas, lung, esophagus and breast (3,8,13,14).

Gastric carcinoma ranks as the world's second leading cause of cancer mortality behind lung cancer despite a sharp worldwide decline in both its incidence and mortality since the second half of the 20th century (15,16). Tumorigenesis and progression of gastric carcinoma is a multistage process with the involvement of a multifactorial etiology, which mainly results from gene-environment interactions. In our study, expression of cortactin and fascin was examined in gastric carcinoma, adenoma, adjacent non-neoplastic mucosa (ANNM) and gastric carcinoma cell lines, and was compared with the clinicopathological parameters of the tumors, as well as prognosis to explore the clinicopathological significance and molecular roles of both actin-binding proteins in the stepwise development of gastric carcinoma.

Materials and methods

Subjects. Gastric carcinomas (n=516), adenoma (n=46) and ANNMs (n=138) were collected from our affiliated hospital and related institutes between 1993 and 2006. The patients with gastric carcinoma were 361 men and 153 women (29-91 years of age, mean 65.9 years). Among them, 185 cases had tumors accompanied by lymph node metastasis. None of the patients underwent chemotherapy or radiotherapy before surgery. They all provided consent for use of tumor tissue for clinical research, and our University Ethics Committee approved the research protocol. Patient follow-up involved consultation of case documents and phone contact.

Pathology. All tissues were fixed in 4% neutralised formaldehyde, embedded in paraffin and incised into 4-μm sections. These sections were stained with haematoxylin and eosin (H&E) to confirm their histological diagnosis and other microscopic characteristics. The staging for each gastric carcinoma was evaluated according to the Union Internationale Contre le Cancer (UICC) system for the extent of tumor spread (17). Histological architecture of gastric carcinoma was expressed in terms of Lauren's classification (18). Furthermore, tumor size, depth of invasion, lymphatic and venous invasion were determined.

Tissue microarray (TMA). Representative areas of solid tumors were identified in H&E-stained sections of the selected tumor cases, and a 2-mm-diameter tissue core per donor block was punched out and transferred to a recipient block with a maximum of 48 cores using a Tissue Microarrayer (Azumaya Kin-1, Japan). Sections (4 μm) were consecutively incised from the recipient block and transferred to poly-lysine-coated glass slides. H&E staining was performed on TMA for confirmation of tumor tissue.

Cell lines and culture. Gastric carcinoma cell lines, MKN28 (well-differentiated adenocarcinoma), AGS (moderately differentiated adenocarcinoma), MNK45 (poorly differentiated adenocarcinoma), KATO-III (poorly differentiated adenocarcinoma) and HGC-27 (undifferentiated adenocarcinoma) were obtained from the Japanese Physical and Chemical Institute. They were maintained in RPMI-1640 (MKN28, MKN45 and KATO-III), MEM (HGC-27) and Ham's F12 (AGS) medium supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin, in a humidified atmosphere of 5% CO_2 at 37˚C. All cells were collected by centrifugation, rinsed with phosphate-buffered saline (PBS), fixed by 10% formalin and then embedded in paraffin.

Immunohistochemistry. Consecutive sections were de-paraffinised with xylene, dehydrated with alcohol, and subjected to antigen retrieval by irradiating in target retrieval solution (TRS, Dako, Carpinteria, CA, USA) for 15 min with a microwave oven (Oriental Rotor Lmt. Co., Tokyo, Japan). Bovine serum albumin (5%) was then applied for 1 min to prevent non-specific binding. The sections were incubated with primary antibodies for 15 min, then treated with anti-rabbit or anti-mouse Envison-PO (Dako) antibodies for 15 min. All the incubations were performed in a microwave oven to allow intermittent irradiation as described previously (19). After each treatment, the slides were washed 3 times for 1 min with TBST (10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20). Rabbit anti-cortactin (Ab-466 and Ab-421, Applied Biological Materials Inc., Vancouver, Canada; 1:50) and mouse anti-fascin (Lab Vision, Fremont, CA, USA; ready-to-use) antibodies were employed to detect the individual proteins. Binding sites were visualized with 3,3'-diaminobenzidine (DAB) with a 5-min reaction. After counterstaining with Mayer's haematoxylin, the sections were dehydrated, cleared and mounted. Omission of the primary antibody was used as a negative control.

One hundred cells were randomly selected and counted from 5 representative fields of each section in a blinded manner by three independent observers (Y. Takano, H.C. Zheng and X.H. Li). The positive percentage of counted cells was graded semi-quantitatively according to a 4-tier scoring system: 0-5% negative (-), 6-25% weakly positive (+), 26-50% moderately positive (++), and 51-100% strongly positive (+++).

