Arp2/3 Overexpression Contributed to Pathogenesis, Growth and Invasion of Gastric Carcinoma

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Abstract. *Background: Tumor metastasis depends on cell adhesion, motility and deformability, resulting from quantitative alterations and rearrangement of actin-related protein (Arp) 2 and 3. The aim of this study was to clarify the roles of both molecules in tumorigenesis and progression of gastric carcinomas. Patients and Methods: Immunohistochemistry (IHC) was employed on tissue microarray containing gastric carcinomas, adjacent metaplasia and gastritis using antibodies against Arp2 and Arp3 with a comparison of their expression with clinicopathological parameters of carcinomas. Gastric carcinoma cell lines (MKN28, AGS, MKN45, KATO-III and HGC-27) were studied for Arp2 and Arp3 protein by IHC. Results: Both proteins were expressed at low levels in gastritis compared with carcinomas ($p<0.05$). Arp2 was more frequently expressed in intestinal metaplasia than in carcinoma and gastritis ($p<0.05$). Most gastric carcinoma cell lines showed expression at different levels. Expression was positively correlated with tumor size, depth of invasion, venous invasion, Union Internationale Contre le Cancer (UICC) staging and expression of cortactin or fascin ($p<0.05$), but not with age, sex, lymphatic invasion or lymph node metastasis ($p>0.05$). There was stronger positivity of Arp3 in intestinal- than diffuse-type carcinomas ($p<0.05$). A positive relationship between Arp2 and Arp3 proteins was noted ($p<0.05$). Univariate analysis indicated that the cumulative survival rate of patients with positive Arp2 or Arp3 expression was not different from those without their expression ($p>0.05$). 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 ($p<0.05$). Conclusion: Aberrant expression of Arp2 and Arp3 is possibly involved in pathogenesis, growth, invasion and progression of gastric carcinomas. Distinct Arp3 expression underlies the molecular mechanisms for the differentiation of intestinal- and diffuse-type carcinomas. They were considered as objective and effective markers to indicate the pathobiological behaviors of gastric carcinomas.*

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 disassembly of actin filaments, coordinated with inactivation of cell–cell adhesion and actin polymerization. Actin-related protein (Arp)2/3 complex with seven subunits has been identified to bind to each other and directly regulates the actin polymerization reaction by interacting with Wiskott-Aldrich-syndrome-related protein (WASP) family proteins downstream of three G proteins of the Rho family including Rho, Cdc42, and Rac (2, 3).

Arp2/3 complex is a seven-subunit protein that plays a major role in the regulation of the actin cytoskeleton. Among these subunits, Arp2 and Arp3 closely resemble the structure of monomeric actin and serve as nucleation sites for new actin filaments to stimulate actin polymerization. The complex is found in cellular regions characterized by dynamic actin filament activity, macropinocytotic cups, the leading edge of motile cells (lamellipodia), and motile actin patches in yeast. The complex has also been shown to be involved in the establishment of cell polarity and the migration of fibroblast monolayer in a wound-healing model (4, 5). Moreover, Arp2/3 complex also regulates the

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intracellular motility of endosomes, lysosomes, pinocytic vesicles and mitochondria (4-7). The Arp2/3 complex might control the networks of actin filaments to drive membrane protrusion during cell migration. Focal adhesion kinase (FAK) was found to form a phosphorylation-regulated complex with Arp2/3, linking integrin signaling directly with the actin polymerization machinery. Therefore, the complex also participates in the regulation of cadherin-mediated cell–cell adhesion (8).

