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Nuclear expression of N-myc downstream regulated gene 1/ Ca^{2+}-associated protein 43 is closely correlated with tumor angiogenesis and poor survival in patients with gastric cancer

AKIHIKO KAWAHARA[1,2], JUN AKIBA[4], SATOSHI HATTORI[3], TOMOHIKO YAMAGUCHI[1], HIDEYUKI ABE[1], TOMOKI TAIRA[1], HIROKI URESHINO[6], YUICHI MURAKAMI[6], KOSUKE WATARI[6], KIKUO KOUFUJI[5], KAZUO SHIROUZU[5], MICHIHIKO KUWANO[7], MAYUMI ONO[6] and MASAYOSHI KAGE[1,2]

[1]Department of Diagnostic Pathology, Kurume University Hospital; [2]Center for Innovative Cancer Therapy, and [3]Biostatistics Center, Kurume University; Departments of [4]Pathology, and [5]Surgery, Kurume University School of Medicine, Kurume; [6]Department of Pharmaceutical Oncology, and [7]Laboratory of Molecular Cancer Biology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

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Abstract. Expression of N-myc downstream regulated gene 1 (NDRG1)/Ca^{2+}-associated protein 43 (Cap43) in cancer cells is a predictive marker of good or poor prognosis depending on tumor type. In this study, we examined whether NDRG1/Cap43 is a marker of good or poor prognosis in gastric cancer patients, and whether it is associated with tumor stromal responses, including angiogenesis and macrophage infiltration. The expression levels of NDRG1/Cap43, the number of CD68-positive macrophages and the CD34-positive microvessel density were analyzed by immunohistochemistry in 129 gastric cancer patients, including 65 with the intestinal type and 64 with the diffuse type. The expression of NDRG1/Cap43 in the nucleus and the membrane was evaluated. Nuclear NDRG1/Cap43 expression was found in 20/65 (30.8%) patients with the intestinal type and in 9/64 (14.1%) patients with the diffuse type of gastric cancer. Nuclear NDRG1/Cap43 expression was significantly associated with pathological stage in the intestinal type (P=0.002), but not in the diffuse type (P=0.039). Nuclear NDRG1/Cap43 expression was also closely associated with infiltrating macrophages (P=0.001) and tumor angiogenesis (P=0.001) in the intestinal type. Furthermore, nuclear NDRG1/Cap43 expression was associated with poor prognosis in both the intestinal (P=0.001) and the diffuse types of gastric cancer (P=0.047). By contrast, membranous NDRG1/Cap43 expression was not associated with the overall survival of gastric cancer patients with either the intestinal or diffuse type of gastric cancer. The expression of NDRG1/Cap43 in the nucleus may be a predictive biomarker for malignant progression in the intestinal type of gastric cancer, preferable to the expression of NDRG1/Cap43 in the membrane.

Correspondence to: Dr Akihiko Kawahara, Department of Diagnostic Pathology, Kurume University Hospital, 67 Asahi-machi, Kurume 830-0011, Japan
E-mail: akihiko4@med.kurume-u.ac.jp

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Introduction

N-myc downstream regulated gene 1 (NDRG1)/Ca^{2+}-associated protein 43 (Cap43) has been identified as a nickel- and calcium-induced gene, identical to the homocysteine-inducible gene, reduced in tumor (RTP/rit42) and to the differentiation-related gene-1 (Drg-1) (1-6). In cancer progression, overexpression of NDRG1/Cap43 was found to reduce cell proliferation and anchorage-independent growth *in vitro* and tumor growth *in vivo* (2). However, NDRG1/Cap43 has no effect on the primary tumor growth of colon and prostate cancer *in vivo* (7,8). NDRG1/Cap43 expression has been found to be increased in numerous types of human cancer in comparison to normal tissues (8). However, other studies have reported that the expression of NDRG1/Cap43 is increased in normal cells and in well-differentiated cancer cells, but decreased in poorly differentiated cancer cells and in cancer of the colon, prostate, breast and pancreas (7-11). This suggests a close association of NDRG1/Cap43 with the cancer differentiation status.

NDRG1/Cap43 is a predictive marker of good prognosis in patients with cancer of the prostate, esophagus, breast, colon and pancreas, and with neuroblastoma (2,8,12-15). However, the expression of NDRG1/Cap43 is a predictive marker of poor prognosis in patients with liver, colon, esophagal and cervical cancer (16-19). Taken together, the effectiveness of NDRG1/Cap43 as a predictive marker of good or poor prognosis in cancer patients may depend on the type of human malignancy and the histological type or differentiation status of the tumor (20).

We previously reported that NDRG1/Cap43 expression suppressed tumor growth and angiogenesis in a human pancreatic cancer xenograft model (11). Overexpression of NDRG1/Cap43 resulted in a marked inhibition of the production of the potent angiogenesis factors VEGF-A and IL-8 (CXCL8) and

of matrix metalloproteinase-9 (MMP-9) by pancreatic cancer cells, suggesting a possible role of NDRG1/Cap43 in angiogenesis and extracellular remodeling in the tumor stroma (11). Our recent study further demonstrated that NDRG1/Cap43 decreased the expression of various chemoattractants, including CXC chemokines (CXCL5, CXCL1 and CXCL8) for inflammatory cells, and the recruitment of macrophages and neutrophils with the suppression of angiogenesis and growth in pancreatic cancer (21). The underlying mechanism whereby NDRG1/Cap43 suppressed tumor growth and angiogenesis as well as the production of CXC chemokines in pancreatic cancer appeared to be due to the attenuation of NF-κB signaling through marked decreases in IKKβ expression and IKBα phosphorylation (21,22). In patients with pancreatic cancer, the NDRG1/Cap43 expression levels were inversely correlated with the number of infiltrating macrophages and tumor angiogenesis in the tumor stroma.