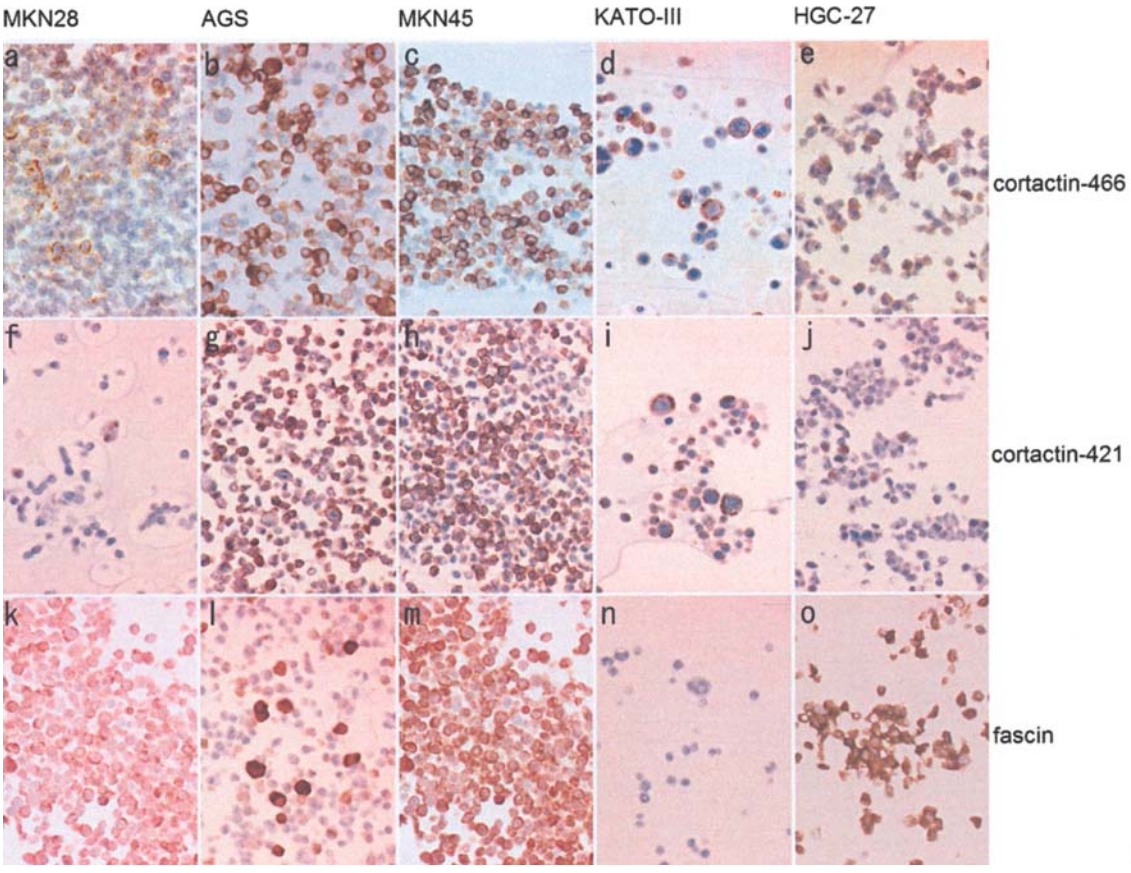

Figure 1. Immunohistochemical staining of cortactin and fascin in gastric carcinoma cell lines. Cortactin-466 was positively immunostained in the cytoplasm and membrane of MKN28 (a), AGS (b), MKN45 (c), KATO-III (d) and HGC-27 (e). Cortactin-421 was positively immunostained in the cytoplasm and membrane of MKN28 (f), AGS (g), MKN45 (h), KATO-III (i), but in the mitotic nuclei of MKN28 (f) and HGC-27 (j), while fascin was positively immunostained in the cytoplasm of MKN28 (k), AGS (l), MKN45 (m), KATO-III (n) and HGC-27 (o).

Table I. Cortactin-466 expression in gastric carcinogenesis.

		Cortactin-466 expression				
Groups	n	-	+	++	+++	PR (%)
Adjacent non-neoplastic mucosa	138	42	60	26	10	69.6
Adenoma	46	7	20	13	6	84.8[a]
Carcinoma	509	228	192	75	14	55.2[b]

[a]Compared with adjacent non-neoplastic mucosa, p=0.019; or with carcinoma, p=0.001. [b]Compared with adjacent non-neoplastic mucosa, p=0.001. PR, positive rate.

Statistical analysis. Statistical evaluation was performed using the Spearman correlation test to analyze the rank data. Kaplan-Meier survival plots were generated, and comparisons between survival curves were made with the log-rank statistics. The Cox's proportional hazards model was employed for multivariate analysis. $p<0.05$ was considered to be statistically significant. SPSS 10.0 software was employed to analyze data.

Results

Expression of cortactin and fascin in gastric tissue samples. As showed in Fig. 1, cortactin-466 was positively immunostained in the cytoplasm or membrane of MKN28, AGS, MKN45, KATO-III and HGC-27, whereas cortactin-421 was positively immunostained in the cytoplasm or membrane of MKN28, AGS, MKN45 and KATO-III, but in the mitotic nuclei of MKN28 and HGC-27. Cortactin (Ab-466, Ab-421) was stained in the cytoplasm or membrane of gastric ANNMs and adenoma, but mainly in the cytoplasm of gastric carcinomas. In ANNMs, cortactin-466 was distributed to the superficial and deep glands, but cortactin-421 only to the latter. Positive immunoreactivity to cortactin-466 was occasionally observed in inflammatory cells as well (Fig. 2). Overall, cortactin-466 expression was detected respectively in

