Correlation of N-myc downstream-regulated gene 1 expression with clinical outcomes of colorectal cancer patients of different race/ethnicity

Minori Koshiji, Kensuke Kumamoto, Keiichirou Morimura, Yasufumi Utsumi, Michiko Aizawa, Masami Hoshino, Shinji Ohki, Seiichi Takenoshita, Max Costa, Thérèse Commes, David Piquemal, Curtis C Harris, Kam-Meng Tchou-Wong

Minori Koshiji, Kam-Meng Tchou-Wong, Department of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987, United States
Kensuke Kumamoto, Shinji Ohki, Seiichi Takenoshita, Second Department of Surgery, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, Japan
Kensuke Kumamoto, Curtis Harris, National Institutes of Health, National Cancer Institute, Building 37, Bethesda, MD 20892, United States
Keiichirou Morimura, Department of Pathology, Osaka City University, Osaka, Japan
Yasufumi Utsumi, Michiko Aizawa, Department of Pathology, Ohara General Hospital, 6-11 Ohmachi, Fukushima 960-8611, Japan
Masami Hoshino, Department of Surgery, Ohara General Hospital, 6-11 Ohmachi, Fukushima 960-8611, Japan
Therese Commes, David Piquemal, University Montpellier II, Montpellier, France
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Correspondence to: Dr. Kam-Meng Tchou-Wong, Department of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987, United States. tchouk02@med.nyu.edu
Telephone: +1-845-7313504 Fax: +1-845-3512218
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Abstract

AIM: To evaluate the role of N-myc downstream-regulated gene 1 (NDRG1) expression in prognosis and survival of colorectal cancer patients with different ethnic backgrounds.

METHODS: Because NDRG1 is a downstream target of p53 and hypoxia inducible factor-1α (HIF-1α), we examined NDRG1 expression together with p53 and HIF-1α by immunohistochemistry. A total of 157 colorectal cancer specimens including 80 from Japanese patients and 77 from US patients were examined. The correlation between protein expression with clinicopathological features and survival after surgery was analyzed.

RESULTS: NDRG1 protein was significantly increased in colorectal tumor compared with normal epithelium in both Japanese and US patient groups. Expression of NDRG1 protein was significantly correlated with lymphatic invasion, venous invasion, depth of invasion, histopathological type, and Dukes' stage in Japanese colorectal cancer patients. NDRG1 expression was correlated to histopathological type, Dukes' stage and HIF-1α expression in US-Caucasian patients but not in US-African American patients. Interestingly, Kaplan-Meier survival analysis demonstrated that NDRG1 expression correlated significantly with poorer survival in US-African American patients but not in other patient groups. However, in p53-positive US cases, NDRG1 positivity correlated significantly with better survival. In addition, NDRG1 expression also correlated significantly with improved survival in US patients with stages Ⅲ and Ⅳ tumors without chemotherapy. In Japanese patients with stages Ⅱ and Ⅲ tumors, strong NDRG1 staining in p53-positive tumors correlated significantly with improved survival but negatively in patients without chemotherapy.

CONCLUSION: NDRG1 expression was correlated with various clinicopathological features and clinical outcomes in colorectal cancer depending on the race/ethnicity of the patients. NDRG1 may serve as a biological basis for the disparity of clinical outcomes of colorectal cancer patients with different ethnic backgrounds.

INTRODUCTION

N-myc downstream-regulated gene 1 (NDRG1) was first

discovered by two groups under differing physiological conditions[1,2]. NDRG1, also termed CAP43, DRG1, NDR1, RIT42, and RTP, has been mapped to human chromosome 8q24 and encodes for a 394-amino acid cytoplasmic protein with a molecular weight of 43 kDa[2-5]. Transcription of the NDRG1 gene is negatively regulated by the MYC family proteins including N-MYC and c-MYC[6]. The expression of NDRG1 could be induced by diverse agents including metals that mimic hypoxia, homocysteine, calcium ionophore, okadaic acid, and androgens[3,4,7-10]. The induction of NDRG1 expression by carcinogenic nickel and hypoxia is mediated by hypoxia inducible factor-1α (HIF-1α)[7,8,11]. DNA damaging agents induced NDRG1 expression in a p53-dependent manner[12,13] and a putative p53 binding site had been reported within the NDRG1 promoter region[13].