The key role infiltrating macrophages play in the tumor stroma, promoting the malignant progression of cancer through interaction with cancer cells, has recently been highlighted (23-25). Tumor-supportive macrophages play an active role in extracellular matrix remodeling, tissue repair and angiogenesis, whereas tumor-suppressive macrophages exert antimicrobial and antitumor activities through immunostimulatory functions (26,27). Tumor-supporting macrophages, also known as tumor-associated macrophages (TAMs), promote invasion, metastasis and angiogenesis through the production of inflammatory cytokines, chemokines, proteases, prostanoids, growth factors and angiogenic factors. Clinical studies have demonstrated a close association between the abundance of TAMs and poor prognosis or tumor angiogenesis in various types of solid tumors (28). A number of chemotactic cytokines are expected to play important roles in the recruitment and accumulation of macrophages in the tumor stroma, and TAMs that are recruited to the tumor play a key role in the angiogenic switch and malignant transition of cancer. We previously reported that depletion of these TAMs, as well as macrophages, by macrophage-targeting bisphosphonate encapsulated in liposomes markedly inhibits tumor growth, angiogenesis and bone metastasis (24-31), suggesting the involvement of macrophages in tumor growth, metastasis and angiogenesis (25).

In the present study, we investigated whether the expression of NDRG1/Cap43 is a biomarker for the favorable or poor prognosis of gastric cancer, as no previous study has focused on the role of NDRG1/Cap43 in the differentiation status of gastric cancer. We also examined whether NDRG1/Cap43 modulates the infiltration of macrophages and angiogenesis in the tumor stroma of gastric cancer, in association with histological type and progression. We also discuss whether NDRG1/Cap43 plays a role in tumor stromal responses, thus affecting tumor progression in gastric cancer.

Materials and methods

Patients and tumor samples. The study comprised 129 patients with advanced gastric cancer whose tumors had been surgically removed at the Department of Surgery, Kurume University, between 2001 and 2004. The age of the gastric cancer patients ranged from 33 to 86 years (median 69); 91 were male and 38 were female. Histological types were classified according to the criteria of the Lauren classification (32), and tumor stage was classified according to the TNM classification. Patient characteristics are summarized in Table I. Cancer stages included 30 (23.2%) cases of stage I (IA + IB), 18 (14.0%) stage II, 27 (20.9%) stage III (IIIA + IIIB) and 54 (41.9%) stage IV. At the time of surgery, 79 (61.2%), 17 (13.2%) and 29 (22.5%) patients had lymph node metastasis, liver metastasis or peritoneal dissemination, respectively. No patients had been administered drugs before surgery, including neo-adjuvant chemotherapy, and the standard chemotherapy was performed after surgery: stage II or III patients were administered TS-1 and stage IV patients were administered a combination of TS-1 and cisplatin.

Table I. NDRG1/Cap43 expression and clinicopathological characteristics in the gastric cancer patients.

	Gastric cancer patients		
	Total (n=129)	Intestinal type (n=65)	Diffuse type (n=64)
Age (years)			
<66	52	21	31
≥66	77	44	33
Gender			
Male	91	56	35
Female	38	9	29
Pathological stage			
I	30	17	13
II	18	9	9
III	27	14	13
IV	54	25	29
Membrane NDRG1/Cap43			
Positive	76	50	26
Negative	53	15	38
Nuclear NDRG1/Cap43			
Positive	29	20	9
Negative	100	45	55

Immunohistochemistry (IHC). Paraffin-embedded tissue samples were cut into 4-μm sections, examined on a coated glass slide and labeled with the following antibodies using the BenchMark XT (Ventana Automated Systems, Inc., Tucson, AZ, USA) and ChemMate Envision (DakoCytomation, Glostrup, Denmark) methods: NDRG1/Cap43 (x200, produced in our laboratory) (11), CD68 (x1,200; KP-1; DakoCytomation) and CD34 (x200; Novo Castra, Newcastle, UK). For NDRG1/Cap43, the BenchMark XT was used. Each slide was heat-treated using Ventana's CC1 retrieval solution for 30 min and incubated with the NDRG1/Cap43 antibody for 30 min. This automated system used the streptavidin biotin complex method with 3,3' diaminobenzidine as the chromogen (Ventana iVIEW DAB detection kit). The ChemMate Envision method was used for CD68 and CD34. Endogenous peroxidase activity was inhibited by incubating the slides in 3% H_2O_2 for 5 min. CD68 and CD34 antigen retrieval was performed by treatment with proteinase K for 5 min. Each slide was incubated for 30 min with the antibody at room