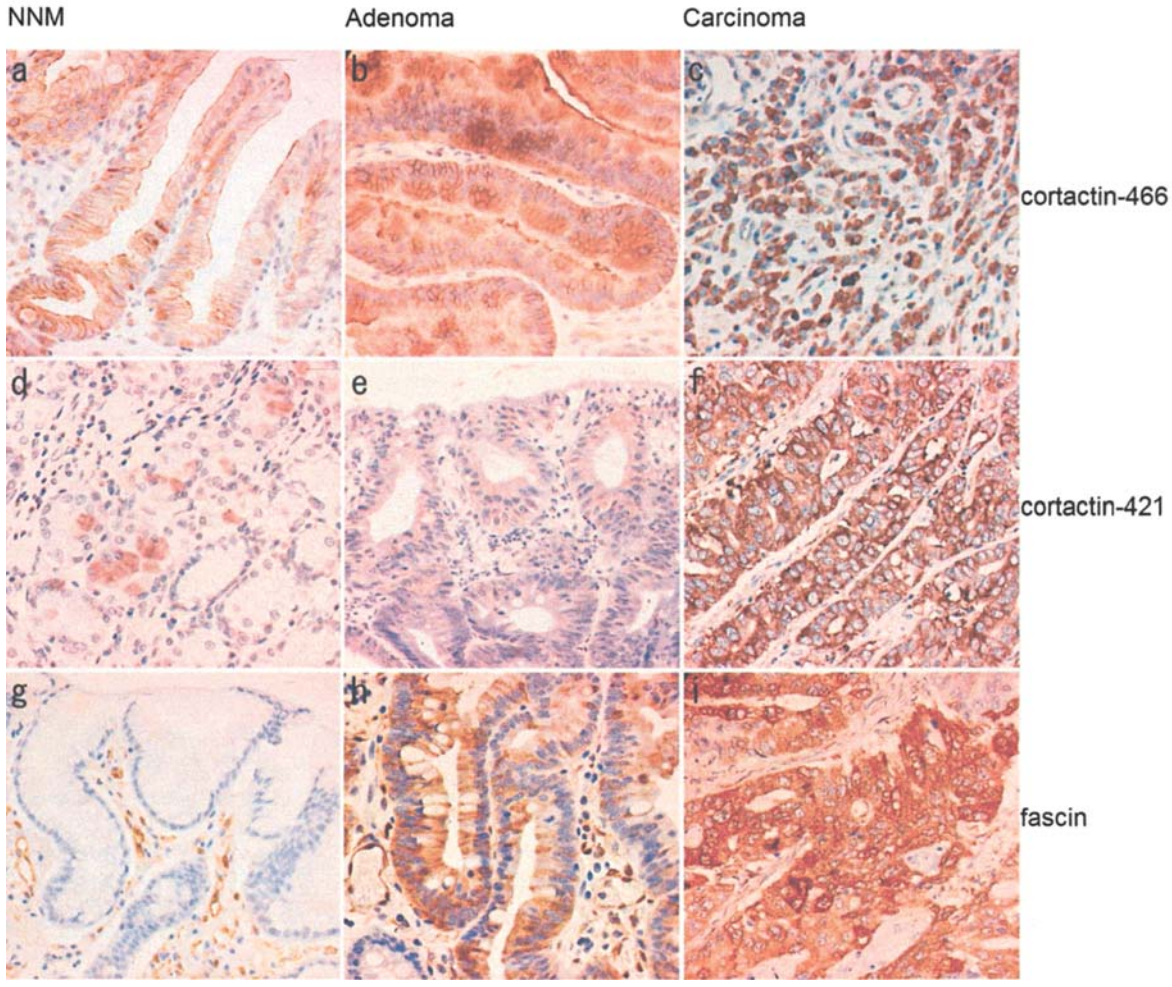

Figure 2. Immunohistochemical staining of cortactin and fascin in gastric non-neoplastic mucosa (NNM), adenoma and carcinoma. Cortactin-466 positivity was observed in the cytoplasm or membrane of gastric superficial NNM (a, +++), adenoma (b, +++) and carcinoma (c, +++). Cortactin-421 positivity was observed in the cytoplasm or membrane of gastric propria gland (d, +), adenoma (e, +) and carcinoma (f, +++), while fascin expression was limited to the cytoplasm of vascular epithelial cells (g, +++), gastric adenoma (h, +++) and carcinoma (i, +++), but not in gastric NNM (g, -).

Table II. Cortactin-421 expression in gastric carcinogenesis.

Groups	n	Cortactin-421 expression				
		-	+	++	+++	PR (%)
Adjacent non-neoplastic mucosa	138	101	36	1	0	26.8
Adenoma	46	32	12	2	0	30.4
Carcinoma	509	255	155	67	32	49.9[a]

[a]Compared with adjacent non-neoplastic mucosa, p=0.001; or adenoma, p=0.004. PR, positive rate.

96 out of 138 ANNMs (69.6%), 39 out of 46 adenoma patients (84.8%) and 281 out of a total of 509 gastric carcinoma patients (55.2%). Cortactin-466 was highly expressed in adenoma, compared with ANNMs or carcinoma ($p<0.05$) and more frequently in ANNMs than in carcinoma ($p<0.05$) (Table I). However, cortactin-421 expression was positive in 254 cases of 509 gastric carcinomas (49.9%), higher than gastric adenoma (30.4%, 12/46) and ANNMs (26.8%, 37/138) ($p<0.05$) (Table II).