Activation of WASPs by guanosine triphosphatase (GTPase) activation or tyrosine phosphorylation exposes a cryptic carboxyl-terminal domain containing an acidic region that binds and activates the Arp2/3 complex. Arp2/3 complex activity results in nascent polymerization of actin filaments at the cell periphery, providing the protrusive force responsible for lamellipodia extension at the leading edge. Regulation of the actin cytoskeleton by Arp2/3 complex activity has been proposed as a mechanism for controlling tumor cell migration, invasion, and metastasis. In addition to WASPs, another protein that binds and activates Arp2/3 complex is cortactin, an Src kinase substrate and filamentous (F)-actin-binding protein that is enriched in lamellipodia (9, 10). Cortactin directly activates Arp2/3 complex actin nucleation activity through the binding of the amino-terminal acidic domain region to Arp2/3 complex and the repeat region to F-actin. The cortactin SH3 domain also binds and activates neuronal (N)-WASP, which in turn promotes Arp2/3 actin nucleation and cell motility. Additionally, cortactin modulates cell movement by the phosphorylation of three specific tyrosine residues by Src and other oncogenic tyrosine kinases (11, 12). Fascin is a globular protein that organizes F-actin into well-ordered, tightly packed parallel bundles *in vitro* and plays an essential role in the cytoskeleton formation, cell migration and signaling pathways (13).

Gastric carcinoma ranks 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. Tumorigenesis and progression of gastric carcinoma is a multistage process with the involvement of a multifactorial etiology, which mainly results from gene–environment interactions (14, 15). In the present study, Arp2 and Arp3 expression was examined in gastric carcinoma, adjacent intestinal metaplasia and gastritis mucosa, and compared with the clinicopathological parameters of tumors, as well as expression of fascin and cortactin to explore the clinicopathological significance and molecular roles of Arp2 and Arp3 in the stepwise development of gastric carcinoma.

Patients and Methods

Patients. Gastric carcinomas (n=420), adjacent intestinal metaplasia (n=100) and gastritis (n=54) samples were collected from our affiliated hospital and related institutes between 1993 and 2006. The patients with gastric carcinoma were 289 men and 131 women (aged 29-91 years, mean=65.4 years). Among them, 161 cases had tumors accompanied with 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 Ethical Committee approved the research protocol. All patients were followed-up by consulting their case documents and *via* telephone communications.

Pathology. All tissues were fixed in 4% neutralised formaldehyde, embedded in paraffin and cut into 4 μm sections. These sections were stained by haematoxylin and eosin (HE) 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 (16). Histological architecture of gastric carcinoma was expressed in terms of Lauren's classification (17). Furthermore, tumor size, depth of invasion, lymphatic and venous invasion were determined.

Tissue microarray (TMA). Representative areas of solid tumors were identified in HE-stained sections of the selected tumor cases and a 2 mm-in-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). Four-μm-thick sections were consecutively incised from the recipient block and transferred to poly-lysine-coated glass slides. HE 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), MKN45 (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), modified Eagle's medium (MEM, HGC-27) and Ham 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 as routinely processed.

Immunohistochemistry. Consecutive sections were deparaffinised with xylene, dehydrated with alcohol, and subjected to antigen retrieval by irradiating in a target retrieval solution, pH 7.2, (TRS, DAKO, Carpinteria CA93013, USA) for 15 min with a microwave oven (Oriental rotor Limited. Co., Tokyo, Japan). Five percent bovine serum albumin 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-mouse or anti-rabbit Envison-PO (DAKO, USA) antibodies for 15 min. All the incubations were performed in a microwave oven to allow intermittent irradiation as described previously (18, 19). After each treatment, the slides were washed with TBST (10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20) three times for 1 min. Rabbit anti-Arp2 (Santa Cruz, USA; 1:50), rabbit anti-Arp3 (Santa Cruz; 1:50), mouse anti-fascin (LAB VISION, Fremont CA 94530, USA; ready-to-use) and rabbit anti-cortactin (Ab-421; Applied Biological Materials Inc., Vancouver, Canada; 1:25) antibodies were employed to detect the individual proteins. Binding sites were visualized with 3, 3'-diaminobenzidine (DAB). 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 blindly by three independent observers (Takano Y., Zheng H.C. and Li X.H.). The percentage of positively stained cells was graded semi-quantitatively according to a four-tier scoring system: negative (–), 0-5% stained cells; weakly positive (+), 6-25; moderately positive (++), 26-50; and strongly positive (+++), 51-100%.