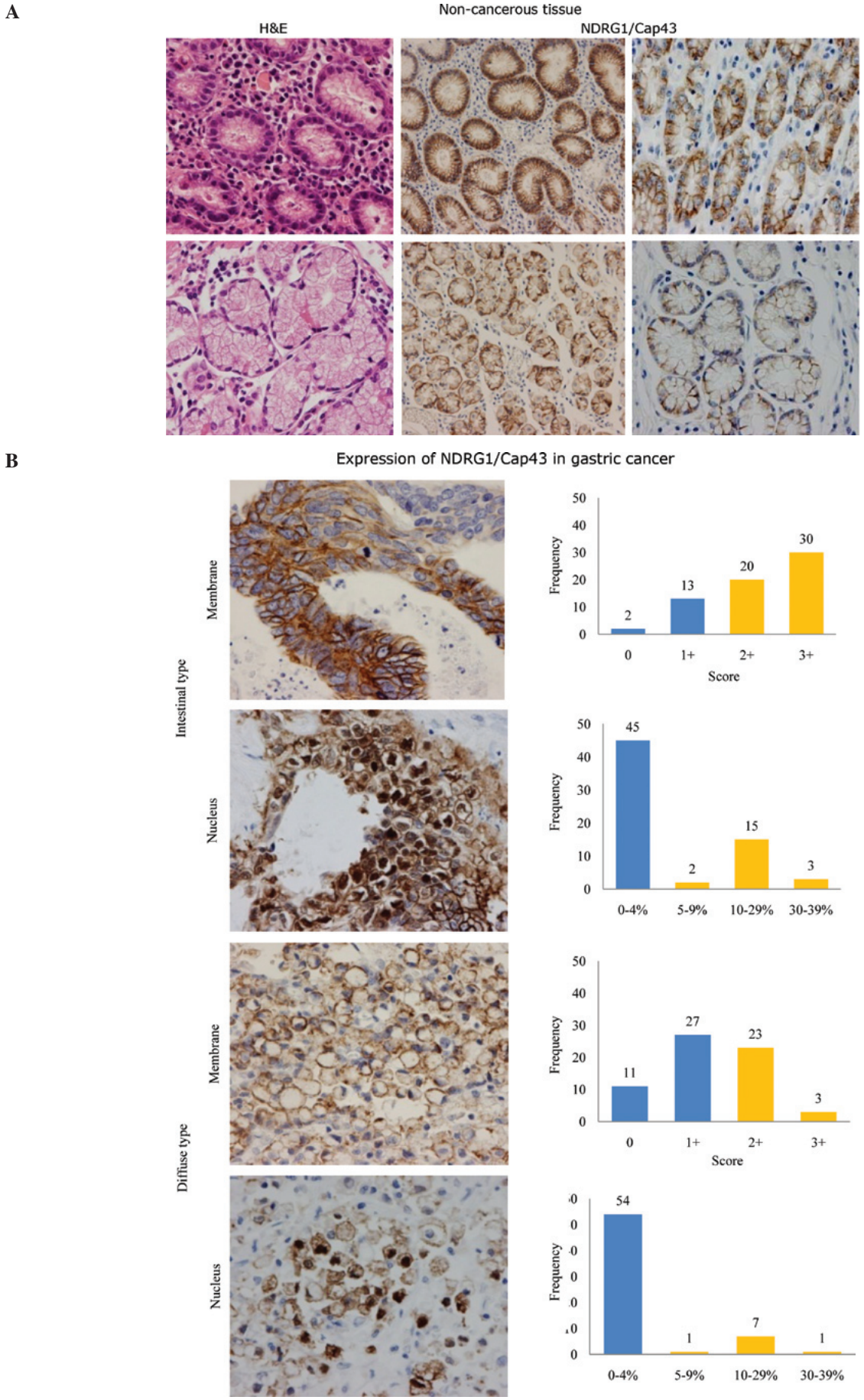

Figure 1. Representative NDRG1/Cap43 immunostaining in gastric cancer of the intestinal and diffuse types. (A) The specific localizations of immunostaining with NDRG1/Cap43 are observed in the membrane and/or cytoplasm in only the non-cancerous gastric mucosal cells. (B) In the histograms indicating the score for the expression of NDRG1/Cap43 in the membrane, the intestinal type exhibited overexpression of NDRG1/Cap43 compared to the diffuse type. Nuclear localization of NDRG1/Cap43 was consistently noted only in the cancer cells.

Table II. Association of nuclear and membranous NDRG1/Cap43 expression with pathological stage.

Histological type	Pathological stage				Total	P-value
	I	II	III	IV		
NDRG1/Cap43 (nucleus)						
Intestinal type, n (%)						
Negative	17 (37.8)	6 (13.3)	10 (22.2)	12 (26.7)	45	0.002
Positive	0 (0.0)	3 (15.0)	5 (25.0)	12 (60.0)	20	
Diffuse type, n (%)						
Negative	13 (23.6)	10 (18.2)	9 (16.4)	23 (41.8)	55	0.039
Positive	0 (0.0)	0 (0.0)	3 (33.3)	6 (66.7)	9	
NDRG1/Cap43 (membrane)						
Intestinal type, n (%)						
Negative	10 (66.7)	1 (6.7)	0 (0.0)	4 (26.7)	15	0.002
Positive	7 (14.0)	8 (16.0)	15 (30.0)	20 (40.0)	50	
Diffuse type, n (%)						
Negative	8 (21.0)	5 (13.1)	9 (23.7)	16 (42.1)	38	0.926
Positive	5 (19.2)	5 (19.2)	3 (11.5)	13 (50.0)	26	

Association between stage and nuclear NDRG1/Cap43 expression tested by the Mantel-Haenszel linear trend test.

temperature. For staining detection, the ChemMate Envision method was used with DAB as the chromogen. Healthy non-cancerous mucosal lesions were used as controls.

