Biochemical and Biophysical Research Communications 441 (2013) 102–107

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Suppressed expression of *NDRG2* correlates with poor prognosis in pancreatic cancer

Akihiro Yamamura [a,b], Koh Miura [a], Hideaki Karasawa [a], Kazuhiro Morishita [c], Keiko Abe [b], Yasuhiko Mizuguchi [b], Yuriko Saiki [b], Shinichi Fukushige [b], Naoyuki Kaneko [a], Tomohiko Sase [a], Hiroki Nagase [d], Makoto Sunamura [a,b,e], Fuyuhiko Motoi [a], Shinichi Egawa [a], Chikashi Shibata [a], Michiaki Unno [a], Iwao Sasaki [a], Akira Horii [b,*]

[a] Department of Surgery, Tohoku University, Graduate School of Medicine, Sendai, Japan
[b] Department of Pathology, Tohoku University, Graduate School of Medicine, Sendai, Japan
[c] Department of Medical Sciences, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan
[d] Department of Advanced Medical Science, Nihon University School of Medicine, Tokyo, Japan
[e] Department of Digestive Tract Surgery and Transplantation Surgery, Tokyo Medical University, Hachioji Medical Center, Tokyo, Japan

ARTICLE INFO

Article history:
Received 2 October 2013
Available online 14 October 2013

Keywords:
NDRG2
Epigenetic silencing
Hypermethylation
Pancreatic cancer
Prognostic factor

ABSTRACT

Pancreatic cancer is a highly lethal disease with a poor prognosis; the molecular mechanisms of the development of this disease have not yet been fully elucidated. N-myc downstream regulated gene 2 (*NDRG2*), one of the candidate tumor suppressor genes, is frequently downregulated in pancreatic cancer, but there has been little information regarding its expression in surgically resected pancreatic cancer specimens. We investigated an association between NDRG2 expression and prognosis in 69 primary resected pancreatic cancer specimens by immunohistochemistry and observed a significant association between poor prognosis and NDRG2-negative staining ($P = 0.038$). Treatment with trichostatin A, a histone deacetylase inhibitor, predominantly up-regulated *NDRG2* expression in the *NDRG2* low-expressing cell lines (PANC-1, PCI-35, PK-45P, and AsPC-1). In contrast, no increased *NDRG2* expression was observed after treatment with 5-aza-2′ deoxycytidine, a DNA demethylating agent, and no hypermethylation was detected in either pancreatic cancer cell lines or surgically resected specimens by methylation specific PCR. Our present results suggest that (1) *NDRG2* is functioning as one of the candidate tumor-suppressor genes in pancreatic carcinogenesis, (2) epigenetic mechanisms such as histone modifications play an essential role in *NDRG2* silencing, and (3) the expression of NDRG2 is an independent prognostic factor in pancreatic cancer.

1. Introduction

Pancreatic cancer is a highly lethal disease; few patients are diagnosed at a state early enough for curative treatments. It is the fourth most common cause of cancer death worldwide [1], and the long-term prognosis remain poor with a 5-year survival rate of less than 5% after the initial diagnosis [2]. One of the major hallmarks of pancreatic cancer is its extensive local tumor invasion and early systemic dissemination. The molecular basis for these characteristics of pancreatic cancer is incompletely understood.

N-Myc downstream regulated gene 2 (*NDRG2*) is a member of *NDRG* gene family that is highly expressed in many normal tissue types, including brain, spinal cord, skeletal muscle, heart, and salivary gland [3–5]. *NDRG* gene family members share 53–65% homologous amino acid sequences with each other. Each member has a distinct tissue specificity of expression and may be intimately involved in cell proliferation, differentiation, development, and stress responses [6].

NDRG2 has been reported to be a candidate tumor suppressor gene, and its expression is downregulated in a number of primary tumors developed in organs of brain and meninges [4,7,8], liver [9,10], pancreas [10], esophagus [11], stomach [12], colorectum [6,13,14], kidney [6], thyroid [15], oral cavity [16], prostate [17], gallbladder [18], blood [19], and lung [20]. *NDRG2* is reported to

Abbreviations: *NDRG2*, N-myc downstream regulated gene 2; 5-aza-dC, 5-aza-2′ deoxycytidine; TSA, trichostatin A; qRT-PCR, quantitative reverse transcription polymerase chain reaction; cDNA, complementary DNA; AU, arbitrary unit; *B2M*, β2-microglobulin; MSP, methylation-specific PCR; HDAC, histone deacetylase.

