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

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.

Key words: NDRG1 expression; Colorectal cancer; Race; Ethnicity; Clinical outcomes

Koshiji M, Kumamoto K, Morimura K, Utsumi Y, Aizawa M, Hoshino M, Ohki S, Takenoshita S, Costa M, Commes T, Piquemal D, Harris CC, Tchou-Wong KM. Correlation of N-myc downstream-regulated gene 1 expression with clinical outcomes of colorectal cancer patients of different race/ethnicity. World J Gastroenterol 2007; 13(20): 2803-2810

http://www.wjgnet.com/1007-9327/13/2803.asp

INTRODUCTION

N-myc downstream-regulated gene 1 (NDRG1) was first
discovered by two groups under differing physiological conditions\cite{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\cite{3-5}. Transcription of the NDRG1 gene is negatively regulated by the MYC family proteins including N-MYC and c-MYC\cite{6}. The expression of NDRG1 could be induced by diverse agents including metals that mimic hypoxia, homocysteine, calcium ionophore, okadaic acid, and androgens\cite{3-4,7-10}. The induction of NDRG1 expression by carcinogenic nickel and hypoxia is mediated by hypoxia inducible factor-1α (HIF-1α)\cite{7,8,11,12}. DNA damaging agents induced NDRG1 expression in a p53-dependent manner\cite{13,14} and a putative p53 binding site had been reported within the NDRG1 promoter region\cite{15}.

Earlier publications had reported down regulation of NDRG1 expression in colon, breast, and prostate cancers\cite{2,16-18}. In contrast, recent studies reported increased expression of NDRG1 protein in malignancy including skin, brain, lung, colon, breast and prostate cancers\cite{17-19,21,22}. The expression of NDRG1 in colon cancer has also been controversial. Wang et al\cite{23} 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\cite{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\cite{24}.

It has been reported that African Americans have a higher incidence and mortality from colorectal cancer than Caucasians\cite{25}. 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\cite{25}. 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\cite{26} demonstrated that African American patients presented with a significantly worse clinical-pathological 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\cite{26-28}. 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 immunohistochemical staining were performed as described\cite{29}. Sections were incubated with a 1:100 dilution of polyclonal anti-NDRG1 antibody\cite{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 region of 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 χ2 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
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 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 1 Correlation of NDRG1 expression with clinicopathologic variables in Japanese cases**

| Variable                  | Total cases (%) | Positive | Negative | P value |
|---------------------------|-----------------|----------|----------|---------|
| Age (yr)                  | n = 80          | n = 55   | n = 25   |         |
| > 66                      | 48 (60.0)       | 34 (61)  | 14 (25)  | 0.622   |
| < 66                      | 32 (40.0)       | 21 (66)  | 11 (34)  |         |
| Gender                    | Male            | 45 (56.3)| 30 (67)  | 0.649   |
| Female                    | 35 (43.7)       | 25 (73)  | 10 (27)  |         |
| Tumor location            | Right-hemicolon | 28 (35.0)| 22 (64)  | 0.164   |
| Left-hemicolon            | 52 (65.0)       | 33 (65)  | 19 (35)  |         |
| Histopathological Type    | Well            | 39 (48.8)| 22 (56)  | 0.067   |
| Moderate                  | 38 (47.6)       | 31 (81)  | 7 (19)   | 0.017   |
| Poor                      | 1 (1.2)         | 0 (0)    | 1 (25)   |         |
| Mucinous                  | 1 (1.2)         | 1 (25)   | 0 (0)    |         |
| Sig                       | 1 (1.2)         | 0 (0)    | 1 (25)   |         |
| Dukes’ stage              | A               | 22 (27.5)| 11 (45)  | 0.036   |
| B                         | 21 (26.3)       | 14 (67)  | 7 (33)   |         |
| C                         | 27 (33.7)       | 20 (74)  | 7 (26)   |         |
| D                         | 10 (12.5)       | 10 (100) | 0 (0)    |         |
| Liver metastasis          | Negative        | 73 (91.3)| 48 (65)  | 0.062   |
| Positive                  | 7 (8.7)         | 7 (9)    | 0 (0)    |         |
| Lymph node metastasis     | Negative        | 48 (60.0)| 30 (62)  | 0.14    |
| Positive                  | 32 (40.0)       | 25 (78)  | 7 (22)   |         |
| Venous invasion           | Negative        | 19 (23.8)| 9 (45)   | 0.021   |
| Positive                  | 61 (76.2)       | 46 (75)  | 15 (25)  |         |
| Depth of invasion         | m               | 5 (6.3)  | 1 (20)   | 0.01    |
| sm                        | 8 (10.0)        | 4 (50)   | 4 (50)   |         |
| mp                        | 14 (17.5)       | 10 (71)  | 4 (29)   |         |
| se, a1                    | 29 (36.3)       | 23 (79)  | 6 (21)   |         |
| se, a2                    | 23 (28.7)       | 16 (69)  | 7 (28)   |         |
| si, ai                    | 1 (1.2)         | 1 (100)  | 0 (0)    |         |