Evaluation of NDRG1/Cap43 expression in the membrane and nucleus of gastric cancer cells. With regard to the expression of NDRG1/Cap43, membranous and/or cytoplasmic and nuclear staining was observed in gastric cancer tissues by IHC. Expression of NDRG1/Cap43 was predominantly found in the membrane and/or cytoplasm of gastric mucosal cells (Fig. 1A), and in the cancerous cells in both the intestinal and diffuse types of gastric cancer (Fig. 1B). By contrast, the expression of NDRG1/Cap43 in the nucleus was consistently observed only in the gastric cancer cells (Fig. 1B). Based on the IHC profiles of membranous and nuclear staining, the presence and absence of NDRG1/Cap43 expression was evaluated. The intensity of membranous NDRG1/Cap43 expression was scored using the following scale: no staining, 0; weak staining, 1+; moderate staining, 2+; and strong staining, 3+ in >10% of cancer cells. Scores of 0 and +1 were classified as negative, and scores of 2+ and 3+ as positive. The expression of nuclear NDRG1/Cap43 was classified based on the percentage of cancer cells with strongly stained nuclei: ≥5% indicated that the cancer tissue was positive and ≤4% indicated that it was negative. NDRG1/Cap43 expression was evaluated by two experienced observers (A.K. and M.K.) blinded to the condition of the patients.

Determination of the number of CD68$^+$ macrophages and CD34$^+$ microvessel density (MVD). Digital expression data were extracted using the following image analysis systems: CD68- and CD34-stained specimens were examined to identify the areas of expression with high density. Images of the areas of expression were selected for clarity from at least 6 fields at x200 for each IHC specimen using a CCD digital camera (Nikon, DXM1200). Expression analysis was performed to measure the areas of expression of the number of macrophages and MVD in all cases using 'Win ROOF' software (version 5.7; Mitani Corporation, Osaka, Japan) (33). The digitized data of the expression areas were measured and averaged.

Statistical analysis. The distribution of CD68$^+$ and CD34$^+$ was compared between NDRG1/Cap43-negative and -positive patients with the Wilcoxon rank-sum test and was displayed with box plots. Associations between CD68$^+$, CD34$^+$ and stage were examined by comparing the distribution of CD68$^+$ and CD34$^+$ among patients of each stage with the Kruskal-Wallis test and displayed with box plots. Associations between NDRG1/Cap43 and stage were examined by the Mantel-Haenszel linear trend test. Overall survival was defined as days from surgery until death due to any cause. The log-rank test and the Kaplan-Meier method were applied to examine the effect of NDRG1/Cap43 on overall survival. The hazard ratio of NDRG1/Cap43-positive patients relative to NDRG1/Cap43-negative patients was estimated by applying the Cox regression model. When adjusting for possible confounding factors in the Cox regression model, stage was not adjusted, since it may be an intermediate variable in evaluating the effects of NDRG1/Cap43 on patient prognosis (34). Statistical analysis was performed by SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), StatXact (Cytel Inc., Cambridge, MA, USA) and R version 2.8.1.

Results

Clinicopathological features and expression of NDRG1/Cap43 in non-cancerous gastric mucosal cells and gastric cancer cells. In non-cancerous gastric mucosal cells, the expression of NDRG1/Cap43 was observed in the membrane and/or cytoplasm of almost all the cells, and no nuclear expression was evident (n=77) (Fig. 1A). Among the 129 gastric cancer specimens analyzed, 65 were classified as the intestinal type