As indicated in Fig. 1, fascin was expressed in five carcinoma cell lines (MKN28, AGS, MKN45, KATO-III and HGC-27) at different levels using immunohistostaining. Fig. 2 shows that fascin was stained mainly in the cytoplasm of vascular endothelial cells, lymphocytes, smooth muscle cells, gastric adenoma and carcinoma. Frequently, carcinoma cells showed no fascin immunoreactivity with its strong positivity in neighboring stromal cells. Fascin expression was detected in 131 out of 509 total gastric carcinoma patients (25.7%)

Table III. Fascin expression in gastric carcinogenesis.

Groups	n	Fascin expression				
		-	+	++	+++	PR (%)
Adjacent non-neoplastic mucosa	138	138	0	0	0	0.0[a]
Adenoma	46	31	9	5	1	32.6
Carcinoma	509	378	67	33	31	25.7

[a]Compared with adenoma or carcinoma, p<0.001. PR, positive rate.

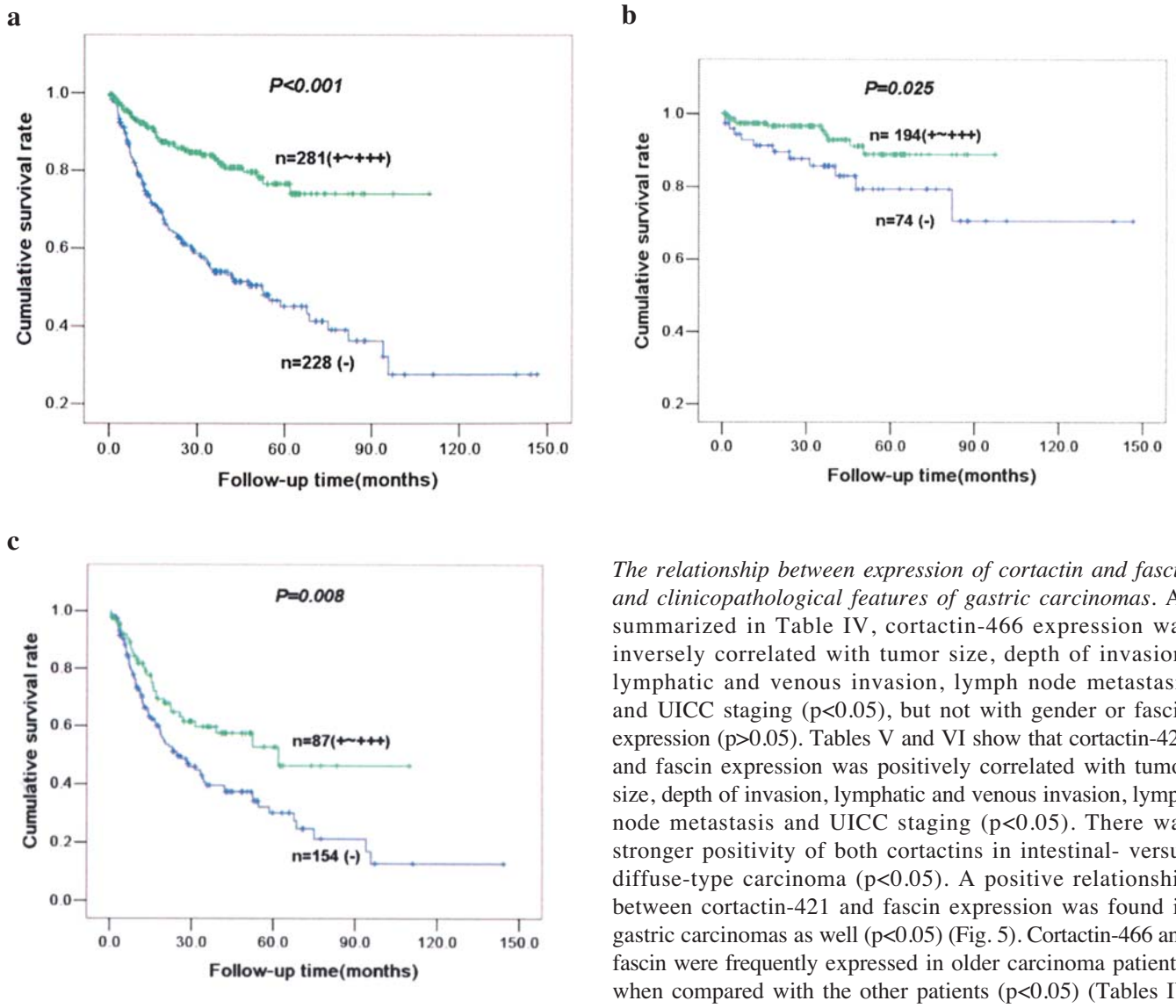

Figure 3. Correlation between status of cortactin-466 and prognosis of the gastric carcinoma patients. Kaplan-Meier curves for the cumulative survival rate of patients with gastric carcinomas according to the expression of cortactin-466 in all (a), early (EGC, b) or advanced (AGC, c) carcinomas.

and 15 out of 46 adenoma patients (32.6%). All gastric non-neoplastic epithelial cells were non-reactive for fascin (0.0%). Statistically, fascin expression was higher in gastric adenoma and carcinoma than in ANNMs (p<0.05) (Table III).