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

---

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

|                | All U.S. cases ($n = 77$) | Caucasian ($n = 43$) | African American ($n = 34$) |
|----------------|----------------------------|----------------------|-----------------------------|
|                | NDRG1                      | NDRG1                | NDRG1                       |
|                | Total (%)                  | $+$                  | $-$ $P$ value               | Total (%)                  | $+$ $-$ | $P$ value |
| Age (yr)       |                            |                      |                             |                            |        |           |
| $>$ 66         | 45 (58.4)                  | 19                   | 26                          | 26 (60.5)                  | 10      | 16        | 0.079     |
| $<$ 66         | 32 (41.6)                  | 20                   | 12                          | 17 (39.5)                  | 11      | 6         | 0.092     |
| Gender         |                            |                      |                             |                            |        |           |
| Male           | 58 (75.3)                  | 26                   | 32                          | 32 (74.4)                  | 14      | 18        | 0.074     |
| Female         | 19 (24.7)                  | 13                   | 6                           | 11 (25.6)                  | 7       | 4         | 0.154     |
| Histopathological type |                    |                      |                             |                            |        |           |
| Well           | 47 (61.0)                  | 20                   | 27                          | 28 (65.1)                  | 11      | 17        | 0.15$^a$ |
| Moderate       | 23 (29.9)                  | 14                   | 9                           | 14 (32.6)                  | 10      | 4         | 0.005     |
| Poor           | 2 (2.6)                    | 1                   | 1                           | 0 (0)                      | 0       | 0         | 0.0063    |
| Mucinous       | 5 (6.5)                    | 4                   | 1                           | 1 (2.3)                    | 0       | 1         | 0.0056    |
| Dukes’ stage   |                            |                      |                             |                            |        |           |
| A              | 7 (9.1)                    | 1                   | 6                           | 4 (9.3)                    | 0       | 4         | 0.39      |
| B              | 28 (36.4)                  | 14                   | 14                          | 16 (37.2)                  | 8       | 8         | 0.39      |
| C              | 23 (29.9)                  | 16                   | 7                           | 14 (32.6)                  | 11      | 3         | 0.39      |
| D              | 19 (24.7)                  | 8                   | 11                          | 9 (20.9)                   | 2       | 7         | 0.39      |
| Liver metastasis |                            |                      |                             |                            |        |           |
| Negative       | 58 (75.3)                  | 31                   | 27                          | 34 (79.1)                  | 19      | 15        | 0.39      |
| Positive       | 19 (24.7)                  | 8                   | 11                          | 9 (20.9)                   | 2       | 7         | 0.39      |
| Lymph node metastasis |          |                      |                             |                            |        |           |
| Negative       | 35 (45.5)                  | 15                   | 20                          | 20 (46.5)                  | 8       | 12        | 0.39      |
| Positive       | 42 (54.5)                  | 24                  | 18                          | 23 (53.5)                  | 13      | 10        | 0.39      |

$^aP < 0.05$ vs moderate.

---

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

|                | P53 | HIF-1$\alpha$ |
|----------------|-----|---------------|
|                | 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-1$\alpha$

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

$P < 0.05$ is considered significant.
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 [30], 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 [21,29,30]. 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...
that NDRG1 expression might be correlated in Japanese colorectal cancer patients (Table 1), suggesting of invasion, and histopathological type, and Dukes’ stage demonstrated that NDRG1 expression was significantly correlated to lymphatic invasion, venous invasion, depth of invasion, and histopathological type, and Dukes’ stage 

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); B: 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[25]. 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 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 mimic 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,
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 III and IV, 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[20], 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.