Earlier publications had reported down regulation of NDRG1 expression in colon, breast, and prostate cancers[2,14-16]. In contrast, recent studies reported increased expression of NDRG1 protein in malignancy including skin, brain, lung, colon, breast and prostate cancers[17-19]. The expression of NDRG1 in colon cancer has also been controversial. Wang et al[19] reported that the level of NDRG1 protein was gradually increased in colorectal cancers and immunohistochemical staining of NDRG1 was correlated with lymph node metastasis in a Chinese patient population. In contrast, Shah et al[20] stated that NDRG1 staining in the primary colorectal tumor was always less than adjacent normal colon in a US patient population. They examined NDRG1 expression in colorectal liver metastases and demonstrated a trend for unilobar metastases with high NDRG1 expression and a suggestion of improved 2-years survival[20].

It has been reported that African Americans have a higher incidence and mortality from colorectal cancer than Caucasians[21]. Another recent study reported that African American patients received less adjuvant chemotherapy than Caucasian patients, providing a plausible reason for the higher mortality in African Americans[22]. However, a biological basis for the existence of a more aggressive colorectal cancer in African American patients remains to be determined. Similarly, disparity in the incidence and clinical progression of prostate cancer between African Americans and Caucasians had also been reported. When access to care as a possible confounding variable in disease outcome was controlled for, Caruso et al[23] demonstrated that African American patients presented with a significantly worse clinicopathological profile than Caucasian patients and that ethnicity was an independent factor in disease recurrence after surgical treatment. Interestingly, different expression patterns of the NDRG1 protein might reflect differences in the response of prostatic epithelium to hypoxia and androgens in African Americans compared with Caucasians, revealing a possible biological basis underlying the disparity in clinical outcomes of prostate cancer patients with different ethnic background[23]. We hypothesized that NDRG1 expression in colorectal cancers, similar to that in prostate cancer, might reflect different race/ethnic backgrounds and underlie the disparity in clinical outcomes of colorectal cancer patients with different race/ethnic backgrounds.

MATERIALS AND METHODS

Patients

The study population consisted of 157 consecutive patients (103 male, 54 female; age range, 37 to 85 years; average 66.5 years) observed between January 1995 and December 2003, with histologically proven colorectal adenocarcinoma. Tumors that met the Bethesda guidelines for hereditary nonpolyposis colorectal cancer and/or carcinomas associated with inflammatory bowel disease were excluded from this study. Patient selection was primarily based on the availability of both adequate clinical follow-up and representative specimens for immunohistochemical analysis. Out of a total of 157 colorectal cancer specimens, 80 were from Japanese patients and 77 were from US patients. Among the 80 Japanese patients, 44 received oral 5-fluorouracil chemotherapy in addition to surgery. The number of stage III and IV US patients totaled 42, among which 19 patients had chemotherapy and 23 patients did not receive chemotherapy.

Immunohistochemical staining and pathological evaluation

Immunohistochemical (IHC) staining was performed by using serial sections of paraffin-embedded tumor specimens. Antigen retrieval and immunochemical staining were performed as described[24]. Sections were incubated with a 1:100 dilution of polyclonal anti-NDRG1 antibody[5], polyclonal anti-HIF-1α antibody (Santa Cruz), and a 1:200 dilution of monoclonal anti-p53 antibody (DAKO). Slides were examined by three independent pathologists blinded to each other's work and with no prior knowledge of clinical and pathological features of tumors. For assessment of IHC staining, the following criteria were used: 0 represented negative staining while positive staining was scored from +1 to +3 (+1, weak staining or moderate to intense staining in the peripheral region of < 10% of the cancer nests; +2, moderate staining in most of the cancer cells or intense staining in the peripheral regions in 10%-40% of the cancer nests; +3, intense staining in almost all the cancer cells). NDRG1 negative staining means 0 while positivity means +1 to +3.

Statistical analysis

Statistical comparisons for significance between protein expression patterns and clinicopathological features were evaluated by Mann Whitney U-test and χ^2 test. Multivariate analyses were performed according to a logistic regression analysis. The survival curves were estimated by the Kaplan-Meier method, and the resulting curves were compared using the log-rank test. Statistical analysis was performed using StatMate (ATMS Co. Ltd., Tokyo, Japan) and $P < 0.05$ was considered statistically significant.

