N-myc downstream regulated gene 1 (NDRG1)/Cap43 enhances portal vein invasion and intrahepatic metastasis in human hepatocellular carcinoma

JUN AKIBA[1,2], SACHIKO OGASAWARA[1,2], AKIHIKO KAWAHARA[2,3], NAOYO NISHIDA[1,2], SAKIKO SANADA[1,2], FUKUKO MORIYA[1,2], MICHIHIKO KUWANO[2], OSAMU NAKASHIMA[1,2] and HIROHISA YANO[1,2]

[1]Department of Pathology, Kurume University School of Medicine, [2]Research Center of Innovative Cancer Therapy of the 21 Century COE Program for Medical Science, [3]Department of Pathology, Kurume University Hospital, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan

Received June 12, 2008; Accepted September 5, 2008

DOI: 10.3892/or_00000148

Abstract. N-myc downstream regulated gene 1 (NDRG1)/Cap43 is a 43 kDa protein that is widely distributed in the body. Its expression is regulated by nickel, cobalt, hypoxic condition and others; it is reported to be weaker in tumors than normal tissues; and NDRG1/Cap43 is considered to act suppressively to tumor metastasis. This current study immunohistochemically examined NDRG1/Cap43 expression in hepatocellular carcinoma (HCC), and analyzed its relationship to clinicopathologic factors and prognosis. The samples were 105 surgically resected HCC tissue blocks, i.e., 18 well-differentiated HCC, 61 moderately differentiated HCC, 10 poorly differentiated HCC, 9 'nodule-in-nodule' type HCC, and 7 sarcomatous HCC. In all cases, NDRG1/Cap43 was not expressed in normal liver cells. Strong expression was found in 65 of the 105 cases (62%), i.e., in 11.1% of well-differentiated HCC, 72.1% of moderately differentiated HCC, 80.0% of poorly differentiated HCC, and 71.4% of sarcomatous HCC. In the 'nodule-in-nodule' type, its expression was found in 55.6% of their well-differentiated component, and this frequency was significantly higher than that in well-differentiated HCC (11.1%). In the cases showing strong NDRG1/Cap43 expression, frequency of portal vein invasion and of intrahepatic metastasis was significantly high. No clear relationship between the expression and prognosis was observed. NDRG1/Cap43 expression that was found in advanced HCC was thought to accelerate tumor invasion and metastasis. NDRG1/Cap43 could act as a useful biomarker of HCC.

Correspondence to: Dr Jun Akiba, Department of Pathology, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan
E-mail: akiba@med.kurume-u.ac.jp

Key words: N-myc, Cap43, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the fifth commonest malignancy worldwide and is the third most common cause of cancer related death. The geographic areas at highest risk are located in Eastern Asia, Middle Africa, and some countries of Western Africa. HCC most commonly develops in patients with chronic liver disease, the etiology of which includes alcohol, viral infection (hepatitis B and C), metabolic diseases and aflatoxin (1,2). Surgery, including transplantation, remains the only curative modality for HCC. The prognosis of HCC is generally poor, and even after surgery, the 5-year survival rate is limited to 25-29% (3). The ability to predict patients at higher risk of recurrence and with a poor prognosis would help to guide surgical and chemotherapeutic treatment. Efforts have been made to predict recurrence and poor prognosis in patients with HCC after hepatectomy using clinicopathological parameters. Tumor size, tumor number, vascular invasion and the presence of satellite lesion were reported to be useful predictors (4-6). With the development of molecular biology, many biomarkers related to invasion, metastasis, recurrence and survival have been explored.

N-myc downstream regulated gene 1 (NDRG1, also known as Drd-1, Cap43 or RTP) was identified as a homocysteine-responsive gene that was induced by sulfhydryl regents such as cysteine and 2-mercaptoethanol in human umbilical vein endothelial cells (7). The NDRG1/Cap43 gene is mapped to chromosome 8q24 (8) and encodes a 3.0 kb mRNA that is translated into a protein with a molecular weight of 43 kDa (7,9). NDRG1/Cap43 expression is regulated with nickel, cobalt, oxidative stress, hypoxia, phorbol esters, vitamin A and D, steroids, histone deacetylase-targeting drugs, lysophosphatidylcholine, oncogene, and tumor suppressor gene (p53 and VHL) products (7,8,10-12). NDRG1/Cap43 acts in maintenance and differentiation (13) of myelin sheath; and in exocytosis (14), maturation (15) and degranulation (16) of mast cells. The NDRG1/CAP43 mRNA is widely expressed in the non-neoplastic tissues and its expression is especially high in the prostate (17), brain (18), kidney (13,19), placenta and

intestine (1,3,4,7,9,10,14,15,20,21). Sincce NDRG1/Cap43 expression level in neoplastic cells of breast cancer, prostatic cancer and colon cancer is lower in comparison to that in non-neoplastic tissues, NDRG1/Cap43 is reported to be suppressive to tumor metastasis (8,9,17,22-24). On the other hand, NDRG1/Cap43 is also reported to be overexpressed in tumor tissue than normal tissue (25), and to act as an accelerating factor of metastasis (26). At present, actions of NDRG1/Cap43 in tumor remain elusive.