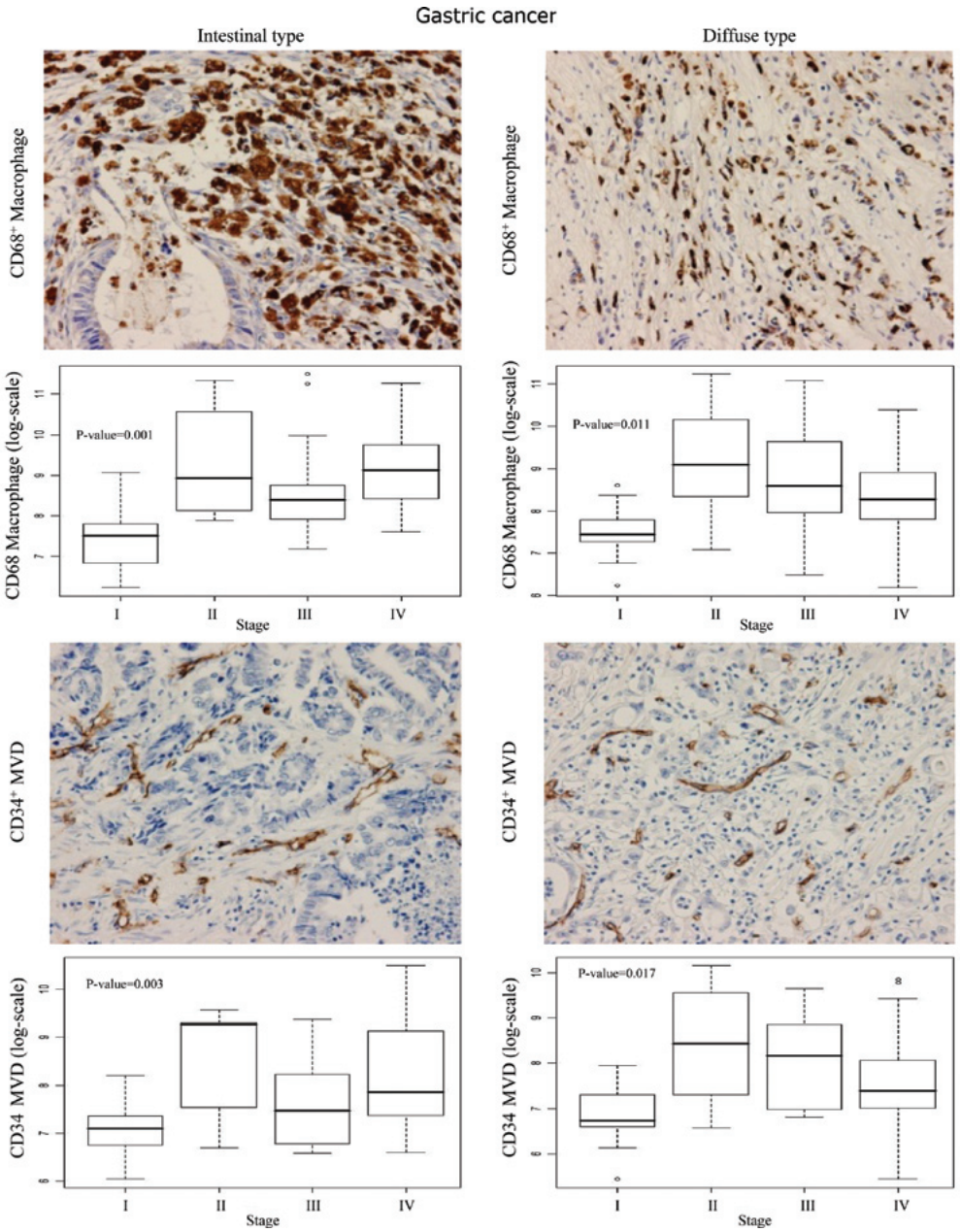

Figure 2. Representative IHC images showing the expression levels of CD68+ macrophages and CD34+ microvessel density at histological stages I, II, III and IV in the intestinal and diffuse types of gastric cancer.

and 64 as the diffuse type. NDRG1/Cap43-positive expression in the membrane was observed in 50 (76.9%) patients with the intestinal type and in 26 (40.6%) patients with the diffuse type. Nuclear expression of NDRG1/Cap43 in gastric cancer cells was evident in 29/129 (22.5%) patients (Fig. 1B).

Increasing nuclear NDRG1/Cap43 expression during progression of pathological stage in gastric cancer patients. Nuclear NDRG1/Cap43 expression was not increased at stage I in the intestinal type and at stages I and II in the diffuse type, but nuclear NDRG1/Cap43 expression was significantly increased at later pathological stages in both the intestinal and diffuse types (Table II). Among the 20 patients with positive nuclear expression of NDRG1/Cap43 in the intestinal type, 17 (85%) were classified as stages III and IV, compared to only 22 (48.9%) of the 45 patients who exhibited negative nuclear NDRG1/Cap43 expression. All 9 patients with nuclear NDRG1/Cap43-positive expression in the diffuse type were at stages III and IV, compared to 32/55 (58.2%) patients with nuclear NDRG1/Cap43-negative expression. The P-value of the linear trend was statistically significant for both the intestinal (P=0.002) and the diffuse type (P=0.039). The linear trend test for membranous NDRG1/Cap43 expression was statistically significant for the intestinal type (P=0.002), but not for the diffused type (P=0.926) (Table II).

Association of NDRG1/Cap43 expression with infiltrating macrophages and tumor angiogenesis in the intestinal, but not in the diffuse type. As shown in Fig. 2, the CD68+ macrophage count and CD34+ MVD were high in the cancerous region in both the intestinal and diffuse types, compared to the non-cancerous gastric mucosal cells. Box