RESULTS

NDRG1 expression in colon cancer

Immunohistochemical analysis demonstrated that NDRG1 was expressed in the cytoplasm and membranes of epithelial cells of colorectal adenocarcinoma from a
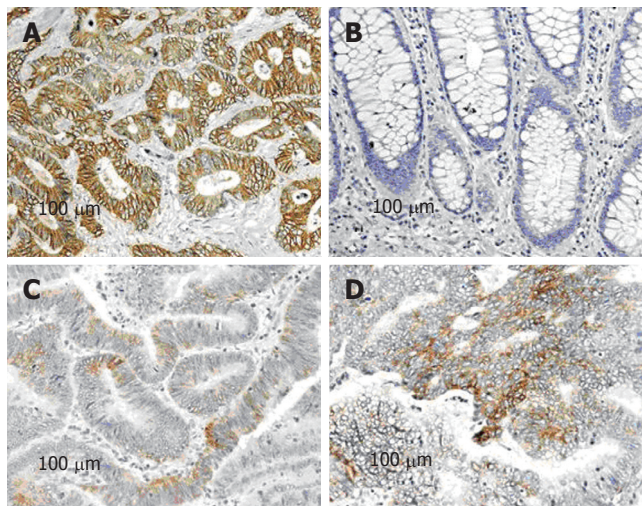
Figure 1 Expression of NDRG1 in colorectal cancer as analyzed by immunohistochemistry. **A**: Moderately differentiated colorectal adenocarcinoma from a Japanese patient demonstrating membranous and cytoplasmic staining with NDRG1 antibody; **B**: No NDRG1 expression was detected in normal adjacent colon mucosa; **C**: Well differentiated colorectal adenocarcinoma from US patient; **D**: Moderately differentiated colorectal adenocarcinoma form US patient.

Japanese patient (Figure 1A) but not in normal adjacent colon mucosa (Figure 1B). Non-epithelial cells including stroma, muscle, and invading lymphocytes were also negative for NDRG1 expression. NDRG1 expression was also detected in well-differentiated (Figure 1C) and moderately differentiated (Figure 1D) colorectal adenocarcinomas from US patients. The correlation of NDRG1 expression with clinicopathologic variables in Japanese and US cases was shown in Table 1 and Table 2. In Japanese colorectal cancer patients, positive NDRG1 expression was correlated with histopathological type (P = 0.017), Dukes' stage (P = 0.036), lymphatic invasion (P = 0.021), venous invasion (P = 0.0077), and depth of invasion (P = 0.01) (Table 1).

Because of the smaller sizes of surgical specimens available from the US patients, pathological information relating lymphatic invasion, venous invasion and depth of invasion could not be obtained. Since the US patient population consisted of two race/ethnic groups, namely, Caucasian and African American, analysis of NDRG1 expression and clinicopathological variables was also performed based on race/ethnicity. As shown in Table 2, while there was a trend in the correlation of NDRG1 expression with Dukes' stage in the overall US cases (P = 0.056), NDRG1 expression was only significantly correlated to Dukes' stage in Caucasians (P = 0.0063) but not in African Americans (P = 0.88). Similarly, when comparing well versus moderate differentiated tumors, significant correlation with NDRG1 positivity was also only observed in Caucasians but not in other populations.

Expression of other biomarkers in colon cancer

Next, we examined the frequency of NDRG1 expression relative to that of p53 and HIF-1α (Table 3). Normal colonic epithelium showed negative to weak nuclear staining of p53 while HIF-1α was not detected in normal colonic mucosa. As shown in Table 4, while there was a

Table 1 Correlation of NDRG1 expression with clinicopathologic variables in Japanese cases

	Total cases (%) n = 80	Positive n = 55	Negative n = 25	*P* value
Age (yr)				0.622
> 66	48 (60.0)	34	14	
< 66	32 (40.0)	21	11	
Gender				0.649
Male	45 (56.3)	30	15	
Female	35 (43.7)	25	10	
Tumor location				0.164
Right-hemicolon	28 (35.0)	22	6	
Left-hemicolon	52 (65.0)	33	19	
Histopathological Type				0.067
Well	39 (48.8)	22	17	0.017
Moderate	38 (47.6)	31	7	
Poor	1 (1.2)	1	0	
Mucinous	1 (1.2)	1	0	
Sig	1 (1.2)	0	1	
Dukes' stage				0.036
A	22 (27.5)	11	11	
B	21 (26.3)	14	7	
C	27 (33.7)	20	7	
D	10 (12.5)	10	0	
Liver metastasis				0.062
Negative	73 (91.3)	48	25	
Positive	7 (8.7)	7	0	
Lymph node metastasis				0.14
Negative	48 (60.0)	30	18	
Positive	32 (40.0)	25	7	
Lymphatic invasion				0.021
Negative	19 (23.8)	9	10	
Positive	61 (76.2)	46	15	
Venous invasion				0.0077
Negative	15 (18.8)	6	9	
Positive	65 (81.2)	49	16	
Depth of invasion				0.01
m	5 (6.3)	1	4	
sm	8 (10.0)	4	4	
mp	14 (17.5)	10	4	
ss, a1	29 (36.3)	23	6	
se, a2	23 (28.7)	16	7	
si, ai	1 (1.2)	1	0	

$P < 0.05$ is considered significant.

trend in the correlation of NDRG1 expression with HIF-1α expression in colorectal tumors from Japanese patients (P = 0.061), the correlation was significant in the overall US patients (P = 0.036). Similar to that observed with Dukes' staging, when analysis was performed based on race/ethnicity, significant correlation between NDRG1 and HIF-1α was only observed in Caucasian patients (P = 0.015) but not in African American patients (P = 0.73). On the other hand, no significant correlation between NDRG1 and p53 expression was observed in all three groups.