To date, NDRG1/Cap43 expression in the liver (25) and its relationship with hepatocellular carcinoma (HCC) were reported (27,28). Chua *et al* showed high NDRG1/Cap43 expression in HCC correlated with shorter overall survival, late tumor stage, vascular invasion, large tumor size, and high histological grade and NDRG1/Cap43 could be a useful predictor (27). In the current study we investigated NDRG1/Cap43 expression and its histopathological features on surgically resected HCC of humans, and evaluated NDRG1/Cap43 as a possible biomarker of HCC.

Materials and methods

Tissue samples. Immunohistochemical examination was performed on formalin-fixed paraffin sections of cancerous and non-cancerous tissues of 105 HCC livers that were surgically resected at Kurume University Hospital in the period between 1989 and 2007. The 105 patients did not receive preoperative anticancer therapies such as a trans-catheter arterial embolization and radiofrequency are summarized in Table I. Among them, 89 cases had a nodule

Table I. Clinicopathological characteristics of 105 HCC cases.

Clinicopathological factors	No. of cases (%)
Age (years, mean ± SD)	64.3±8.5
Sex (M/F)	80/25
Tumor size (mm, mean ± SD)	38.2±25.2
Differentiation	
Well-differentiated	18 (17.1)
Moderately differentiated	61 (58.1)
Poorly differentiated	10 (9.5)
HCC with sarcomatous change	7 (6.7)
Nodule-in-nodule appearance	9 (8.6)
Portal vein invasion	46 (43.8)
Venous invasion	7 (6.7)
Bile duct invasion	4 (3.8)
Intrahepatic metastasis	8 (26.7)
Virus marker	
Hepatitis B virus (HBV) associated	10 (9.5)
Hepatitis C virus (HCV) associated	75 (71.4)
HBV and HCV associated	9 (8.6)
HBV and HCV negative	6 (5.7)
Unknown	5 (4.8)

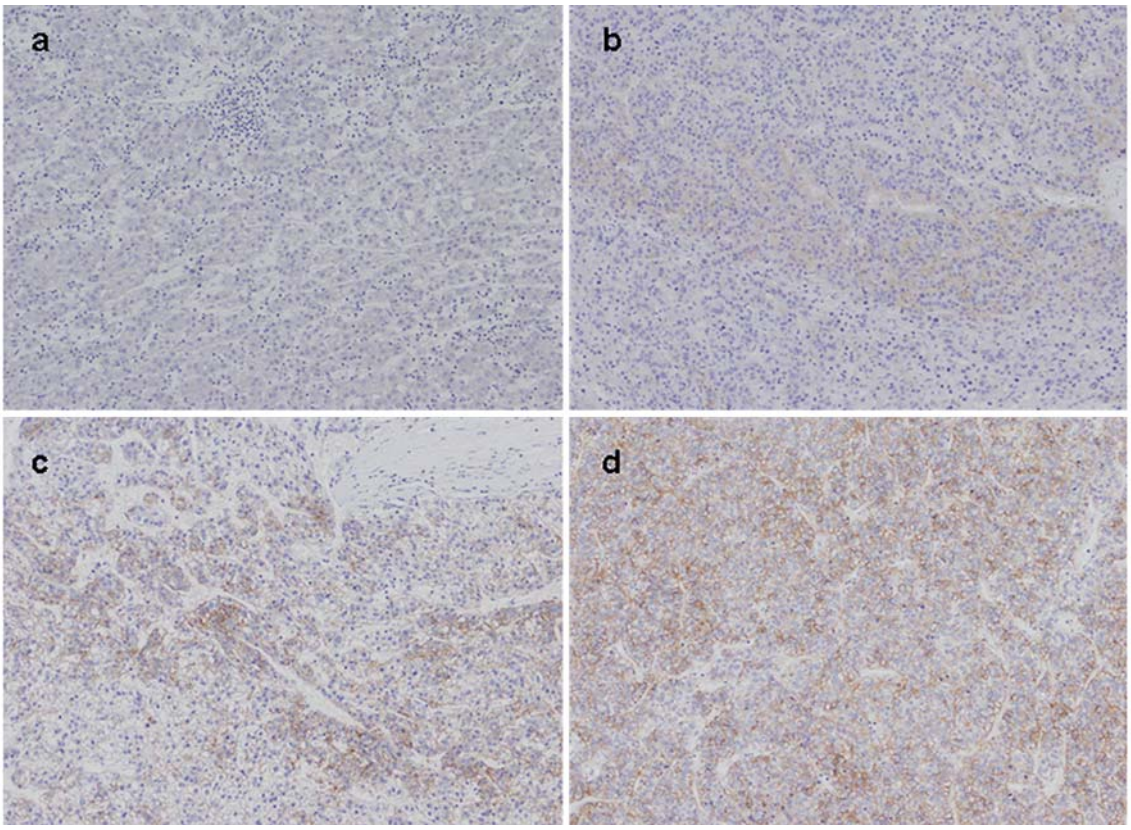

Figure 1. Grading of NDRG1/Cap43 staining distribution. (a) Grade 0: NDRG1/CAP43 positive cells were present <10% of the entire area. (b) Grade +1: the area was 10-40%. (c) Grade +2: the area was 40-70%. (d) Grade +3: the area was 70-100%.