The relationship between expression of cortactin and fascin and clinicopathological features of gastric carcinomas. As summarized in Table IV, cortactin-466 expression was inversely correlated with tumor size, depth of invasion, lymphatic and venous invasion, lymph node metastasis and UICC staging (p<0.05), but not with gender or fascin expression (p>0.05). Tables V and VI show that cortactin-421 and fascin expression was positively correlated with tumor size, depth of invasion, lymphatic and venous invasion, lymph node metastasis and UICC staging (p<0.05). There was stronger positivity of both cortactins in intestinal- versus diffuse-type carcinoma (p<0.05). A positive relationship between cortactin-421 and fascin expression was found in gastric carcinomas as well (p<0.05) (Fig. 5). Cortactin-466 and fascin were frequently expressed in older carcinoma patients, when compared with the other patients (p<0.05) (Tables IV and V). Cortactin-421 was preferentially expressed in the male patients with gastric carcinoma in comparison with the female patients (p<0.05) (Table VI).

Univariate and multivariate survival analysis. Follow-up information was available on 509 gastric carcinoma patients for periods ranging from 0.2 months to 12.2 years (mean 35.9 months). Figs. 3 and 4 respectively show survival curves stratified according to cortactin-466 and fascin expression for all (a), early (b) and advanced (c) carcinomas. Univariate analysis using the Kaplan-Meier method indicated the

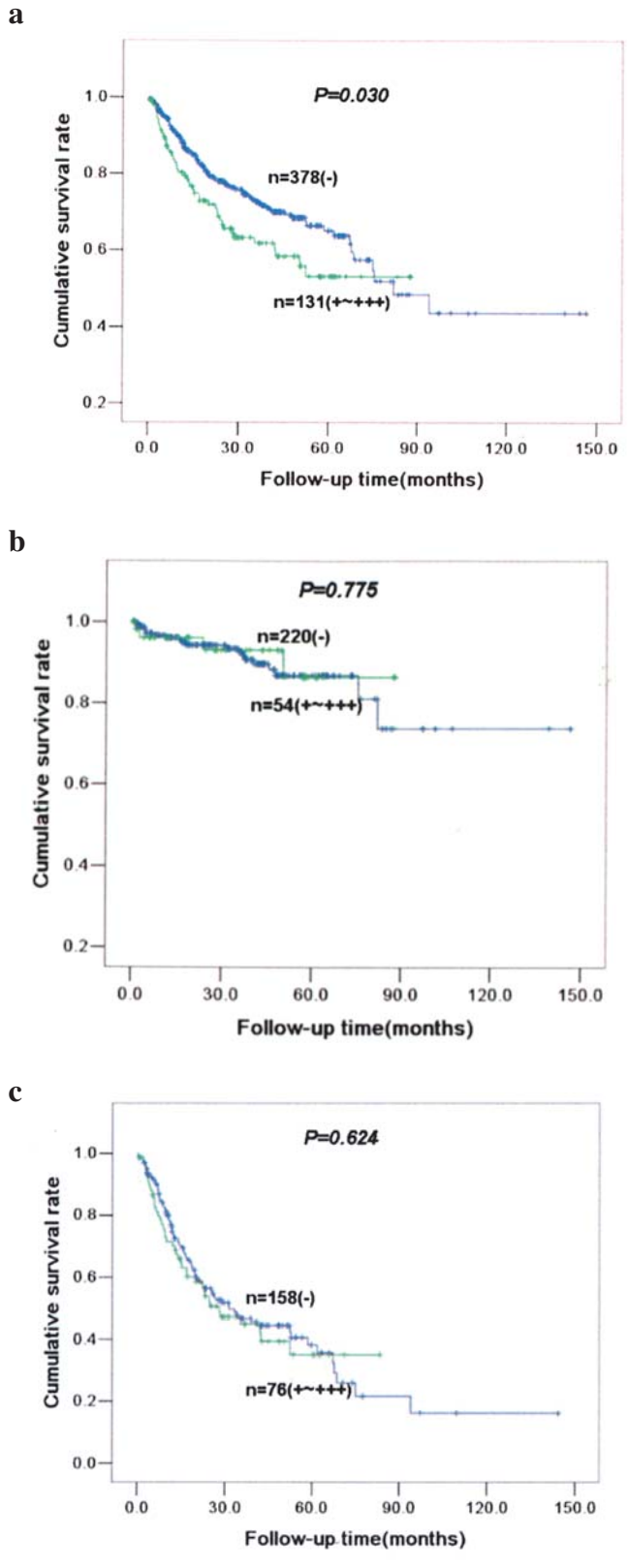

Figure 4. Correlation between status of fascin and prognosis of the gastric carcinoma patients. Kaplan-Meier curves for the cumulative survival rate of patients with gastric carcinomas according to the expression of fascin in all (a), early (EGC, b) or advanced (AGC, c) carcinomas.

cumulative survival rate of patients with weak, moderate or strong cortactin-466 expression to be obviously higher than without its expression ($p<0.05$). There was even statistical