Clinical outcomes of colorectal patients

As shown in Table 1 and Table 2, NDRG1 expression was significantly correlated with advanced Dukes' stages in Japanese and Caucasian colorectal cancer patients (P = 0.036 and 0.0063, respectively), but not in African American patients (P = 0.88). As expected, Kaplan-Meier survival curves revealed that prognosis was associated with Dukes' stage in both Japanese and US groups (Figure 2A). To examine if NDRG1 expression correlated with survival

Table 2 Correlation of NDRG1 expression with clinicopathologic variables in U.S. (Caucasian and African American) cases

	All U.S. cases (n = 77) NDRG1				Caucasian (n = 43) NDRG1				African American (n = 34) NDRG1			
	Total (%)	+	-	P value	Total (%)	+	-	P value	Total (%)	+	-	P value
Age (yr)				0.079				0.092				0.464
> 66	45 (58.4)	19	26		26 (60.5)	10	16		19 (55.9)	9	10	
< 66	32 (41.6)	20	12		17 (39.5)	11	6		15 (44.1)	9	6	
Gender				0.074				0.255				0.153
Male	58 (75.3)	26	32		32 (74.4)	14	18		26 (76.5)	12	14	
Female	19 (24.7)	13	6		11 (25.6)	7	4		8 (23.5)	6	2	
Histopathological type				0.31				0.21				0.18
Well	47 (61.0)	20	27	0.15[a]	28 (65.1)	11	17	0.049[a]	19 (55.9)	9	10	0.88[a]
Moderate	23 (29.9)	14	9		14 (32.6)	10	4		9 (26.5)	4	5	
Poor	2 (2.6)	1	1		0 (0)	0	0		2 (5.9)	1	1	
Mucinous	5 (6.5)	4	1		1 (2.3)	0	1		4 (11.8)	4	0	
Dukes' stage				0.056				0.0063				0.88
A	7 (9.1)	1	6		4 (9.3)	0	4		3 (8.8)	1	2	
B	28 (36.4)	14	14		16 (37.2)	8	8		12 (35.3)	6	6	
C	23 (29.9)	16	7		14 (32.6)	11	3		9 (26.5)	5	4	
D	19 (24.6)	8	11		9 (20.9)	2	7		10 (29.4)	6	4	
Liver metastasis				0.39				0.072				0.59
Negative	58 (75.3)	31	27		34 (79.1)	19	15		24 (70.6)	12	12	
Positive	19 (24.7)	8	11		9 (20.9)	2	7		10 (29.4)	6	4	
Lymph node metastasis				0.212				0.28				0.52
Negative	35 (45.5)	15	20		20 (46.5)	8	12		15 (44.1)	7	8	
Positive	42 (54.5)	24	18		23 (53.5)	13	10		19 (55.9)	11	8	

[a]P < 0.05 vs moderate.

Table 3 Frequency of expression of p53 and HIF-1α in Japanese and U.S. patients n (%)

	p53		HIF-1α	
	Positive	Negative	Positive	Negative
Japanese (n = 80)	43 (54.0)	37 (46.0)	42 (52.5)	38 (47.5)
All U.S. cases (n = 77)	48 (62.3)	29 (37.7)	18 (23.4)	59 (76.6)
Caucasian (n = 43)	28 (65.1)	15 (34.9)	13 (30.2)	30 (69.8)
African American (n = 34)	20 (58.8)	14 (41.2)	5 (14.7)	29 (85.3)

Table 4 Correlation of expression of NDRG1 with p53 and HIF-1α

		NDRG1		P value
		Positive	Negative	
Japanese (n = 80)				
p53	+	31	12	0.49
	-	24	13	
HIF-1α	+	25	17	0.061
	-	30	8	
All U.S. cases (n = 77)				
p53	+	28	20	0.083
	-	11	18	
HIF-1α	+	13	5	0.036
	-	26	33	
Caucasian (n = 43)				
p53	+	16	12	0.14
	-	5	10	
HIF-1α	+	10	3	0.015
	-	11	19	
African American (n = 34)				
p53	+	12	8	0.32
	-	6	8	
HIF-1α	+	3	2	0.73
	-	15	14	