Table II. Relationship between NDRG1/Cap43 expressions and clinicopathological factors.

Clinicopathological factors		NDRG1/Cap43 expression		p value (χ^2 test)
		Strong (n=65)	Weak (n=40)	
Age (years, mean ± SD)		63.8±9.3	65.1±7.2	NS
Gender	Male	48	32	NS
	Female	17	8	
Tumor size (mm, mean ± SD)			41.3±22.9	33.4±28.4
NS				
Histological grade				
Well-differentiated[(a)]		2	16	
Moderately differentiated		44	17	p<0.0001, vs. (a)
Poorly differentiated		8	2	p=0.0003, vs. (a)
HCC with sarcomatous change		5	2	p=0.0032, vs. (a)
Nodule-in-nodule appearance				
Well-differentiated component		5	4	p=0.0145, vs. (a)
Moderately differentiated component		6	3	p=0.003, vs. (a)
Portal vein invasion	(+)	37	9	p=0.0004
	(-)	28	31	
Venous vein invasion	(+)	6	1	NS
	(-)	59	39	
Bile duct invasion	(+)	4	1	NS
	(-)	61	39	
Intrahepatic metastasis	(+)	23	5	p=0.0074
	(-)	42	35	
Hepatitis B virus	(+)	15	4	NS
	(-)	50	36	
Hepatitis C virus	(+)	49	35	NS
	(-)	16	5	

of single histological grade, i.e., 18 well-differ-entiated HCC, 61 moderately differentiated HCC, and 10 poorly differentiated HCC. Another 9 cases had 2 different histological grades in a single tumor nodule, i.e., well-differentiated component and moderately differentiated component with a clear boundary between them ('nodule-in-nodule' type). The remaining 7 cases presented sarcomatous changes (sarcomatous HCC). Pathological features of HCC were evaluated according to the World Health Organization (WHO) classification (29).

Immunohistochemistry. Formalin-fixed, paraffin-embedded sections (4 μm) were mounted on 3-aminoprophl-triethoxysilane-coated slides (Matsunami Glass Inc., Ltd., Osaka, Japan), deparaffinized in xylene, and re-hydrated in graded alcohol. The sections were soaked in 10 mmol/l of sodium citrate buffer (pH 6.0) and treated in microwave for 20 min for antigen retrieval. NDRG1/Cap43 expression was immunohistochemically examined with rabbit polyclonal anti-NDRG1/Cap43 antibody (gift from Professor K. Kohno, Department of Molecular Biology, University of Occupation and Environmental Health, Fukuoka, Japan. Diluted 1:2000) as the primary antibody (12,30), and using Histofine SAB-PO kit (Nichirei, Tokyo, Japan) according to the manufacturer's protocol. The sections were incubated with primary antibody for 60 min at room temperature after blocking endogenous biotin and peroxidase activities. Negative control was prepared by replacing the primary antibody with normal rabbit serum. The peroxidase reaction was developed with the addition of 3, 3-diaminobenzidine and H_2O_2 substrate solution. After counterstaining with hematoxylin, the slides were dehydrated, coverslipped, and observed under a microscope (Olympus BX41, Olympus Optical, Tokyo, Japan). The immunohistochemical staining was evaluated independently by two pathologists (J.A. and H.Y.). Immunoreactivity of NDRG1/Cap43-positive cells was compared among the tissue sections, and the ratio of the area where positive cells were present to the area of entire specimen was calculated. NDRG1/Cap43

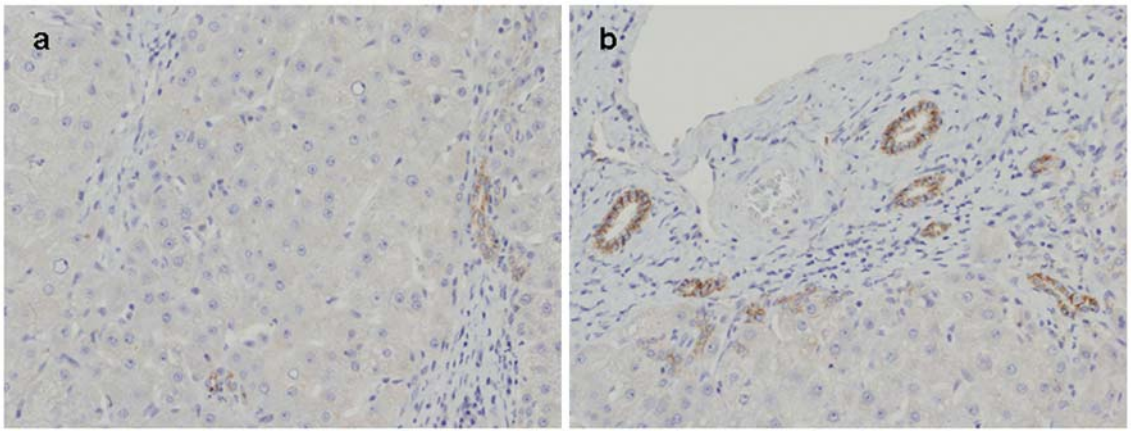

Figure 2. NDRG1/Cap43 expression in non-neoplastic tissues. (a) NDRG1/Cap43 was always negative in normal liver cells. (b) Bile duct epithelium in portal tract was always positive.