Statistical analysis. Statistical evaluation was performed using Fisher's test to compare the positive rates and Spearman's correlation test to analyze the rank data. Kaplan-Meier survival plots were generated and comparisons between survival curves were made with the log-rank statistic. The Cox's proportional hazards model was employed for multivariate analysis. $P<0.05$ was considered as statistically significant. SPSS 10.0 software (SPSS, Chicago, IL, USA) was employed to analyze all data.

Results

Expression of Arp2 and Arp3 in gastric carcinogenesis. As shown in Figure 1, Arp3 was positively immunostained in the cytoplasm of MKN28, AGS, MKN45, KATO-III and HGC-27, whereas Arp2 was found in the cytoplasm of MKN28, AGS, KATO-III and HGC27, but not in MKN45. Arp2 and Arp3 were positively stained in the cytoplasm of gastric carcinoma, occasionally in gastric deep propria glands, but not in superficial epithelium (Figure 2). Gastric intestinal metaplasia and signet ring cell carcinomas also showed Arp2 expression (Figure 2). Overall, Arp2 expression was detected in 17 out of 54 gastritis (31.5%), 100 out of 100 intestinal metaplasia (100.0%) and 226 out of total 415 gastric carcinoma patients (54.5%). Arp2 was more frequently expressed in intestinal metaplasia, compared with gastritis and carcinomas ($p<0.05$, Table I). Its expression was also stronger in carcinomas than gastritis ($p<0.05$, Table I). Among various subtypes of gastric carcinomas, the positive rate for Arp2 staining was higher in signet ring cell carcinoma (72.2%, 52/72) than those in other subtypes of carcinomas (46.6%, 169/363; $p<0.05$). However, Arp3 expression was positive in 358 cases of 412 gastric carcinomas (86.9%), higher than gastritis (42.0%, 21/50; $p<0.05$, Table II).

The relationship between Arp2 and Arp3 expression and clinicopathological features of gastric carcinomas. As summarized in Table III, Arp2 expression was positively correlated with tumor size, depth of invasion, venous invasion, UICC staging, expression of cortactin and fascin ($p<0.05$), but not with sex, age, lymphatic invasion, lymph node metastasis or Lauren's classification ($p>0.05$). Table IV shows that Arp3 expression was positively correlated with tumor size, depth of invasion, venous invasion, UICC staging, expression of cortactin, fascin and Arp2 ($p<0.05$), but not with age, sex, lymphatic invasion or lymph node metastasis ($p>0.05$). There was stronger positivity of Arp3 in intestinal-type than diffuse-type carcinomas ($p<0.05$).

Table I. *Arp2 expression in gastric tissue samples*.

Group	n	Arp2 expression				
		–	+	++	+++	PR (%)
Gastritis	54	37	9	6	2	31.5
Intestinal metaplasia	100	0	45	35	20	100.0*
Carcinoma	415	189	162	51	13	54.5**

*Compared with gastritis or carcinoma, $p<0.001$; **compared with gastritis, $p<0.05$; PR, positive rate.

Table II. *Arp3 expression in gastric tissue samples*.

Group	n	Arp3 expression				
		–	+	++	+++	PR (%)
Gastritis	50	29	21	0	0	42.0
Carcinoma	412	54	159	104	95	86.9*

*Compared with gastritis, $p<0.001$; PR, positive rate.

Univariate and multivariate survival analysis. Follow-up information was available on 415 gastric carcinoma patients for periods ranging from 0.2 months to 12.2 years (median=65.6 months). Figure 3 shows survival curves stratified according to Arp2 and Arp3 expression for gastric carcinomas. Univariate analysis using the Kaplan-Meier method indicated that the cumulative survival rate of patients with positive Arp2 or Arp3 expression was not different from those without their expression ($p>0.05$). Multivariate analysis using Cox's proportional hazard model indicated that age, depth of invasion, lymphatic invasion, lymph node metastasis, UICC staging and Lauren's classification ($p<0.05$), but not gender, tumor size, venous invasion, expression of Arp2 or Arp3, were independent prognostic factors for overall gastric carcinomas (Table V).