P < 0.05 is considered significant.

of patients after surgery, Kaplan-Meier analysis was performed comparing NDRG1-positive with NDRG1-negative tumors from Japanese and US patients. As depicted in Figure 2B, no significant correlation between NDRG1 expression and survival was observed in Japanese patients (Log rank, P = 0.36) while NDRG1 positivity was significantly correlated with poorer survival compared to NDRG1 negativity in US patients (Log rank, P = 0.025). To examine if there was differential correlation based on race/ethnicity, NDRG1 expression was further examined in Caucasian and African American populations. Interestingly, NDRG1 positivity was significantly correlated with poorer survival only in African Americans but not Caucasians (P = 0.035 and P = 0.31, respectively) (Figure 3A). Since NDRG1 is a possible downstream target of p53, survival was further correlated with expression of the p53 protein, generally indicative of p53 mutations leading to a longer protein half-life. In p53-negative tumors in US cases, NDRG1 expression had no correlation with survival (Figure 3B). In contrast, in p53-positive tumors, NDRG1 positivity was significantly correlated with better survival (P = 0.005), suggesting that p53 and NDRG1 could potentially be used as biomarkers for prognosis in
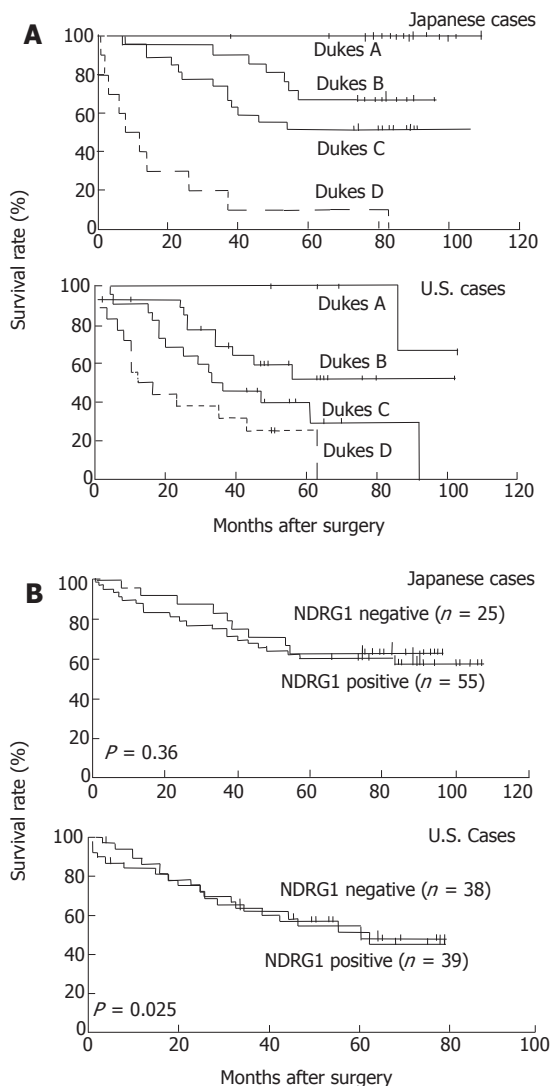
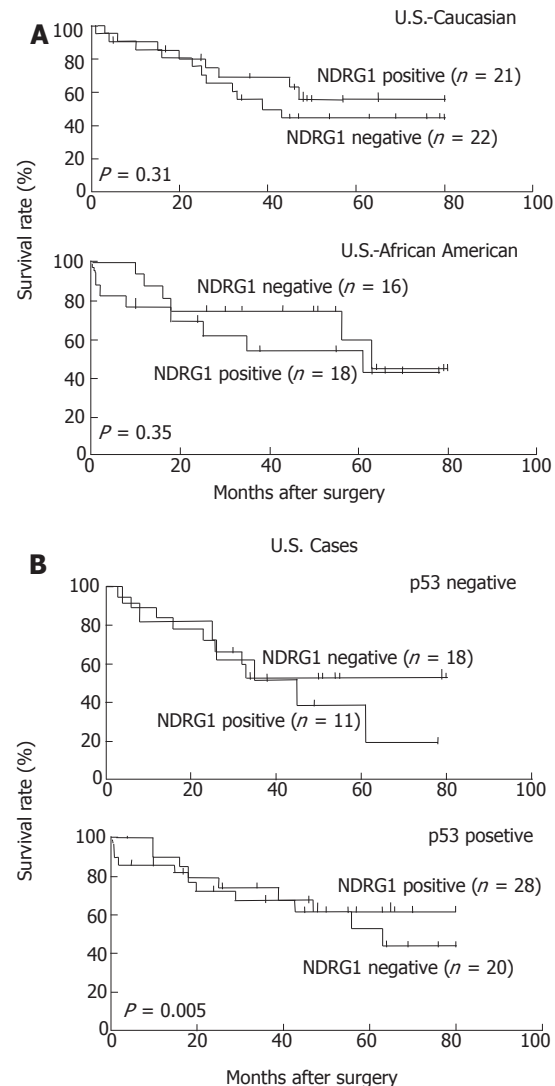US colorectal cancer patients.