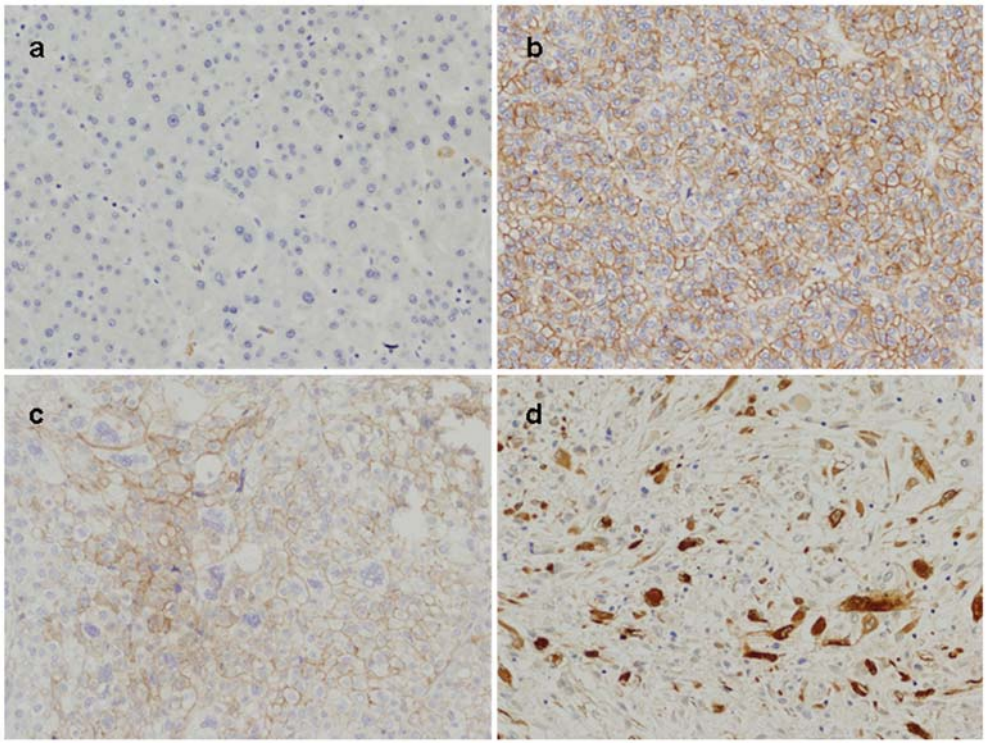

Figure 3. Photomicrograph of immunohistochemical staining. (a) NDRG1/Cap43 in well-differentiated HCC, (b) in moderately differentiated HCC, (c) in poorly differentiated HCC, (d) in HCC with sarcomatous change.

expression in bile duct epithelium was used as an internal positive control because it is always positive. NDRG1/Cap43 expression was graded into 4 levels according to the distribution of immunoreactive HCC cells (Fig. 1), i.e., 0 when NDRG1/Cap43-positive cells were present in <10% of the entire area, +1 when the area was 10-40%, +2 when 40-70%, and +3 when 70-100%. Staining intensity for NDRG1/Cap43 was graded into 3 levels, i.e., 0 when the intensity in HCC area was less than that of bile duct epithelium, +1 when the intensity was almost equal to that of bile duct epithelium, and +2 when the intensity was stronger than that of bile duct epithelium. Total score was obtained as the expression grade multiplied by the staining intensity score. This total score was then evaluated into 2 levels, i.e., 0-2, weak expression; 3-6, strong expression, and the relationship between NDRG1/Cap43 expression and clinicopathological features was examined. Statistical significance was examined with χ^2 test.

The relationship between NDRG1/Cap43 expression and postoperative course was examined in 72 of 105 cases who were monitored up to 9.1 years after surgery. The survival rates were calculated by using the Kaplan-Meier method, and the differences were compared by the log-rank test.

Results

NDRG1/Cap43was always negative in non-cancerous liver cells, and its positivity was not affected by the condition of