* Corresponding author. Address: Department of Pathology, Tohoku University, Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan. Fax: +81 22 717 8047.
E-mail address: horii@med.tohoku.ac.jp (A. Horii).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.010

suppress proliferation and metastasis, and expressional inactivation of *NDRG2* may play an important role in carcinogenesis [4,9,11,12,21,22]. Several possible mechanisms, including promoter hypermethylation [7–9,13,16,23] and/or repression by MYC [4,11,14,15,24], are responsible for such expressional suppression. However, the precise mechanisms that lead to inactivation of *NDRG2* remain largely unknown, and role of *NDRG2* in human carcinogenesis is not yet well understood.

In the present study using immunohistochemistry, we found a significant association between poor prognosis and suppressed expression of NDRG2 in primary pancreatic cancer. Furthermore, *NDRG2* gene expression was up-regulated by histone deacetylase inhibitor in pancreatic cancer. It is notable that this histone modification has never previously been demonstrated in suppression of *NDRG2* expression in human cancer. These findings suggest that *NDRG2* is likely to be a novel prognostic marker and important indicator for a possible role of *NDRG2* in pancreatic cancer.

2. Materials and methods

2.1. Tissue specimens

A total of 69 pancreatic cancer tissues obtained from surgically resected specimens at Tohoku University Hospital (Sendai, Miyagi, Japan) during the period from 1997 to 2006 were analyzed. The clinical and histopathological characteristics of the pancreatic cancer patients are summarized in Table 1. Staging followed the TNM Classification of Malignant Tumor (6th edition) [25]. None of the patients had received any preoperative adjuvant therapy. The resected tissue specimens from these patients were fixed in 10% formalin and embedded in paraffin. Written informed consent was obtained from all patients. The study was approved by the Ethics Committee of Tohoku University School of Medicine.

Table 1
Relationship between NDRG2 expression and clinicopathological features of pancreatic cancer patients.

	NDRG2 expression		*P*-value
	Positive *n* = 18	Negative *n* = 51	
Gender			
Male	14	32	0.24
Female	4	19	
Age (mean, years)	61.2	64.0	0.25
Tumor size[a] (mm)	38.3	42.3	0.45
UICC Stage			
I	2	2	0.70
II	6	21	
III	6	16	
IV	4	12	
Differentiation			
Wel	2	1	0.14
Mod	15	41	
Por	1	9	
Lymph node metastasis			
N0	6	18	0.88
N1	12	33	
Lymphatic invasion			
Absent	3	5	0.61
Present	15	46	
Venous invasion			
Absent	0	4	0.22
Present	18	47	
Intrapancreatic neural invasion			
Absent	1	2	0.77
Present	17	49	

[a] Average longitudinal diameter.

2.2. Tissue array analysis and immunohistochemistry

A tissue array consisting of 69 paired pancreatic cancer and their corresponding normal tissues was constructed using TISSUE MICROPROCESSOR (AZUMAYA, Tokyo, Japan). Each paraffin-embedded block was cored out at a diameter of 3 mm, and the cored columns were re-embedded in paraffin. For further analyses, 4 μm slide sections were prepared. The immunohistochemical assay was done by the avidin–biotin–peroxidase method described previously [26]. Rabbit polyclonal anti-NDRG2 (1:3000, Atlas Antibodies AB, Stockholm, Sweden) and anti-rabbit (1:1000, Amersham Biosciences, Little Chalfont, UK) secondary antibodies were used. Immunoreactivity was evaluated by two pathologists. NDRG2 immmunoreactivity was detected in both the cytoplasm and the plasma membrane. Normal epithelial cells showed expression of NDRG2 in all specimens. NDRG2 immunoreactivity was defined by comparison the signal intensities of normal and cancerous tissues; strong, moderate, and weak designations denote signals with cancerous tissue that were stronger, similar, or weaker than the normal tissues, respectively. When no NDRG2 signal was detected, we defined the tumor as negative.

2.3. Cell lines analyzed in this study

Nine human pancreatic cancer cell lines (PANC-1, PCI-35, PK-45P, AsPC-1, BxPC-3, PK-1, MIAPaCa-2, PK8 and PK9) and two colorectal cancer cell lines (Clone A and LS174T) were used. These cell lines were also used in our previous studies and were maintained as described [27,28].