When similar studies were performed on Japanese cases, survival was not significantly correlated with NDRG1 and p53 expression. Hence, we analyzed NDRG1 expression in various stages of colorectal cancer in Japanese patients. As shown in Figure 4A, no significance in correlation of NDRG1 expression with survival was observed in stages II and III colorectal cancers. However, when NDRG1 expression was re-classified as weak

Figure 2 Kaplan-Meier survival analysis of colorectal cancer patients. **A**: Survival (months after surgery) of Japanese and US patients stratified according to Dukes' staging; **B**: Correlation of NDRG1 expression (negative, 0; positive, +1 to +3) with survival in Japanese patients and US patients (including Caucasians and African Americans).
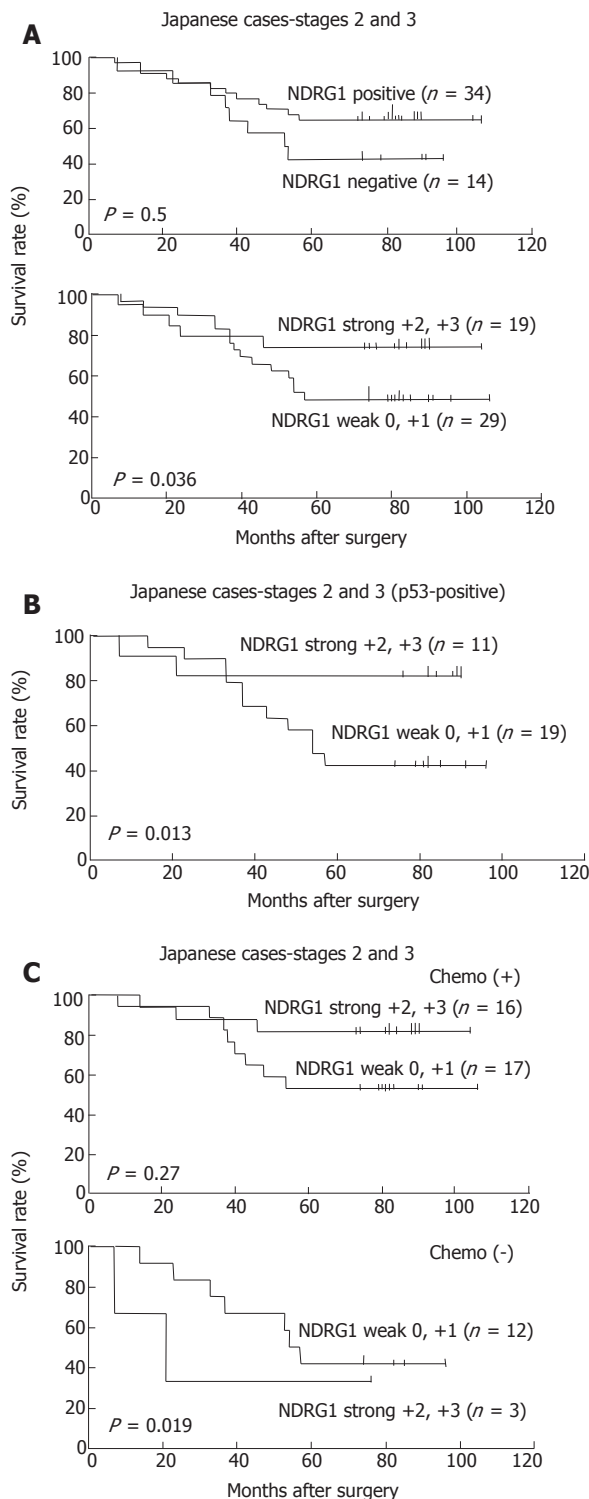

Figure 3 Kaplan-Meier survival analysis of US colorectal cancer patients according to NDRG1 expression. **A**: Comparison of survival according to race/ethnicity, i.e., Caucasian and African American; **B**: Correlation of survival in p53-negative and p53-positive staining tumors.