Discussion

In the present study using more than 400 cases of gastric tumors, we found that Arp2 expression underwent up-regulation and then down-regulation from gastritis to carcinoma through intestinal metaplasia, an adaptive condition for gastric epithelium with injury and inflammation as a precancerous lesion of gastric carcinoma (20). In contrast, overexpression of Arp3 was observed in gastric

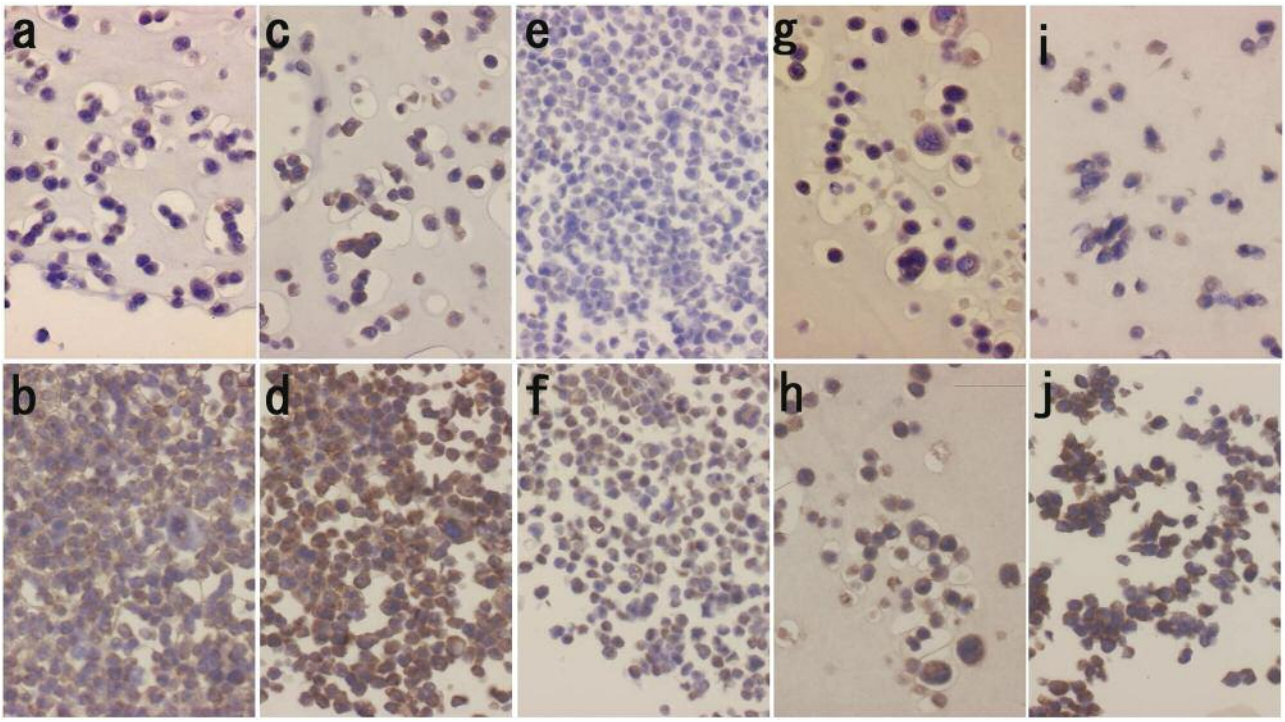

Figure 1. *Immunohistochemical staining of Arp2 and Arp 3 in gastric carcinoma cell lines. Arp2 was positively immunostained in the cytoplasm of MKN28 (a), AGS (c), KATO-III (g) and HGC-27(i), but not in MKN45 (e). Arp3 was expressed in the cytoplasm of MKN28 (b), AGS (d), MKN45 (f), KATO-III (h) and HGC-27(j).*

carcinoma in comparison with gastritis, similar to a previous report (21). These findings suggested that aberrant expression of Arp2 and Arp3 was involved in malignant transformation of gastric epithelial cells. In particular, signet ring cell carcinomas displayed a higher positive rate of Arp2 expression than the other types. Gastric intestinal metaplasia could develop into globoid dysplasia, which is closely linked to signet ring cell carcinomas, as evidenced by morphological and biological characteristics (22-24). High Arp2 expression in intestinal metaplasia and signet ring cell carcinoma indicated that Arp2 might be considered as a good maker for intestinal metaplasia and may be involved in the molecular mechanisms of gastric carcinogenesis from intestinal metaplasia, globoid dysplasia to signet ring cell carcinomas.