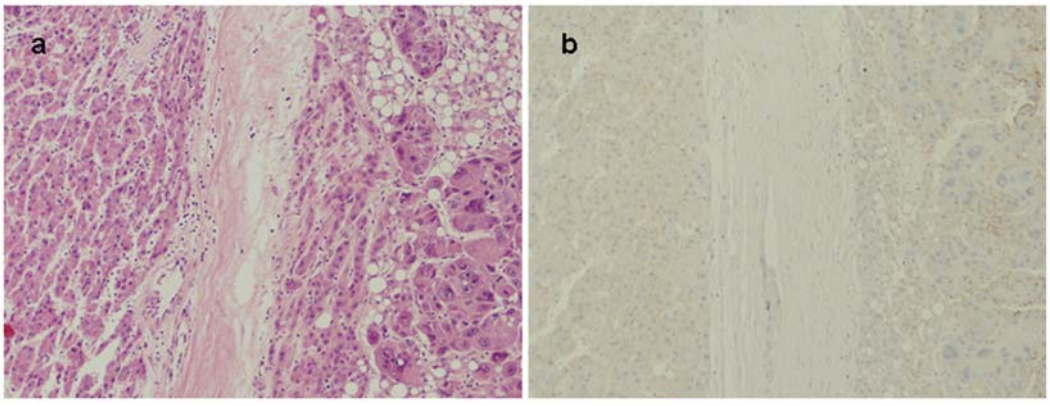

Figure 4. Photomicrographs. (a) The boundary between well-differentiated component (left) and moderately differentiated component (right) with a nodule-in-nodule appearance. (b) NDRG1/Cap43 was immunohistochemically stained in well-differentiated component (left) and moderately differentiated component (right).

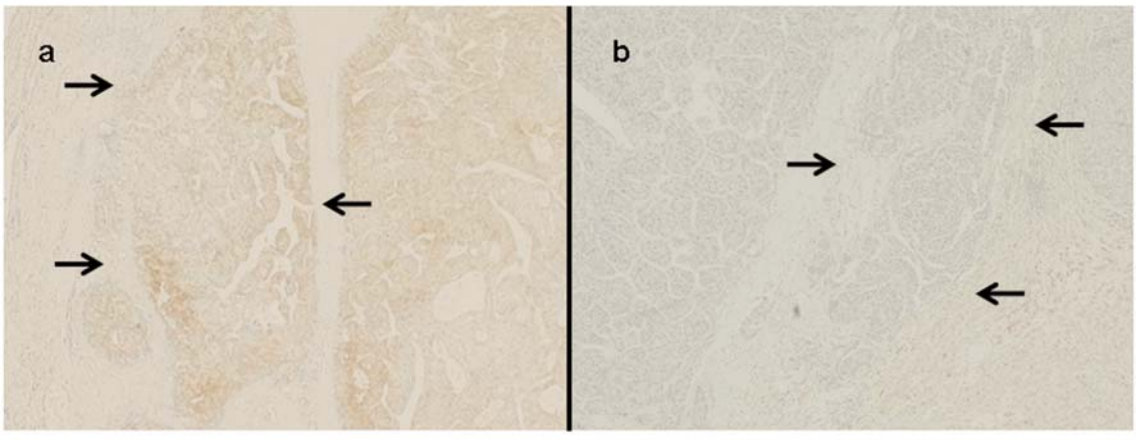

Figure 5. The area of portal vein invasion. (a) Strong NDRG1/Cap43 expression was found in tumor body and in the tumor casts of portal vein (arrows). (b) In one case, NDRG1/Cap43 expression was not found tumor body and in the tumor casts of portal vein (arrows).

the liver (e.g., hepatitis and cirrhosis) or the type of infected hepatitis virus. On the other hand, NDRG1/Cap43 was always positive on the bile duct membrane in the portal tract (Fig. 2), and this picture was used as the internal positive control in this study. Among the 105 cases, NDRG1/Cap43 was expressed at a high level in 65 (62.0%) cases. Their cytoplasm and/or cellular membrane were NDRG1/Cap43 positive, but nuclei were not. There was no significant relationship between the location of positively stained area and clinicopathologic factors. In the 89 cases that HCC nodules were in a single histological grade, strong NDRG1/Cap43 expression was found in 11.1% (2/18) of the well-differentiated HCC, 72.1% (44/61) of moderately differentiated HCC ($p<0.0001$, vs. well-differentiated), and 80.0% (8/10) of the poorly differentiated HCC ($p=0.0003$) (Table II). Among the 7 sarcomatous HCC cases, strong NDRG1/Cap43 expression was observed in 5 (71.4%) and this was significantly higher than the well-differentiated HCC ($p=0.0032$, Fig. 3). In the 9 cases with 'nodule-in-nodule' appearance that contained 2 or more components of different histological grades, NDRG1/Cap43 was strongly expressed in 55.6% (5/9) of well-differentiated component and 66.7% (6/9) of moderately differentiated component (Table II). The frequency in well-differentiated component was significantly higher than that in the well-differentiated HCC of a single histological grade ($p=0.0145$, Fig. 4). In the relationship with clinicopathologic factors, frequencies of portal vein invasion and intrahepatic metastasis were significantly high in the cases of strong NDGR1/Cap43 expression ($p=0.0004$ and $p=0.0074$, respectively). Among the 17 cases who were evaluable for NDRG1/Cap43 expression in the tumor casts of portal vein, 16 cases showed strong expression in the entire tumor body as well as in the tumor casts of portal vein, but one of the 17 cases showed low expression in the tumor body as well as in the tumor casts of portal vein (Fig. 5). Post-operative course was monitored in 72 cases, and NDRG1/Cap43 expression was not clearly related to their survival time. However, among clinicopathological factors the presence of portal vain invasion was significantly associated with shorter survival (log-rank test; $p=0.003$) and intrahepatic metastasis also influenced shorter survival (log-rank test; $p=0.0575$) (Fig. 6).