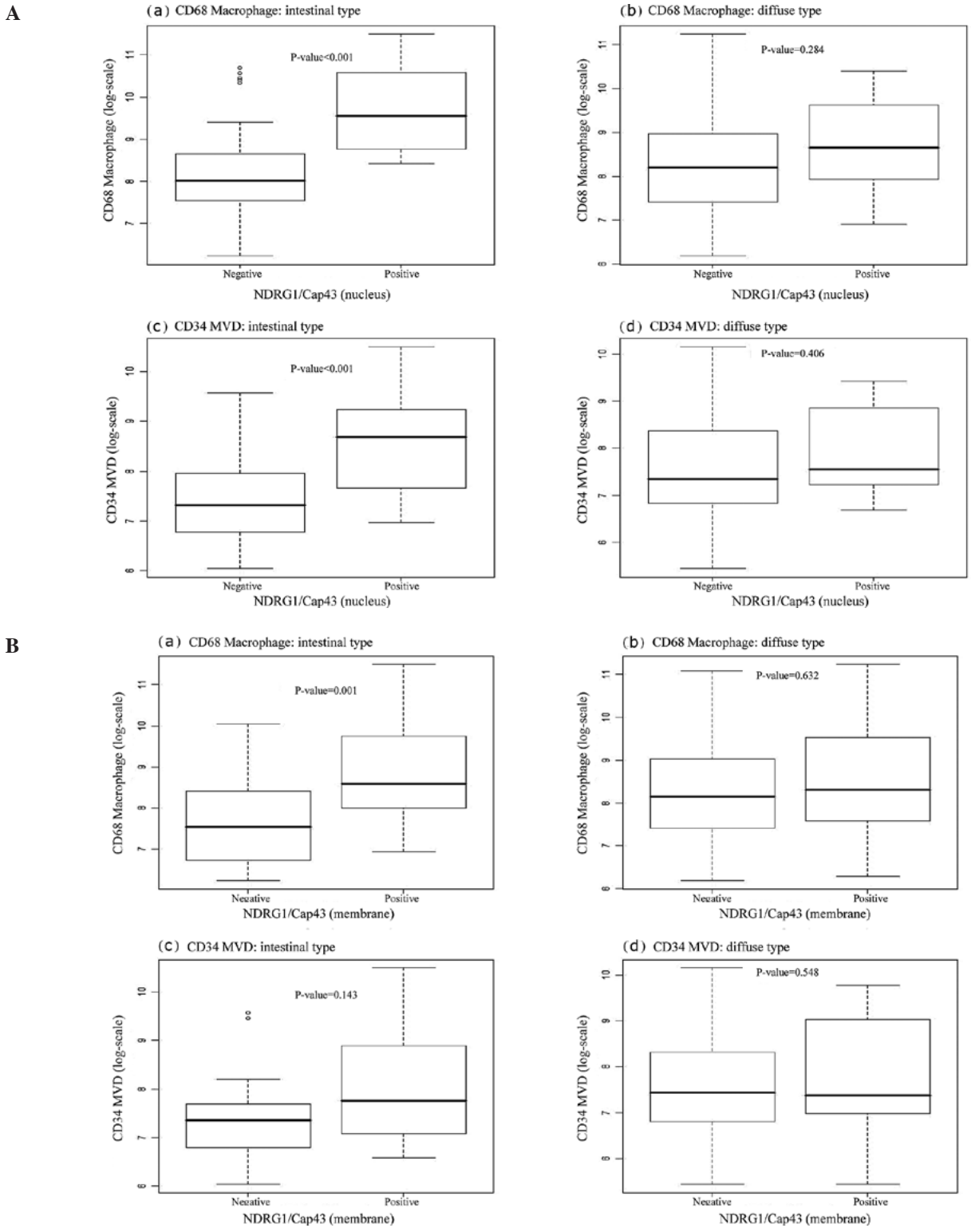

Figure 3. (A) Association of nuclear NDRG1/Cap43 expression with the number of infiltrating CD68$^+$ macrophages and MVD in the intestinal type (a and c) and the diffuse type (b and d) of gastric cancer. (B) Association of membranous NDRG1/Cap43 expression with the number of infiltrating CD68$^+$ macrophages and MVD in the intestinal type (a and c) and the diffuse type (b and d) of gastric cancer.

blots showed significantly higher numbers of infiltrating CD68$^+$ macrophages (P<0.001) and higher MVD (P=0.003) at stages II, III and IV compared to stage I in the intestinal type (Fig. 2). A stage-dependent increase in both macrophage count (P=0.011) and MVD (P=0.017) was also observed in the diffuse type of gastric cancer.

We next examined whether nuclear or membranous NDRG1/Cap43 expression was correlated with the infiltrating CD68$^+$ macrophage count and MVD. Box plots indicated that the number of infiltrating CD68$^+$ macrophages was significantly correlated with nuclear NDRG1/Cap43 expression in the intestinal type (P<0.001) (Fig. 3A-a), but not in the diffuse type