Here, overexpression of Arp2 and Arp3 was found to be closely linked to the tumor size, depth of invasion, venous invasion and UICC staging, indicating that they might have impact on the growth, invasion, metastasis and progression of gastric carcinomas and be employed to indicate the aggressive behaviors of carcinomas. It was interesting that Arp2 and Arp3 were more frequently expressed in gastric carcinomas with lymphatic invasion and lymph node metastasis, but the correlation was of no statistical significance. This finding indicated that Arp2 and Arp3 might play some weak role in lymphatic invasion and metastasis. Iwaya *et al*. (25) reported that the colocalization of Arp2 and WAVE2 is an independent risk factor for liver metastasis of colorectal carcinoma. Coexpression of Arp2 and WAVE2 was increased in breast and lung carcinomas with lymph node metastasis (25, 26). Otsubo *et al*. (21) found that the frequency of expression of Arp2 and 3 was higher in invasive carcinoma than that in intramucosal carcinoma of the colon. A recent investigation indicated that Src tyrosine kinase and Rac1 promote recruitment of cortactin and activation of Arp2/3 at the Listeria entry site, mimicking events that occur during adherens junction formation (12). Yoo *et al*. (27) reported that the Arp2/3 complex was physically associated with RNA polymerase II and was involved in the RNA polymerase II-dependent transcriptional regulation both *in vivo* and *in vitro*, indicating that the Arp2/3 complex regulates transcription, similar to the N-WASP through the regulation of nuclear actin polymerization in a manner similar to their function in the cytoplasm (28). The exocystic component Exo70 was also found to interact with the Arp2/3 complex to regulate actin at the leading edges of migrating cells and therefore coordinate cytoskeleton and membrane traffic during cell migration, which is regulated by epidermal growth factor (EGF) signaling (27). Combined together, it was suggested up-regulated Arp2 and Arp3 expression might contribute to