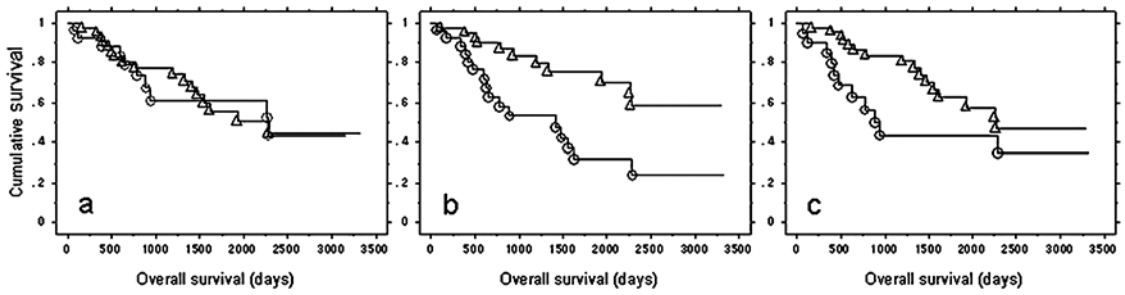

Figure 6. Kaplan-Meier analysis for overall survival time. (a) There was no significant difference between HCC with strong NDRG1/Cap43 expression (Δ) and HCC with weak NDRG1/Cap43 expression (○). (b) Significant difference was observed between HCC with portal vein invasion (○) and HCC without portal vein invasion (Δ) (log-rank test; p=0.003). (c) The period tended to be shorter in cases with intrahepatic metastasis (○) than those without intrahepatic metastasis (Δ) (log-rank test; p=0.0575).

Discussion

Cangul (25) reported no or very mild positivity in non-neoplastic liver cells. Chua *et al* (27) found no NDRG1/Cap43 expression in normal liver cells, but 6% of cirrhosis and benign liver lesions had the expression. On the other hand, in our cases, NDRG1/Cap43 was not expressed in non-neoplastic liver cells even though many of our cases were positive to hepatitis B and/or C virus and had conditions of chronic hepatitis or cirrhosis. This difference in the findings would be attributable to the differences of antibody and immunostaining kits used in each study, disease condition of the patients examined, and evaluation method. Since it was not expressed in non-neoplastic liver cells but shown with the development of tumor, NDRG1/Cap43 could be a marker of HCC.

NDRG1/Cap43 was strongly expressed in 62.0% of the lesions of our cases, and the frequency of strong expression was significantly higher in the moderately and poorly differentiated HCC in comparison to well-differentiated HCC. This indicated that NDRG1/Cap43 is not related to early event of carcinogenesis in the liver but to the growth and development into advanced HCC. This point is supported by the findings that the cases with strong NDRG1/Cap43 expression highly associated with portal vein invasion or intrahepatic metastasis, and tended to have a larger diameter of the nodule. The relationship between NDRG1/Cap43 expression and cancer has not yet been fully elucidated, but previous studies reported that NDRG1/Cap43 acts suppressively to metastasis in prostatic cancer, breast cancer, colon cancer and pancreatic cancer; and is also a useful prognostic factor (22-24,31). In our current study, the relationship between NDRG1/Cap43 and prognosis was not clear, but the results indicated that NDRG1/Cap43 accelerates vascular invasion and metastasis of cancer. Chua *et al* (27) also showed in their HCC study that NDRG1/Cap43 is an indicator of poor prognosis and related to such features as vascular invasion, large tumor size and high histological grade. On the other hand, in our cases portal vain invasion and intrahepatic metastasis was associated with short survival, and frequencies of portal vain invasion and intrahepatic metastasis was significantly high in the cases with NDRG1/Cap43 high expression. This suggests NDRG1/Cap43 high expression is indirectly associated with short survival.

HCC develops in the liver with such chronic diseases as hepatitis and cirrhosis. It occurs as a well-differentiated cancer without having a capsule or distinct margin, and then de-differentiate to present 'nodule-in-nodule' appearance that contains moderately or poorly differentiated component within the nodule. At that stage, tumor growth is accelerated. In our findings, frequencies of NDRG1/Cap43 expression in the well-differentiated component of a 'nodule-in-nodule' type HCC was higher than in a well-differentiated HCC that consists of a single grade HCC. In our cases, NDRG1/Cap43 may act as a promoter of dedifferentiation. Another possible explanation for this difference in the expression rates is that we examined well-differentiated HCC that was in the early-stage of development, contained a single nodule of a single histological grade, and had indistinct margin; therefore the environment such as vascular structure is different from that of 'nodule-in-nodule' type HCC resulting in a different staining pattern to NDRG1/Cap43 even when their histological grade was the same as the corresponding component in a 'nodule-in-nodule' type HCC.