2.4. RNA and DNA extraction

Total RNAs from cultured cells were extracted using RNeasy Mini Kit (Qiagen, Valencia, CA), and their concentrations were determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Genomic DNAs from cultured cells were extracted using DNeasy Blood & Tissue Kits (Qiagen) according to the manufacturer's instructions, and their concentrations were measured with a NanoDrop ND-1000 Spectrophotometer. All the processes were carried out according to the manufacturers' instructions.

2.5. Quantitative reverse transcription PCR (qRT-PCR)

Each aliquot of 2 μg total RNA was reverse transcribed to synthesize cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. qRT-PCR analyses were performed using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems) following the manufacturer's instructions. Expression of β2-microglobulin (*B2M*) was used as the internal control [29]. The nucleotide sequences for primers, probes, and PCR conditions are listed in Table 2. Amplifications were carried out in the 15 μl reaction mixtures according to methods described previously [30]. The expression ratios of *NDRG2*/*B2M* were calculated and used for characterization. Each experiment was performed in triplicate.

2.6. 5-Aza-2′-deoxycytidine (5-aza-dC) and trichostatin A (TSA) treatment

Cells were seeded at a density of 2×10^6 cells per 100 mm dish and were maintained for 72 h while replacing the culture medium containing 1 μM 5-aza-dC (Sigma, St. Louis, MO) every 24 h. Subse-

Table 2
Nucleotide sequences of the primers and probes.

	Forward primer (5′–3′)	Reverse primer (5′–3′)	Probe (5′–3′)	Annealing temperature (°C)	PCR cycles	Product size (bp)
qRT-PCR						
NDRG2	GAAGATGCAGTGGTGGAATG	TCAGCTTGCCTGGCTGAGT	TTCCTCAAGATGGCTGACTCCGG	60	30	109
B2M[a]	TTTCAGCAAGGACTGGTCTTT	CCAAATGCGGCATCTTCAAAC	CTGAAAAAGATGAGTATGCCTGCCGTGTG	60	30	171
MSP						
Methylation specific primer	GTTTGCGGGAAGTTCGAGTC	CCGCCGACCCGACTAACG		70	35	134
Unmethylation specific primer	GTGGGTTTGTGGGAAGTTTGAGTTG	CCACCCACCAACCCAACTAACA		70	30	142

[a] Nucleotide sequences for *B2M* primers and probe were previously reported by Ogawa et al. [29].