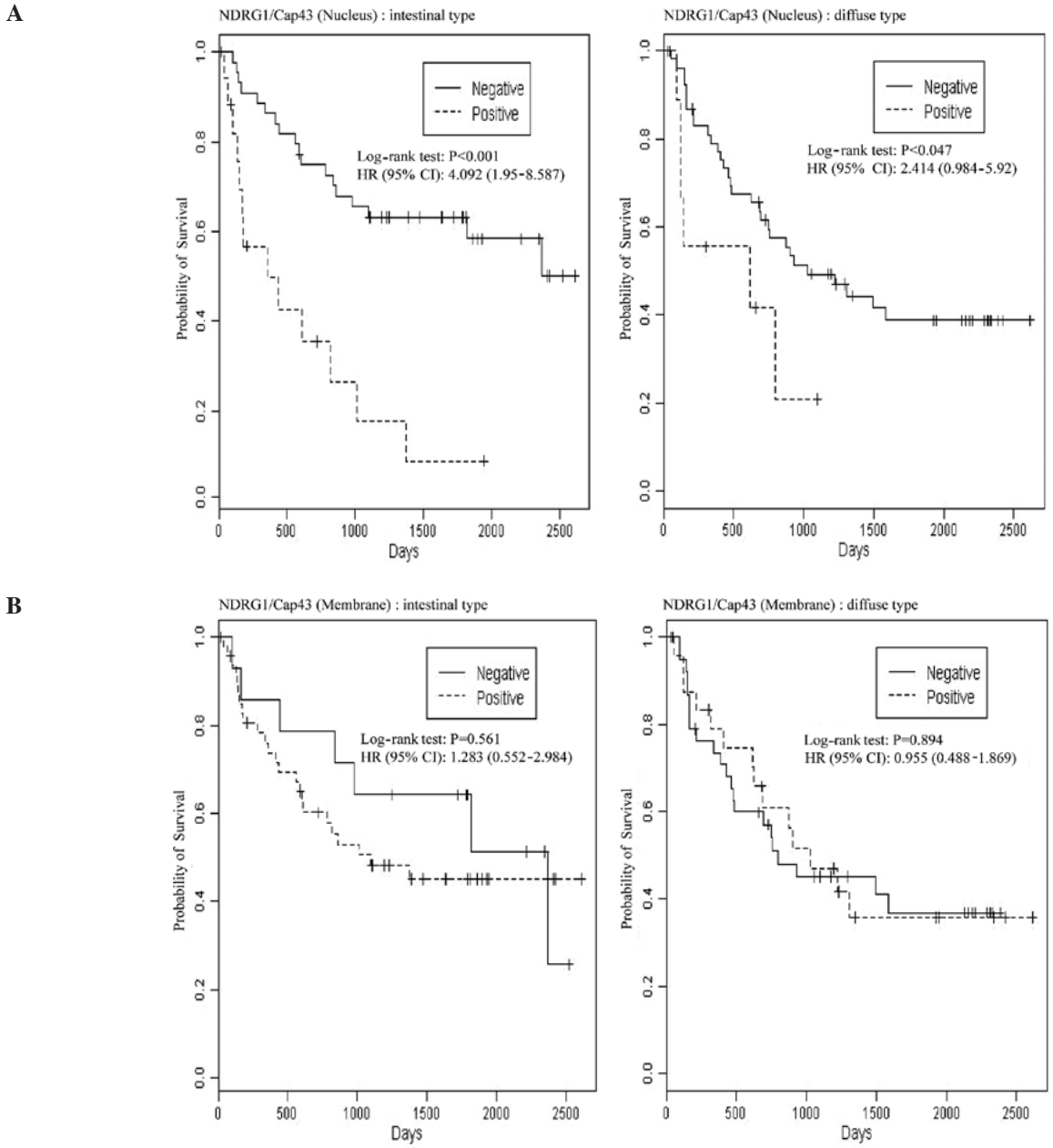

Figure 4. (A) Kaplan-Meier estimators for overall survival in the intestinal and diffuse types of gastric cancer according to positive (---) and negative (–) NDRG1/Cap43 expression in the nucleus with P-value by the log-rank test. Patients with NDRG1/Cap43-positive expression in the nucleus had a statistically significantly poor prognosis for overall survival in both the intestinal and diffuse types. (B) Kaplan-Meier estimators for overall survival in the intestinal and diffuse types of gastric cancer according to positive (---) and negative (–) NDRG1/Cap43 expression in the membrane with P-value by the log-rank test. Membranous NDRG1/Cap43 expression was not associated with overall survival in both the intestinal and diffuse types.

(Fig. 3A-b). There was also a significant association of nuclear NDRG1/Cap43 expression with MVD, in the intestinal type (P=0.001) (Fig. 3A-c), but not in the diffuse type (Fig. 3A-d). Furthermore, membranous NDRG1/Cap43 expression was found to be significantly correlated with the number of infiltrating $CD68^+$ macrophages (P=0.001), but not with MVD in the intestinal type (Fig. 3B-a and -c). There was no significant association between membranous NDRG1/Cap43 expression and the number of infiltrating $CD68^+$ macrophages or MVD in the diffuse type of gastric cancer (Fig. 3B-b and -d).

Association of NDRG1/Cap43 expression with the survival of patients with intestinal type gastric cancer. We further examined whether NDRG1/Cap43 expression in the membrane and nucleus was associated with the overall survival of gastric cancer patients. Log-rank P-values for nuclear expression of NDRG1/Cap43 and Kaplan-Meier plots are shown in Fig. 4A. Among patients with the intestinal type, those who were nuclear NDRG1/Cap43-positive had significantly shorter survival than those who were nuclear NDRG1/Cap43-negative [P<0.001, hazard ratio (HR)=4.092, confidence interval (CI) 1.950-8.547]. Among patients with the diffuse type, a similar tendency was observed, with statistical significance (P=0.047, HR=2.414, CI 0.984-5.920). The results remained similar even after adjustment for possible confounding factors, such as age and gender, by Cox regression (P=0.002, HR=4.163, CI 1.970-8.797 for

the intestinal type and P=0.111, HR=2.086, CI 0.845-5.145 for the diffuse type). By contrast, membranous NDRG1/Cap43 expression was not associated with overall survival in either the intestinal or the diffuse type of gastric cancer (Fig. 4B).

Discussion

In the present study, we observed that the expression of NDRG1/Cap43 in the nucleus was specifically increased during stage progression of both the intestinal and diffuse types of gastric cancer. We evaluated whether nuclear or membranous NDRG1/Cap43 expression affected tumor angiogenesis, the infiltration of macrophages and patient survival, and demonstrated that nuclear NDRG1/Cap43 expression, rather than its membranous expression, was significantly correlated with the number of infiltrating macrophages and tumor angiogenesis in the intestinal, but not in the diffuse, type of gastric cancer. Furthermore, nuclear NDRG1/Cap43 expression was associated with a poor prognosis in both the intestinal and diffuse types of gastric cancer.