REFERENCES
1. Kokame K, Kato H, Miyata T. Homocysteine-responsive genes in vascular endothelial cells identified by differential display analysis. GRP78/Bip and novel genes. J Biol Chem 1996; 271: 29659-29665
2. van Belzen N, Dinjens WN, Diesveld MP, Groen NA, van der Made AC, Nozawa Y, Vlieistra R, Trapman J, Bosman FT. A novel gene which is up-regulated during colon epithelial cell differentiation and down-regulated in colorectal neoplasms. Lab Invest 1997; 77: 85-92.
3. Zhou D, Salnikow K, Costa M. Cap43, a novel gene specifically induced by N2+ compounds. Cancer Res 1998; 58: 2182-2189.
4. Kokame K, Kato H, Miyata T. Nonradioactive differential display cloning of genes induced by homocysteine in vascular endothelial cells. Methods 1998; 16: 434-443.
5. Piquemal D, Jouila D, Balaguer P, Basset A, Marti J, Commes T. Differential expression of the RTP/Drg-1 gene product in proliferating and growth arrested cells. Biochem Biophys Acta 1999; 1450: 364-373.
6. Shimono A, Okuda T, Kondoh H. N-myc-dependent repression of NDRG1, a gene identified by direct subtraction of whole mouse embryogenesis cDNAs between wild type and N-myc mutant. Mech Dev 1999; 83: 39-52.
7. Park H, Adams MA, Lachat P, Bosman F, Pang SC, Graham CH. Hypoxia induces the expression of a 43-kDa protein (PROXY-1) in normal and malignant cells. Biochem Biophys Res Commun 2000; 276: 321-328.
8. Salnikow K, Blagosklonny MV, Ryan H, Johnson R, Costa M. Carcinogenic nickel induces genes involved with hypoxic stress. Cancer Res 2000; 60: 38-41.
9. Salnikow K, Kluza T, Costa M, Piquemal D, Demidenko ZN, Xie K, Blagosklonny MV. The regulation of hypoxic genes by calcium involves c-Jun/AP-1, which cooperates with hypoxia-inducible factor 1 in response to hypoxia. Mol Cell Biol 2002; 22: 1734-1741.
10. Ulijn SW, Swinnen JV, Heyns W, Verhoogen G. The differentiation-related gene Drg-1 expressed in breast cancer. A novel integrin alpha 1 gene leading to its overexpression in various human cancers. BMC Genetics 2004; 5: 27.
11. Koshiji M et al. NDRG1 expression in colorectal cancer. www.wjgnet.com
a potential aid to cancer diagnosis. *Cell Biol Toxicol* 2002; 18: 87-96

19 Wang Z, Wang F, Wang WQ, Gao Q, Wei WL, Yang Y, Wang GY. Correlation of N-myc downstream-regulated gene 1 overexpression with progressive growth of colorectal neoplasm. *World J Gastroenterol* 2004; 10: 550-554

20 Shah MA, Kemeny N, Hummer A, Drobnjak M, Motwani M, Cordon-Cardo C, Gonen M, Schwartz GK. Drg1 expression in 131 colorectal liver metastases: correlation with clinical variables and patient outcomes. *Clin Cancer Res* 2005; 11: 3296-3302

21 Polite BN, Dignam JJ, Olopade OI. Colorectal cancer and race: understanding the differences in outcomes between African Americans and whites. *Med Clin North Am* 2005; 89: 771-793

22 Baldwin LM, Dobie SA, Billingsley K, Cai Y, Wright GE, Dominitz JA, Barlow W, Warren JL, Taplin SH. Explaining black-white differences in receipt of recommended colon cancer treatment. *J Natl Cancer Inst* 2005; 97: 1211-1220

23 Caruso RP, Levinson B, Melamed J, Wieczorek R, Taneja S, Polsky D, Chang C, Zeleniuch-Jacquotte A, Salnikow K, Yee H, Costa M, Osman I. Altered N-myc downstream-regulated gene 1 protein expression in African-American compared with Caucasian prostate cancer patients. *Clin Cancer Res* 2004; 10: 222-227

24 Plaschke J, Krüger S, Pistorius S, Theissig F, Sæger HD, Schackert HK. Involvement of hMSH6 in the development of hereditary and sporadic colorectal cancer revealed by immunostaining is based on germline mutations, but rarely on somatic inactivation. *Int J Cancer* 2002; 97: 643-648

25 Guan RJ, Ford HL, Fu Y, Li Y, Shaw LM, Pardee AB. Drg-1 as a differentiation-related, putative metastatic suppressor gene in human colon cancer. *Cancer* 2000; 60: 749-755

26 Jessup JM, Stewart A, Greene FL, Minsky BD. Adjuvant chemotherapy for stage III colon cancer: implications of race/ethnicity, age, and differentiation. *JAMA* 2005; 294: 2703-2711