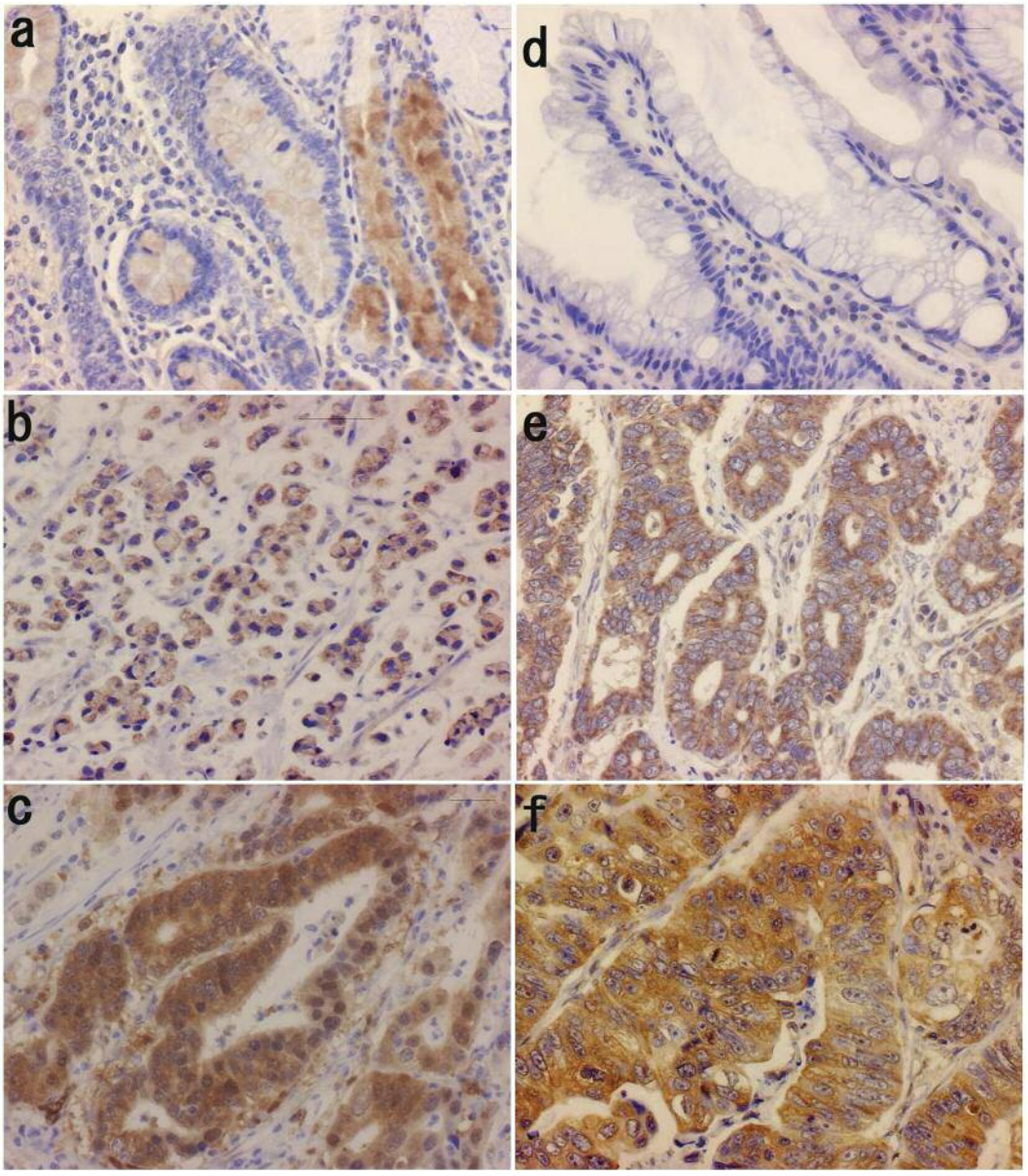

Figure 2. *Immunohistochemical staining in gastric epithelial cells, metaplasia or carcinoma. Note: Arp2, Arp3, fascin or cortactin positivity was localized in the cytoplasm. Arp2 was positively expressed in the gastric epithelial goblet cells, intestinal metaplasia and gastric propria gland (a), whereas Arp3 expression was seldom observed in the superficial epithelium (d). There appeared to be strong expression of Arp2 (b), Arp3 (e), fascin (c), and cortactin (f) in gastric carcinomas.*

invasion and metastasis of malignancies by their nuclear and cytoplasmic ability to control actin polymerization and even transcription. Furthermore, the more frequent expression of Arp3 in intestinal-type than diffuse-type carcinomas suggested that the protein might underlie the molecular basis for differentiation of both carcinomas.

Arp2/3 complex produces a normal dendritic array which then becomes rearranged into parallel bundles through intermediate formation of Λ-precursors. Fascin recruitment to the clustered barbed ends of Λ-precursors initiates filament bundling. Cortactin can directly activate Arp2/3 complex through the binding of the amino-terminal acidic domain region to Arp2/3 complex and the repeat region F-actin and stabilize newly generated filament branch points (4, 11, 12, 29, 30). In the immunohistochemical study, we found that gastric carcinomas exhibited coexpression of these actin-related or -binding proteins, such as Arp2, Arp3, fascin and cortactin, which are involved in carcinoma cell motility,

Table III. *Relationship between Arp2 expression and clinicopathological features of gastric carcinoma.*