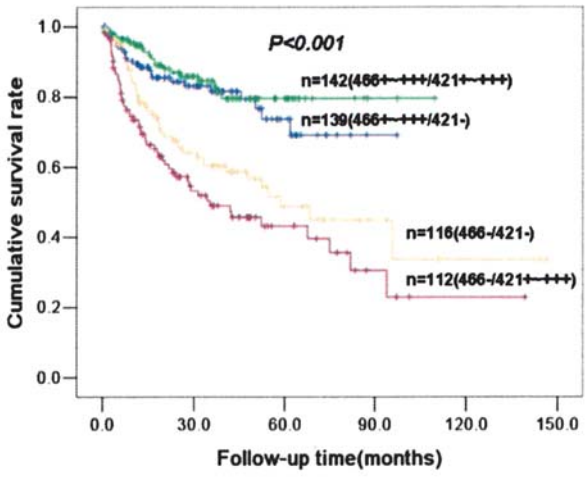

Figure 5. Correlation between status of both cortactins and prognosis of the gastric carcinoma patients. Kaplan-Meier curves for the cumulative survival rate of patients with gastric carcinomas according to the expression of cortactin-466 and -421.

difference stratified according to the depth of invasion (for early gastric carcinoma, EGC, $p<0.05$; advanced gastric carcinoma, AGC, $p<0.05$). However, no difference was observed stratified according to the depth of invasion ($p>0.05$) although the patients with positive fascin expression had significantly lower survival rates than without its expression in overall gastric carcinomas ($p<0.05$). Contrasting expression of cortacin-466 (negative) and -421 (positive) was closely linked to the low survival rate of carcinoma patients ($p<0.05$) (Fig. 5). Multivariate analysis using Cox's proportional hazards model indicated that age, depth of invasion, lymphatic invasion, lymph node metastasis, UICC staging and Lauren's classification, but not gender, tumor size, expression of cortactin or fascin were independent prognostic factors for overall gastric carcinomas ($p<0.05$) (Table VII).

Discussion

Cortactin was found to stabilize F-actin networks and promote actin polymerization by activating the Arp2/3 complex. Its overexpression, as observed in several human cancers including esophageal cancer, head and neck cancer, hepatocellular carcinoma and salivary gland adenoid cystic carcinoma (4-7) stimulates cell migration, invasion, and experimental metastasis by reducing cell spreading and intercellular adhesive strength (2,4,20). It was found that cortactin in invadopodia regulates the secretion of matrix metalloproteinases and points to a novel mechanism coupling dynamic actin assembly to the secretory machinery, producing enhanced extracellular matrix (ECM) degradation and invasiveness. A study by Luo *et al* indicated that the protective role of cortactin in anoikis resistance enhanced cell migration, tumor growth and lung metastasis of esophageal squamous cell carcinoma (ESCC) (7). Cortactin also underpins CD44-promoted invasion and adhesion of breast cancer cells to bone marrow endothelial cells (21). According to van Rossum *et al*, cortactin might sustain epidermal growth factor receptor (EGFR) signaling by preventing ligand-induced receptor degradation in human carcinoma cells (22).

Table IV. Relationship between cortactin-466 expression and clinicopathological features of gastric carcinomas.

Clinicopathological features	n	Cortactin-466 expression					p-value
		-	+	++	+++	PR (%)	
Age (years)							0.034
<65	210	103	77	25	5	51.0	
≥65	299	125	115	50	9	58.2	
Gender							0.891
Male	356	159	137	50	10	55.3	
Female	153	69	55	25	4	54.9	
Tumor size (cm)							<0.001
<4	268	86	114	57	11	67.9	
≥4	241	142	78	18	3	41.1	
Depth of invasion							<0.001
T_{is-1}	268	74	125	59	10	72.2	
T_{2-4}	241	154	67	16	4	36.0	
Lymphatic invasion							<0.001
-	332	119	144	58	11	64.2	
+	177	109	48	17	3	38.4	
Venous invasion							<0.001
-	443	185	174	70	14	58.2	
+	66	43	18	5	0	34.9	
Lymph node metastasis							<0.001
-	324	105	147	60	12	67.6	
+	185	123	45	15	2	33.5	
UICC staging							<0.001
0-I	296	91	134	61	10	69.3	
II-IV	213	137	58	14	4	35.7	
Lauren's classification							<0.001
Intestinal-type	291	101	128	52	10	65.3	
Diffuse-type	218	127	65	23	4	41.7	
Fascin expression							0.636
-	378	171	145	52	10	54.8	
+ - +++	131	57	47	23	4	56.5	

PR, positive rate; T_{is}, carcinoma *in situ*; T_1, lamina propria and submucosa; T_2, muscularis propria and subserosa; T_3, exposure to serosa; T_4, invasion into serosa; UICC, Union Internationale Contre le Cancer.

Phosphorylation of cortactin tyrosine 421 and 466 was elevated in response to Src, EGFR and Rac1 activation, and tyrosine 421 phosphorylated cortactin localized with F-actin in lamellipodia and podosomes. Tyrosine phosphorylation of cortactin occurs in a progressive manner, with initial phosphorylation at tyrosine 421 followed by tyrosine 466 (23). Therefore, we employed both anti-cortactin antibodies produced against synthesized murine cortactin non-phosphopeptide around the phosphorylation site of tyrosine 466 or 421 because both antibodies can cross-react with human samples. Regarding the antibodies used in this study, immunogenic peptide sequences of cortactin-421 are the same as the human sequences, whereas those of cortactin-466 are slightly different (Fig. 6A). Paradoxically, it was found that cortactin-466 expression underwent up-regulation and then down-regulation from ANNMs to carcinoma through adenoma, and was inversely correlated with invasion, metastasis and tumor staging of gastric carcinoma. Consequently, cortactin-466 expression was found to be positively linked to a favorable prognosis of tumors independent of the depth of tumor invasion. In line with other reports (4-7), a gradual increase in cortactin-421 expression

Table V. Relationship between cortactin-421 expression and clinicopathological features of gastric carcinomas.