During normal postnatal development, NDRG1/Cap43 is expressed in the membrane and/or cytoplasm of cells in the rat kidney and brain (35). NDRG1/Cap43 is also a membrane and/or cytoplasm protein in human tissues, but its cellular localization is dependent on cell type (35). For instance, epithelial cells of the prostate predominantly show membranous expression of NDRG1/Cap43. Analysis of human NDRG1/Cap43 localization has demonstrated that the probability of NDRG1/Cap43 expression in the membrane and/or cytoplasm, nucleus and mitochondria is 47.8, 26.1 and 8.7%, respectively (36). Overexpression of NDRG1/Cap43 is an indicator of poor prognosis in hepatocellular carcinoma (17) and cervical adenocarcinoma (18), and NDRG1/Cap43 is specifically expressed in the membrane and/or cytoplasm. In hepatic cancer cells, NDRG1/Cap43 is localized in the membrane and/or cytoplasm both *in vivo* and *in vitro* (37). In the present study, NDRG1/Cap43 was localized in the membrane and/or cytoplasm of normal gastric mucosal cells and in early-stage gastric cancer. By contrast, NDRG1/Cap43 was not expressed in the nucleus of normal gastric mucosal cells or in cancer cells at earlier stages, but its nuclear expression was markedly increased in cancer cells at later stages of progression. Thus, in gastric cancer, nuclear NDRG1/Cap43 expression may play a role in tumor progression.

NDRG1/Cap43 is a specific differentiation-related gene first identified by van Belzen *et al* (1). NDRG1/Cap43 expression suppresses the expression of angiogenic factors and MMP-9 in pancreatic cancer cells, and also suppresses tumor growth and angiogenesis in human pancreatic cancer (11,21). Furthermore, macrophage infiltration and tumor angiogenesis have been shown to be significantly correlated with the expression level of NDRG1/Cap43 in patients with pancreatic cancer (20). Contrary to the inverse association of NDRG1/Cap43 expression with tumor angiogenesis and prognosis in pancreatic cancer, our present study demonstrated that NDRG1/Cap43 expression was positively correlated with tumor angiogenesis in the intestinal type of gastric cancer. Thus, depending on tumor type, NDRG1/Cap43 may be a positive or negative biomarker of malignant progression, including tumor angiogenesis, infiltration of TAMs and the prognosis of cancer patients. Nuclear NDRG1/Cap43 may positively regulate the infiltration of macrophages, including TAMs in tumors, as well as tumor angiogenesis, in the intestinal type of gastric cancer. We favor the idea that NDRG1/Cap43 induces the accumulation and activation of macrophages/monocytes, resulting in an angiogenic switch in the tumor stroma (25), probably in close connection with the differentiation status. Further study is required to ascertain how NDRG1/Cap43 functions in association with histological type in gastric cancer.

In gastric cancer, tumor angiogenesis and lymphangiogenesis are known to be closely associated with malignant progression and poor prognosis (38). Macrophage infiltration and tumor angiogenesis, which are stimulated by nuclear NDRG1/Cap43, may play roles in the promotion of metastasis to the lymph nodes and liver in intestinal type gastric cancer cells. NDRG1/Cap43 may specifically modulate tumor angiogenesis and metastasis in close correlation with the recruitment of macrophages and TAMs, depending on the histological type of gastric cancer. In both the intestinal and diffuse types of gastric cancer, infiltration of macrophages and tumor angiogenesis were found to be increased during progression of pathological stages (Fig. 2). However, there was no such significant correlation between infiltrating macrophages or MVD and NDRG1/Cap43 expression in the nucleus and membrane in the diffuse type of gastric cancer (Fig. 3). By contrast, our present study showed that the expression of NDRG1/Cap43 in the nucleus significantly affected the survival of patients with intestinal type gastric cancer and those with the diffuse type after surgical treatment (Fig. 4). Although it remains unclear which biological function of NDRG1/Cap43 is specifically responsible for survival in gastric cancer, the localization of NDRG1/Cap43 expression to the nucleus rather than the membrane appears to be a better indicator of poor prognosis in gastric cancer patients.

Inagaki *et al* (39) recently reported that nuclear localization of NDRG1/Cap43 is significantly related to lymph node metastasis as well as to the survival of patients with diffuse type gastric cancer, and also that nuclear localization of NDRG1/Cap43 is significantly correlated with p53 expression in the nucleus. Of the various suppressor genes linked to the expression of NDRG1/Cap43 (40), p53 is known to be closely associated with NDRG1/Cap43 in tumor growth and/or cancer cell apoptosis (2,41,42), but the molecular interaction between p53 and NDRG1/Cap43 remains unclear. In the present study, we did not examine whether any suppressor gene was linked to NDRG1/Cap43 expression in gastric cancer cells. Consistent with the study by Inagaki *et al* (39), nuclear NDRG1/Cap43 expression was found to be significantly correlated with lymph node metastasis. Further study is required to understand how the expression of NDRG1/Cap43 affects the peritoneal dissemination of gastric cancer.

In conclusion, the present study demonstrated for the first time a close association of nuclear NDRG1/Cap43 expression with the infiltration of macrophages and tumor angiogenesis in the intestinal type of gastric cancer, whereas no such association was evident in the diffuse type. The present findings suggest that the nuclear expression of NDRG1/Cap43 may serve as a novel biomarker for the molecular diagnosis of gastric cancer, and also for the development of new therapeutic strategies. Further study is required to ascertain how the nuclear localization of NDRG1/Cap43 is controlled at the molecular level.

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