staining (0, +1) and strong staining (+2, +3), strong NDRG1 staining was significantly correlated with better survival in patients with stage Ⅱ and Ⅲ colorectal cancers ($P = 0.036$). In combination with p53-positive staining, strong NDRG1 staining (+2, +3) also significantly correlated with improved survival ($P = 0.013$) (Figure 4B). Since high NDRG1 expression in patients with colorectal liver metastases was associated with relative resistance to irinotecan [20], NDRG1 expression and survival was compared in patients with or without chemotherapy. Interestingly, despite the small sample size, strong NDRG1 staining was significantly correlated with poorer survival in stages Ⅱ and Ⅲ colorectal cancer patients without chemotherapeutic treatment ($P = 0.019$) (Figure 4C).

When stages Ⅱ and Ⅲ colorectal cancer patients from the US were similarly evaluated, no significance was observed between weak or strong NDRG1 staining with survival (data not shown). We therefore evaluated if NDRG1 expression correlated with survival of US patients with stages Ⅲ and Ⅳ colorectal cancers. As shown

in Figure 5A, although it was not statistically significant ($P = 0.058$), a trend for improved survival was observed with NDRG1-positive staining. Interestingly, when these stages Ⅲ and Ⅳ tumors were analyzed based on the status of chemotherapy, NDRG1 positivity was significantly correlated with improved survival in stages Ⅲ and Ⅳ patients without chemotherapeutic treatment ($P = 0.04$) while no significant correlation was observed in patients who had received chemotherapy (Figure 5B).
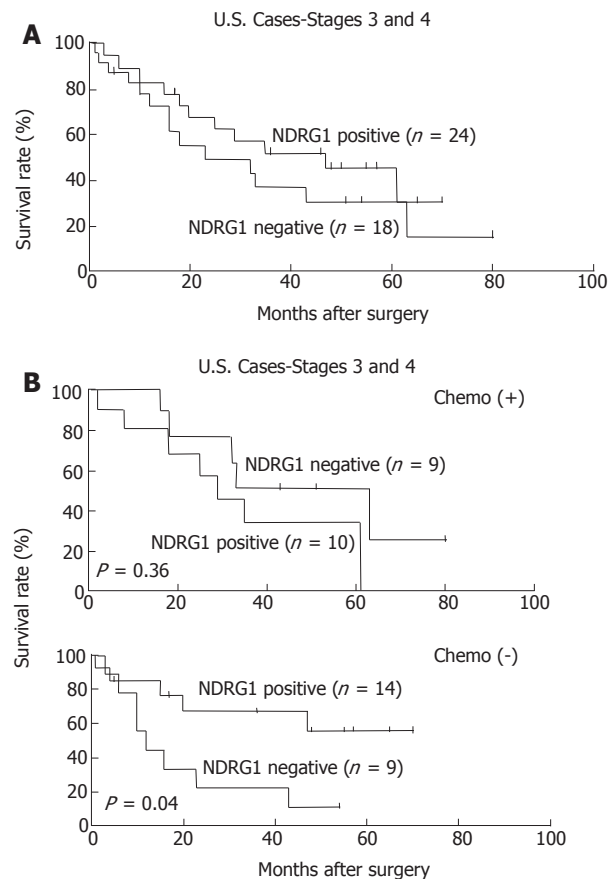
DISCUSSION

Despite NDRG1 upregulation in many other tumor types[10,17,18,23], NDRG1 expression in colorectal cancer has been controversial[2,19,20]. Wang *et al*[19] demonstrated that NDRG1 expression was increased in colorectal carcinogenesis and correlated with lymph node metastasis in a Chinese population and suggested that NDRG1 might be a possible marker for prediction of early metastasis of colorectal cancers. Consistent with the latter, we

Figure 4 Kaplan-Meier survival analysis of NDRG1 expression in Japanese patients with stages II and III colorectal cancers. **A**: Correlation of survival with NDRG1 expression stratified as negative (0) or positive (+1 to +3) and weak (0, +1) or strong (+2, +3); **B**: Correlation of survival with weak or strong NDRG1 staining in p53-positive tumors; **C**: Correlation of survival with weak or strong NDRG1 staining in patients with or without chemotherapeutic treatment, chemo (+) or chemo (-), respectively.