Clinicopathological features	n	Cortactin-421 expression					
		-	+	++	+++	PR (%)	p-value
Age (years)							0.344
<65	210	108	66	25	11	48.6	
≥65	299	147	89	42	21	49.3	
Gender							0.021
Male	356	170	106	51	29	52.2	
Female	153	85	49	16	3	44.4	
Tumor size (cm)							0.007
<4	268	146	81	33	8	45.5	
≥4	241	109	74	34	24	54.8	
Depth of invasion							<0.001
T_{is-1}	270	155	80	28	5	42.2	
T_{2-4}	239	100	75	39	27	58.5	
Lymphatic invasion							<0.001
-	332	185	94	38	15	44.3	
+	177	70	61	29	17	60.5	
Venous invasion							<0.001
-	443	235	136	52	20	47.0	
+	66	20	19	15	12	70.0	
Lymph node metastasis							0.005
-	324	173	100	39	12	46.6	
+	185	82	55	28	20	55.7	
UICC staging							0.003
0-I	296	160	93	32	11	46.0	
II-IV	213	95	62	35	21	55.4	
Lauren's classification							0.015
Intestinal-type	291	132	95	43	21	55.0	
Diffuse-type	218	123	60	24	11	43.6	
Cortactin-466 expression							0.876
-	228	116	57	32	23	49.1	
+ - +++	281	139	98	35	9	50.5	

PR, positive rate; T_{is}, carcinoma *in situ*; T_1, lamina propria and submucosa; T_2, muscularis propria and subserosa; T_3, exposure to serosa; T_4, invasion into serosa; UICC, Union Internationale Contre le Cancer.

was observed in gastric carcinogenesis and was positively related to invasion, metastasis and aggressive staging of carcinoma. These findings indicate that aberrant expression of cortactins and fascin play an important role in the pathogenesis and subsequent aggressiveness of gastric carcinoma. Thus, cortactin-466 may be a marker for gastric adenoma, involved in the gastric adenoma-carcinoma sequence and considered as a promising prognostic factor of gastric carcinomas. Furthermore, the more frequently observed expression of cortactin-466 and -421 in intestinal-type gastric carcinoma versus diffuse-types suggestss that both cortactins might underlie the molecular basis for the differentiation of these carcinomas.

Cortactin overexpression was only detected in salivary gland adenoid cystic carcinoma and hepatocellular carcinomas (5,6) by immunohistochemistry with anti-cortactin antibodies 4F11 (non-specific) and sc11408 (amino acids 309-499 of C-terminal) respectively. The present study, for the first time, applied both anti-cortactin antibodies (Ab-466 and -421) to immunohistochemistry. If it was developed against the N-terminal, the mRNA variation of cortactin might have caused its protein expression loss similar to its transcript variant 2

Table VI. Relationship between fascin expression and clinicopathological features of gastric carcinomas.

Clinicopathological features	n	Fascin expression					
		-	+	++	+++	PR (%)	p-value
Age (years)							0.006
<65	206	163	26	9	8	20.9	
≥65	303	215	41	24	23	29.0	
Gender							0.261
Male	356	270	42	23	21	24.2	
Female	153	108	25	10	10	29.4	
Tumor size (cm)							0.044
<4	275	213	37	12	13	23.6	
≥4	234	165	30	21	18	29.5	
Depth of invasion							0.001
T_{is-1}	275	220	33	10	12	19.9	
T_{2-4}	234	158	34	23	19	32.6	
Lymphatic invasion							<0.001
-	337	267	41	13	16	20.8	
+	172	111	26	20	15	35.5	
Venous invasion							0.024
-	443	336	58	23	26	24.2	
+	66	42	9	10	5	36.4	
Lymph node metastasis							0.024
-	329	254	44	15	16	22.8	
+	180	124	23	18	15	31.1	
UICC staging							0.001
0-I	303	240	38	11	14	20.8	
II-IV	206	138	29	22	17	33.0	
Lauren's classification							0.908
Intestinal-type	291	216	36	19	20	25.8	
Diffuse-type	218	162	31	14	11	25.7	
Cortactin-421 expression							<0.001
-	255	211	25	11	8	17.3	
+ - +++	254	167	42	22	23	34.3	

PR, positive rate; T_{is}, carcinoma *in situ*; T_1, lamina propria and submucosa; T_2, muscularis propria and subserosa; T_3, exposure to serosa; T_4, invasion into serosa; UICC, Union Internationale Contre le Cancer.

(Fig. 6B). Additionally, there was no alteration of tyrosine phosphorylation sites in the encoding products of the cortactin variants which have been detected until now (Fig. 6C), which demonstrates that both antibodies recognized the identical well-known cortactin protein. Up-regulation and then down-regulation of cortactin-466 expression in the pathogenesis of gastric carcinomas indicate that after the carcinogenesis and subsequent progression of gastric carcinomas, the encoding product of a novel cortactin mRNA isoform lacks the epitope, with which anti-cortactin-466 antibody can react. To verify this hypothesis, we plan to sequence the suspect fragments of cortactin cDNA in gastric tissue samples using RT-PCR and direct cycle sequence, and then produce another antibody against human PVY^{470}ETT peptide for subsequent immunohistochemical staining.