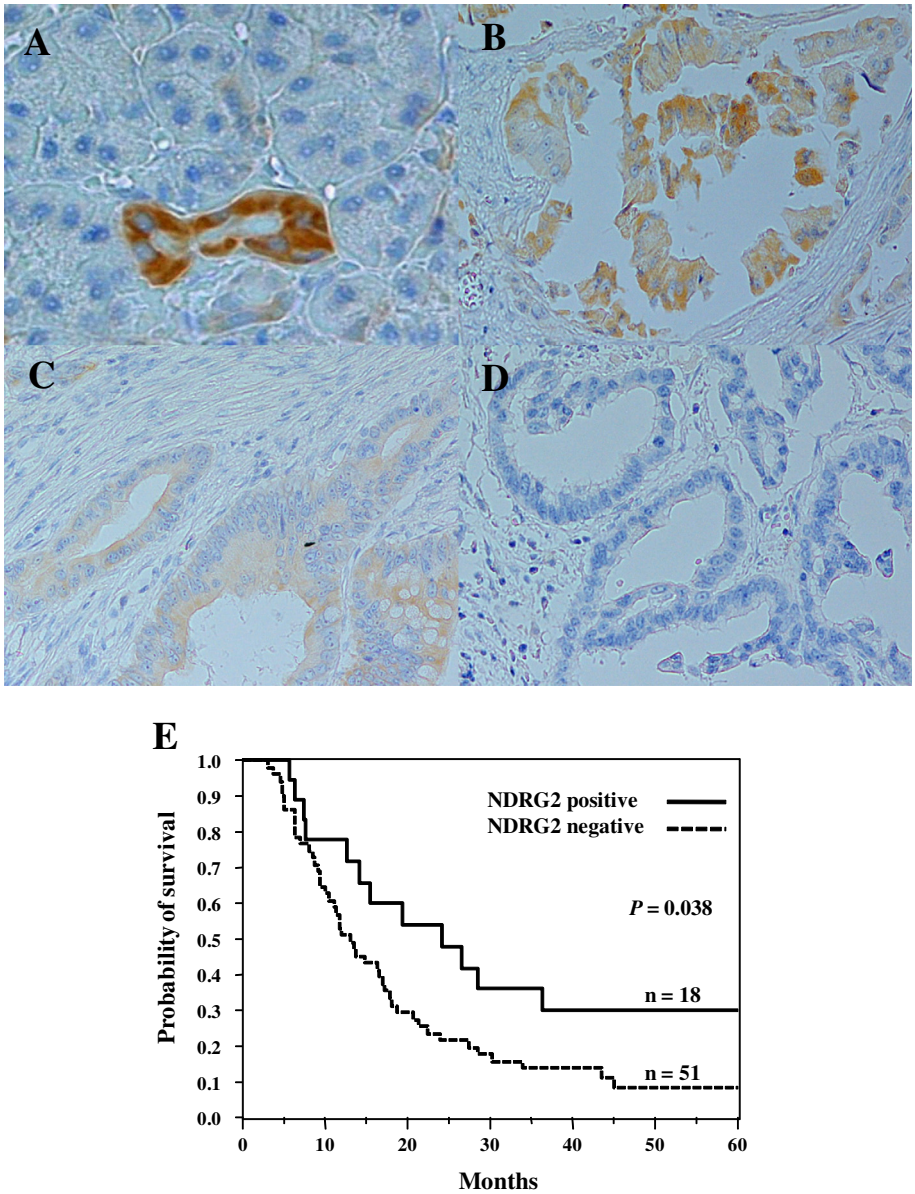

Fig. 1. (A)–(D) Results of representative immmunohistochemical analyses of NDRG2. (A) Strong immunoreactivity was observed in the cytoplasm and plasma membranes of non-neoplastic pancreatic duct (×400 magnification), and pancreatic ductal adenocarcinoma with moderate (B), weak (C), and negative (D) staining (×200 magnification). (**E**) Results of the Kaplan–Meier method indicate a poor overall survival rate of pancreatic cancer patients with negative NDRG2 expression ($P = 0.038$). Solid and dotted lines denote prognoses of NDRG2-positive and -negative patients, respectively.

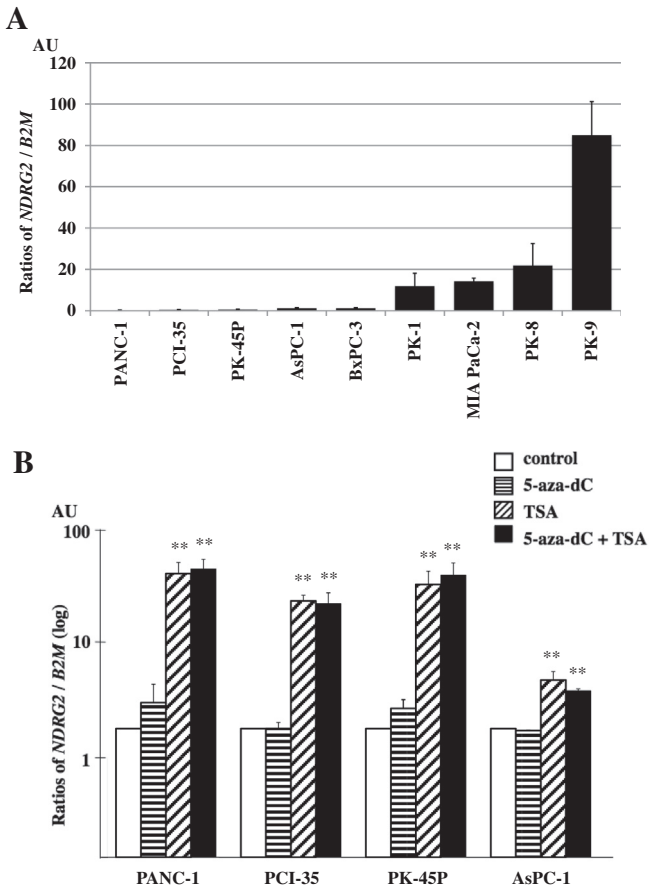

Fig. 2. Expression of *NDRG2* in human pancreatic cancer cell lines by qRT-PCR. Triplicate experiments were done, and the relative expression levels were normalized by the control *B2M* expression (arbitrary units, AU). (A) Results of 9 pancreatic cancer cells are shown. (B) Results of the four low-expressing cells are shown after 5-aza-dC and/or TSA treatments. TSA treatment up-regulated *NDRG2* expression. $^{**}P < 0.01$.

quently, cells were treated with 1 μM 5-aza-dC or 1 μM TSA (Wako, Osaka, Japan) for another 24 h. In TSA only treatment, 2×10^6 cells were plated in a 100 mm dish, and 1 μM TSA was added and cultured for 24 h. All these cells were harvested for qRT-PCR analysis.