Vascular structure of HCC changes from portal vein to arterial vessel along with tumor growth, and in this course ischemic condition temporally occurs (32). On the other hand, NDRG1/Cap43 expression is upregulated in hypoxia in several cancer types (19,25,28,33) including HCC (28). In our current study, not many of our well-differentiated nodules, i.e., the early-stage HCC, expressed NDRG1/Cap43, whereas the well-differentiated component of 'nodule-in-nodule' type that would be under hypoxic stress in its growth process expressed NDRG1/Cap43 at a high frequency. This suggests that hypoxia would be one of the factors that regulate NDRG1/Cap43 expression in HCC.

Many of our cases that showed strong NDRG1/Cap43 expression in the area of portal tract invasion also showed strong expression in the tumor body. On the other hand, there was no expression in the area of portal tract invasion in the cases that showed weak expression in the tumor body. HCC with strong NDRG1/Cap43 expression had significantly high frequency of portal tract invasion, and they also showed NDRG1/Cap43 expression in the area around the invasion. This indicated a mechanism where NDRG1/Cap43-positive cells with high invasive capability invaded the portal tract and then started to express NDRG1/Cap43.

Our findings showed that NDRG1/Cap43 expression occurs in developed HCC and it promotes invasion and metastasis. More details of the mechanism that accelerates proliferation and metastasis should be examined in future studies.

Acknowledgements

We gratefully thank Ms. Sachiyo Maeda, Akiko Tanaka, Akemi Fujiyoshi and Misato Shiraishi for their excellent technical assistance.