Fascin is a globular protein that organizes F-actin into well-ordered, tightly packed parallel bundles *in vitro*. Vertebrate genomes encode three forms of fascin. Fascin-1 (also known as fascin) contributes to the organization of cell protrusions which mediates cell interactions and migration and cytoplasmic microfilament bundles which contribute to cell architecture and to intracellular movements (3,8). To

Table VII. Multivariate analysis of clinicopathological variables for survival with gastric carcinomas.

Clinicopathological parameters	Relative risk (95% CI)	p-value
Age (≥65 years)	1.031 (1.012-1.050)	0.001
Gender (female)	1.295 (0.849-1.977)	0.230
Tumor size (≥4 cm)	1.374 (0.833-2.268)	0.213
Depth of invasion (T_{2-4})	6.657 (2.977-14.886)	<0.001
Lymphatic invasion (+)	2.504 (1.627-3.852)	<0.001
Venous invasion (+)	1.066 (0.705-1.611)	0.762
Lymph node metastasis (+)	3.177 (1.692-5.963)	<0.001
UICC staging (II-IV)	0.242 (0.110-0.532)	<0.001
Lauren's classification (diffuse-type)	1.822 (1.226-2.706)	0.003
Fascin expression (+ - +++)	1.328 (0.902-1.954)	0.150
Cortactin-466 expression (+ - +++)	0.695 (0.466-1.038)	0.075
Cortactin-421 expression (+ - +++)	0.796 (0.548-1.155)	0.230

CI, confidence interval; UICC, Union Internationale Contre le Cancer.

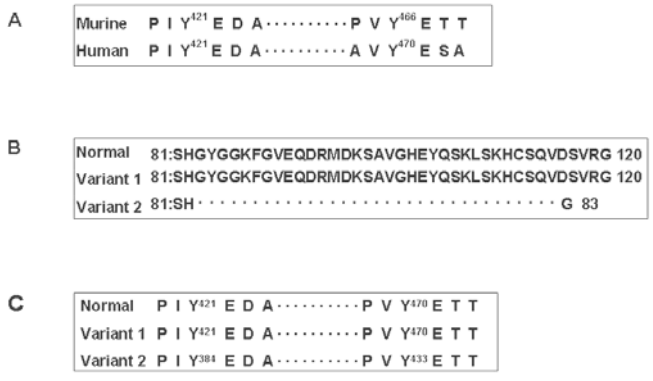

Figure 6. Comparison of different cortactins in the human and the mouse. (A) Alignment of sequences surrounding tyrosine 421 and 466 of murine cortactin (GeneBank accession no. U03184) with homologous regions from human (M98343) cortactin. (B and C) Alignment of sequences surrounding the N-terminal or C-terminal of different human cortactin isoforms (Normal, AK222613; Variant 1, NM_005231; Variant 2, AK291097).

clarify the relationship between expression of fascin and expression of the cortactins in gastric carcinomas, we examined fascin expression using a large number of carcinoma samples and found that it was not expressed in adjacent non-neoplastic mucosa in line with studies of other normal epithelium, such as breast, colon, lung and ovary. We found that fascin expression increased with the progression of gastric carcinomas and was statistically correlated with tumor size, depth of invasion, lymphatic invasion, venous invasion, lymph node metastasis and UICC staging of the tumors, suggesting that fascin is an invasive phenotype in the progression of gastric carcinomas. Therefore, fascin expression was found to positively correlate with a poor prognosis of gastric carcinomas in agreement with other reports (3,9,24). However, the prognostic significance of fascin was found to depend on the depth of invasion of gastric carcinoma in the current study.

In cell culture, colonic adenocarcinoma cells transfected with recombinant fascin display increased proliferation, altered ß1 integrin distribution, enhanced invasive capacity and an aberrant differentiation status (12). One *in vitro* study revealed that all 33 ESCC cell lines expressed fascin protein at a certain level, and down-regulation of fascin expression reduced the motile and invasive properties in a fascin-over-expressed cell line (3). Fascin overexpression was positively linked to invasion, metastasis or staging in oral squamous, esophageal, colorectal, pancreatic and renal cell carcinoma at the level of protein or mRNA (3,13,24-26). Moreover, ki-67, present in the nuclei of cells in the G_1, S and G_2 phases of the cell cycle as well as in mitosis (27), was found to be highly expressed in fascin-positive areas, compared with fascin-negative areas (28). These findings indicate that fascin may greatly contribute to a more aggressive tumor phenotype by promoting cell proliferation, migration and invasion. Investigators report that amplification or overexpression of c-erbB-2/HER-2 is responsible for the fascin up-regulation, and wnt (wingless-type) signaling influences the fascin activity, both of which are common in gastric carcinomas (10,29-31).

In summary, aberrant cortactin and fascin expression plays an important role in the pathogenesis, growth, invasion, metastasis and a poor prognosis of gastric carcinomas. Expression of cortactin-466 and -421 could be employed to differentiate the intestinal- and diffuse-type carcinomas and may underlie the molecular mechanisms associated with the differentiation of both carcinomas. Expression of cortactin-466 and -421 may be objective and effective markers indicating the pathobiological behavior and prognosis of gastric carcinomas.

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