Figure 5 Kaplan-Meier survival analysis of NDRG1 expression in US with stages III and IV colorectal cancers. **A**: Correlation of survival with NDRG1 expression stratified as negative (0) or positive (+1 to +3); **B**: Correlation of survival with weak or strong NDRG1 staining in patients with or without chemotherapeutic treatment, chemo (+) or chemo (-), respectively.

aggressive phenotypes in both Japanese and Chinese populations[19]. Although we did not have a significant number of Japanese cases with liver metastasis for the analysis of the correlation between NDRG1 expression and hepatic metastasis, NDRG1 was expressed in the tumors of all 7 Japanese patients that developed liver metastasis (Table 1). On the other hand, compared with Japanese and Chinese patients, the opposite trend was observed in Caucasian patients with NDRG1 expression correlated negatively with liver metastasis (Table 2). The latter was consistent with a previous report suggesting that NDRG1 suppressed colon cancer metastasis, possibly by inducing colon cancer cell differentiation[25]. In addition, Shah *et al*[20] demonstrated NDRG1 expression in all 131 colorectal liver metastases from US patients and observed a trend for unilobar metastases with high NDRG1 expression which was associated with relative resistance to irinotecan. However, the race/ethnicity of the US patient population was not analyzed in these studies.

Although NDRG1 expression is inducible by hypoxia and its mimetic and is responsive to p53 *in vitro*[7,8,12,13], there was no significant correlation of NDRG1 expression with p53 expression but significant correlation was observed with HIF-1α expression in US patients, attributed to Caucasian patients, while a trend for association was observed in Japanese patients (Table 4). On the other hand,

demonstrated that NDRG1 expression was significantly correlated to lymphatic invasion, venous invasion, depth of invasion, and histopathological type, and Dukes' stage in Japanese colorectal cancer patients (Table 1), suggesting that NDRG1 expression might be correlated with more

no significant correlation was observed between NDRG1 and HIF-1α expression in African American patients, suggesting that there may be other hypoxia-independent pathway(s) contributing to NDRG1 expression in African American patients. Nevertheless, NDRG1 expression in African Americans but not Caucasians was significantly correlated with poorer prognosis in African American patients (Figure 3A). However, when the overall US cases were analyzed based on p53 and NDRG1 positivity, patients with p53-positive and NDRG1-positive tumors have improved survival independent of race/ethnicity (Figure 3B). In p53-negative tumors, no significant correlation of NDRG1 expression with survival was observed, suggesting the potential use of both NDRG1 and p53 as prognostic markers for US colorectal cancer patients. Because of the limitation in small sample sizes, the latter was not further analyzed in Caucasian and African American patients. Future studies with a larger cohort will be needed to determine if NDRG1 and p53 expression can serve reliably as prognostic factors for survival after surgery for US patients, especially in African American patients. Moreover, NDRG1 inducibility may provide a biological basis underlying the ethnic disparity in clinical outcomes between African American and Caucasian patients.

Interestingly, when we analyzed NDRG1 expression in US patients with various stages of colorectal cancers including stages I and Ⅱ, stages Ⅱ and Ⅲ, and stages Ⅲ and Ⅳ, we only observed a trend for NDRG1 expression with improved survival (Figure 5A) while significant association of NDRG1 positivity with better survival was observed in US patients who had not undergone chemotherapy (Figure 5B). Since adjuvant chemotherapy for stage Ⅲ colon cancer had been associated with a 5-year survival of 16% but the benefits of adjuvant chemotherapy seemed to be lower in African American patients[26], future studies with larger sample sizes are needed for determining the utility of NDRG1 expression either alone or in combination with p53 expression as prognostic marker(s) for adjuvant therapies including chemotherapy or radiotherapy to improve survival.

In Japanese patients with stage Ⅱ and Ⅲ colorectal cancers, significant positive association of survival with NDRG1 expression was only observed when the latter was analyzed as weak (0, +1) or strong (+2, +3) staining (Figure 4A). Similar to US cases, strong NDRG1 staining and p53 positivity in stages Ⅱ and Ⅲ Japanese tumors correlated significantly with improved survival (Figures 3B and 4B). However, unlike US stages Ⅲ and Ⅳ patients without chemotherapy, strong NDRG1 staining was significantly correlated with poorer survival in Japanese patients with stages Ⅱ and Ⅲ colorectal cancers without chemotherapy (Figures 4C and 5B). These differences can be due to differences in stages and or race/ethnicity. Nevertheless, NDRG1 expression may potentially be used as a clinical marker for prognosis and adjuvant therapies.

In summary, the correlation of NDRG1 expression with various clinicopathological features and clinical outcomes in colorectal cancers from patients with different race/ethnicity suggested that it may serve not only as a potential prognostic marker but may provide a biological basis underlying the disparity of clinical outcomes among different race/ethnic groups.

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