Clinicopathological features	n	Arp2 expression					
		–	+	++	+++	PR (%)	*p*-value
Age (years)							>0.05
<65	171	77	74	16	4	55.0	
≥65	244	112	88	35	9	54.1	
Gender							>0.05
Male	288	133	109	35	11	53.8	
Female	127	56	53	16	3	55.9	
Tumor size (cm)							<0.01
<4	216	110	80	22	4	49.1	
≥4	199	79	82	29	9	60.3	
Depth of invasion							<0.01
T_{is-1}	212	108	81	21	2	49.1	
T_{2-4}	203	81	81	30	11	60.1	
Lymphatic invasion							>0.05
–	266	128	100	32	6	51.9	
+	149	61	62	19	7	59.1	
Venous invasion							<0.01
–	357	170	139	39	9	52.4	
+	58	19	23	12	4	67.2	
Lymph node metastasis							>0.05
–	259	125	101	29	4	51.7	
+	156	64	61	22	9	59.0	
UICC staging							<0.001
0-I	229	119	87	21	2	48.0	
II-IV	186	70	75	30	11	62.4	
Lauren's classification							>0.05
Intestinal-type	223	103	87	27	6	53.8	
Diffuse-type	182	80	71	24	7	56.0	
Cortactin expression							<0.01
–	201	107	73	18	3	46.8	
+ to +++	214	82	89	33	10	61.7	
Fascin expression							<0.001
–	307	153	118	31	5	50.2	
+ to +++	108	36	44	20	8	66.7	

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.

Table IV. *Relationship between Arp3 expression and clinicopathological features of gastric carcinoma.*

Clinicopathological features	n	Arp3 expression					
		–	+	++	+++	PR (%)	*p*-value
Age (years)							>0.05
<65	167	25	68	42	32	85.0	
≥65	245	29	91	62	63	88.2	
Gender							>0.05
Male	286	39	104	74	69	86.4	
Female	126	15	55	30	26	88.1	
Tumor size (cm)							<0.05
<4	209	33	84	50	42	84.2	
≥4	203	21	75	54	53	89.7	
Depth of invasion							<0.01
T_{is-1}	207	33	88	48	38	84.1	
T_{2-4}	205	21	71	56	57	89.8	
Lymphatic invasion							>0.05
–	259	35	109	61	54	86.5	
+	153	19	50	43	41	87.6	
Venous invasion							<0.01
–	351	51	141	85	74	85.5	
+	61	3	18	19	21	95.1	
Lymph node metastasis							>0.05
–	251	35	102	63	51	86.1	
+	161	19	57	41	44	88.2	
UICC staging							<0.01
0-I	222	35	94	53	40	84.2	
II-IV	190	19	65	51	55	90.0	
Lauren's classification							<0.01
Intestinal-type	228	27	77	57	67	88.2	
Diffuse-type	180	26	81	45	28	85.6	
Cortactin expression							<0. 01
–	197	36	96	44	21	81.7	
+~+++	215	18	63	60	74	91.6	
Fascin expression							<0.001
–	304	43	128	77	56	85.9	
+ to +++	108	11	31	27	39	89.8	
Arp2 expression							<0.001
–	160	33	83	34	10	79.4	
+ to +++	214	10	57	65	82	95.3	

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.

spreading and metastasis by modulating the construction of branched actin filament arrays to form various shaped protrusions. It was suggested that these proteins might concordantly prompt invasion and metastasis of gastric carcinoma cells.

In this investigation, for the first time, we analyzed the relation of Arp2 and Arp3 expression with the survival of more than 400 patients with gastric carcinoma and failed to find any significant relationship between their expression and survival although reports indicated that co-expression of Arp2 and WAVE2 could be employed to indicate the poor prognosis of patients with breast or lung carcinomas (25, 31). Our findings suggest that Arp2 and Arp3 expressions are not good indicators for the unfavorable prognosis of gastric carcinoma patients. The multivariate analysis demonstrated that age, depth of invasion, lymphatic invasion, lymph node metastasis, UICC staging and Lauren's classification were independent prognostic factors for patients with carcinomas. Although Arp2 and Arp3 expressions were closely linked to depth of invasion, lymphatic invasion and UICC staging, a lack of any association of their expression with such independent prognostic factors as lymph nodal involvement might explain their unexpected failure as indicators of prognosis.