References

1. Yamamoto J, Kosuge T, Takayama T, Shimada K, Yamasaki S, Ozaki H, Yamaguchi N and Makuuchi M: Recurrence of hepatocellular carcinoma after surgery. Br J Surg 83: 1219-1222, 1996.
2. Chen XP, Qiu FZ, Wu ZD, Zhang ZW, Huang ZY and Chen YF: Long-term outcome of resection of large hepatocellular carcinoma. Br J Surg 93: 600-606, 2006.
3. Kondo M, Yamamoto H, Nagano H, Okami J, Ito Y, Shimizu J, Eguchi H, Miyamoto A, Dono K, Umeshita K, Matsuura N, Wakasa K, Nakamori S, Sakon M and Monden M: Increased expression of COX-2 in nontumor liver tissue is associated with shorter disease-free survival in patients with hepatocellular carcinoma. Clin Cancer Res 5: 4005-4012, 1999.
4. Hanazaki K, Kajikawa S, Koide N, Adachi W and Amano J: Prognostic factors after hepatic resection for hepatocellular carcinoma with hepatitis C viral infection: univariate and multivariate analysis. Am J Gastroenterol 96: 1243-1250, 2001.
5. Yeh CN, Chen MF, Lee WC and Jeng LB: Prognostic factors of hepatic resection for hepatocellular carcinoma with cirrhosis: univariate and multivariate analysis. J Surg Oncol 81: 195-202, 2002.
6. Tsai TJ, Chau GY, Lui WY, Tsay SH, King KL, Loong CC, Hsia CY and Wu CW: Clinical significance of microscopic tumor venous invasion in patients with resectable hepatocellular carcinoma. Surgery 127: 603-608, 2000.
7. Kokame K, Kato H and Miyata T: Homocysteine-respondent genes in vascular endothelial cells identified by differential display analysis. GRP78/BiP and novel genes. J Biol Chem 271: 29659-29665, 1996.
8. Kurdistani SK, Arizti P, Reimer CL, Sugrue MM, Aaronson SA and Lee SW: Inhibition of tumor cell growth by RTP/rit42 and its responsiveness to p53 and DNA damage. Cancer Res 58: 4439-4444, 1998.
9. van Belzen N, Dinjens WN, Diesveld MP, Groen NA, van der Made AC, Nozawa Y, Vlietstra R, Trapman J and Bosman FT: A novel gene which is up-regulated during colon epithelial cell differen-tiation and down-regulated in colorectal neoplasms. Lab Invest 77: 85-92, 1997.
10. Zhou D, Salnikow K and Costa M: Cap43, a novel gene specifically induced by $Ni2^{+}$ compounds. Cancer Res 58: 2182-2189, 1998.
11. Shimono A, Okuda T and Kondoh H: N-myc-dependent repression of ndr1, a gene identified by direct subtraction of whole mouse embryo cDNAs between wild type and N-myc mutant. Mech Dev 83: 39-52, 1999.
12. Masuda K, Ono M, Okamoto M, Morikawa W, Otsubo M, Migita T, Tsuneyoshi M, Okuda H, Shuin T, Naito S and Kuwano M: Downregulation of Cap43 gene by von Hippel-Lindau tumor suppressor protein in human renal cancer cells. Int J Cancer 105: 803-810, 2003.
13. Okuda T, Higashi Y, Kokame K, Tanaka C, Kondoh H and Miyata T: Ndrg1-deficient mice exhibit a progressive demyelinating disorder of peripheral nerves. Mol Cell Biol 24: 3949-3956, 2004.
14. Sugiki T, Murakami M, Taketomi Y, Kikuchi-Yanoshita R and Kudo I: N-myc downregulated gene 1 is a phosphorylated protein in mast cells. Biol Pharm Bull 27: 624-627, 2004.
15. Sugiki T, Taketomi Y, Kikuchi-Yanoshita R, Murakami M and Kudo I: Association of N-myc downregulated gene 1 with heat-shock cognate protein 70 in mast cells. Biol Pharm Bull 27: 628-633, 2004.
16. Taketomi Y, Sunaga K, Tanaka S, Nakamura M, Arata S, Okuda T, Moon TC, Chang HW, Sugimoto Y, Kokame K, Miyata T, Murakami M and Kudo I: Impaired mast cell maturation and degranulation and attenuated allergic responses in Ndrg1-deficient mice. J Immunol 178: 7042-7053, 2007.
17. Guan RJ, Ford HL, Fu Y, Li Y, Shaw LM and Pardee AB: Drg-1 as a differentiation-related, putative metastatic suppressor gene in human colon cancer. Cancer Res 60: 749-755, 2000.
18. Qu X, Zhai Y, Wei H, Zhang C, Xing G, Yu Y and He F: Characterization and expression of three novel differentiation-related genes belong to the human NDRG gene family. Mol Cell Biochem 229: 35-44, 2002.
19. Zhou RH, Kokame K, Tsukamoto Y, Yutani C, Kato H and Miyata T: Characterization of the human NDRG gene family: a newly identified member, NDRG4, is specifically expressed in brain and heart. Genomics 73: 86-97, 2001.
20. Lachat P, Shaw P, Gebhard S, van Belzen N, Chaubert P and Bosman FT: Expression of NDRG1, a differentiation-related gene, in human tissues. Histochem Cell Biol 118: 399-408, 2002.
21. Piquemal D, Joulia D, Balaguer P, Basset A, Marti J and Commes T: Differential expression of the RTP/Drg1/Ndr1 gene product in proliferating and growth arrested cells. Biochim Biophys Acta 1450: 364-373, 1999.
22. Bandyopadhyay S, Pai SK, Gross SC, Hirota S, Hosobe S, Miura K, Saito K, Commes T, Hayashi S, Watabe M and Watabe K: The Drg-1 gene suppresses tumor metastasis in prostate cancer. Cancer Res 63: 1731-1736, 2003.
23. Bandyopadhyay S, Pai SK, Hirota S, Hosobe S, Takano Y, Saito K, Piquemal D, Commes T, Watabe M, Gross SC, Wang Y, Ran S and Watabe K: Role of the putative tumor metastasis suppressor gene Drg-1 in breast cancer progression. Oncogene 23: 5675-5681, 2004.
24. Shah MA, Kemeny N, Hummer A, Drobnjak M, Motwani M, Cordon-Cardo C, Gonen M and Schwartz GK: Drg1 expression in 131 colorectal liver metastases: correlation with clinical variables and patient outcomes. Clin Cancer Res 11: 3296-3302, 2005.
25. Cangul H: Hypoxia upregulates the expression of the NDRG1 gene leading to its overexpression in various human cancers. BMC Genet 5: 27, 2004.
26. Wang Z, Wang F, Wang WQ, Gao Q, Wei WL, Yang Y and Wang GY: Correlation of N-myc downstream-regulated gene 1 overexpression with progressive growth of colorectal neoplasm. World J Gastroenterol 10: 550-554, 2004.
27. Chua MS, Sun H, Cheung ST, Mason V, Higgins J, Ross DT, Fan ST and So S: Overexpression of NDRG1 is an indicator of poor prognosis in hepatocellular carcinoma. Mod Pathol 20: 76-83, 2007.
28. Sibold S, Roh V, Keogh A, Studer P, Tiffon C, Angst E, Vorburger SA, Weimann R, Candinas D and Stroka D: Hypoxia increases cytoplasmic expression of NDRG1, but is insufficient for its membrane localization in human hepatocellular carcinoma. FEBS Lett 581: 989-994, 2007.
29. Hirohashi S, Ishak KG, Kojiro M, *et al*: World Health Organization Classification of Tumours. Pathology & Genetics of Tumours of Digestive System. Hepatocellular carcinoma. Hamilton SR and Aaltonen LA (eds). IARC Press, Lyon, pp159-172, 2000.
30. Nishie A, Masuda K, Otsubo M, Migita T, Tsuneyoshi M, Kohno K, Shuin T, Naito S, Ono M and Kuwano M: High expression of the Cap43 gene in infiltrating macrophages of human renal cell carcinomas. Clin Cancer Res 7: 2145-2151, 2001.
31. Maruyama Y, Ono M, Kawahara A, Yokoyama T, Basaki Y, Kage M, Aoyagi S, Kinoshita H and Kuwano M: Tumor growth suppression in pancreatic cancer by a putative metastasis suppressor gene Cap43/NDRG1/Drg-1 through modulation of angiogenesis. Cancer Res 66: 6233-6242, 2006.
32. Kutami R, Nakashima Y, Nakashima O, Shiota K and Kojiro M: Pathomorphologic study on the mechanism of fatty change in small hepatocellular carcinoma of humans. J Hepatol 33: 282-289, 2000.
33. Salnikow K, Blagosklonny MV, Ryan H, Johnson R and Costa M: Carcinogenic nickel induces genes involved with hypoxic stress. Cancer Res 60: 38-41, 2000.