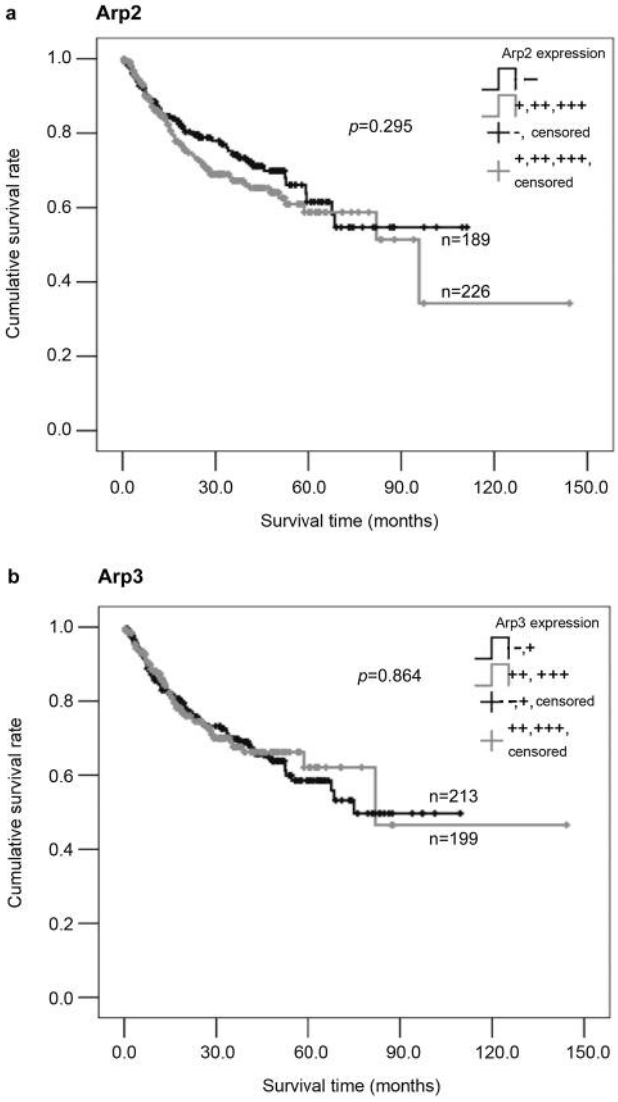

Figure 3. *Kaplan-Meier curves for cumulative survival rate of patients with gastric carcinomas according to the expression of Arp2 (a) and Arp3 (b).*

In summary, elevated Arp2 and Arp3 expression might play an important role in malignant transformation of gastric epithelial cells and be closely related to growth, invasion and high UICC staging of patients with gastric carcinomas. Arp3 expression could be employed to differentiate between the intestinal- and diffuse-type carcinomas and underlay the molecular mechanism of the differentiation of both carcinomas. Arp2 and Arp3 are considered as objective and effective markers to indicate the pathobiological behaviors and prognosis of gastric carcinomas.

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Table V. *Multivariate analysis of clinicopathological variables for survival of patients with gastric carcinoma.*

Clinicopathological feature	Relative risk (95% CI)	*p*-value
Age (≥65 years)	1.925 (1.263-2.934)	<0.01
Gender (female)	1.218 (0.753-1.971)	>0.05
Tumor size (≥4 cm)	1.357 (0.796-2.315)	>0.05
Depth of invasion (T_{2-4})	7.393 (2.714-20.144)	<0.01
Lymphatic invasion (+)	2.229 (1.307-3.800)	<0.01
Venous invasion (+)	1.009 (0.629-1.618)	>0.05
Lymph node metastasis (+)	3.300 (1.676-6.497)	<0.01
UICC staging (II-IV)	0.277 (0.102-0.752)	<0.05
Lauren's classification (diffuse-type)	2.102 (1.3416-3.294	<0.01
Arp2 expression (+ to +++)	0.784 (0.442-1.391)	>0.05
Arp3 expression (+ to +++)	0.662 (0.548-1.617)	>0.05

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

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