2.7. Methylation specific PCR (MSP)

Each aliquot of 2 μg genomic DNA was modified with sodium bisulfite using an Epitect Bisulfite Kit (Qiagen) according to the manufacturer's instructions. The nucleotide sequences of primers for MSP are shown in Table 2. MSP analyses were done by methods described previously [31], and the PCR products were analyzed on 3% agarose gels. Clone A (low *NDRG2* expressing cell line) and LS174T (high *NDRG2* expressing cell line) were used as methylated and unmethylated control cells, respectively.

2.8. Statistical analysis

The Chi-square test was used to examine the correlation between *NDRG2* expression and clinicopathological factors. Survival curves were plotted using the Kaplan–Meier product-limit method, and differences between survival curves were tested using the log-rank test. The gene expression levels before and after 5-aza-dC and/or TSA treatments were analyzed by *t*-test. These statistical analyses were calculated using JMP v9.0 software (SAS Institute Inc., Cary, NC), and results were considered statistically significant when $P < 0.05$.

3. Results

3.1. NDRG2 negative staining correlated with poor prognosis in primary pancreatic cancer

We investigated the expression level of NDRG2 in surgically resected paired cancerous and corresponding normal tissues by immunohistochemical examination. Typical examples are shown in Fig. 1A–D. The spatial distribution of NDRG2 was mainly confined to the cytoplasm and plasma membrane with moderate to strong staining in noncancerous pancreatic ductal cells (Fig. 1A). According to the immunohistochemical results, of the 69 pancreatic cancer specimens examined, one exhibited a moderate NDRG2 expression in tumor cells (Fig. 1B), 17 specimens were weak (Fig. 1C), but no tumor showed stronger NDRG2 expression than normal tissue. The remaining 51 tumors were negative, as shown in Fig. 1D. One moderate and 17 weakly staining tumors were categorized as positive NDRG2 staining (18/69, 26.1%), and 51 tumors (73.9%) were negative. No significant associations were observed in clinicopathological features between positive and negative staining groups (see Table 1). However, the Kaplan–Meier analysis indicated a significant association ($P = 0.038$) between poor prognosis and negative NDRG2 expression in pancreatic cancer patients (Fig. 1E).

3.2. Restoration of NDRG2 expression after 5-aza-dC and/or TSA treatment

To determine whether epigenetic silencing contributes to suppression of the *NDRG2* transcription, we analyzed *in vitro* studies using pancreatic cancer cell lines. The mRNA expressions of *NDRG2* in 9 pancreatic cancer cell lines were determined by

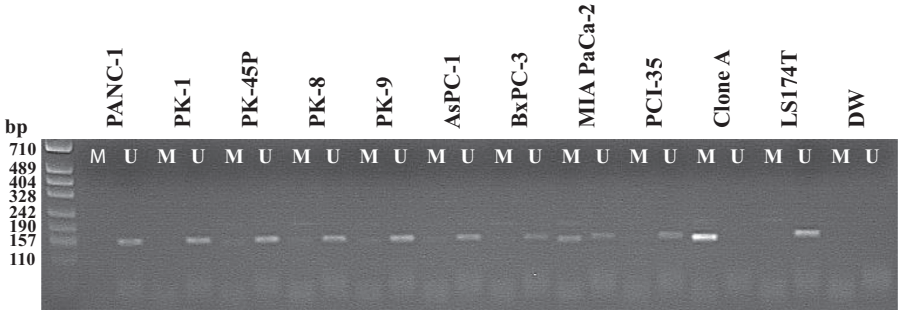

Fig. 3. MSP analyses in pancreatic cancer cell lines. Results of methylation- and unmethylation-specific PCR are indicated by M and U, respectively. All of the cell lines showed unmethylation with one exception: MIA PaCa-2 was partially methylated.

qRT-PCR analyses and found that different cell lines showed different levels of *NDRG2* expression (Fig. 2A). PANC-1, PCI-35, PK45P, AsPC-1 and BxPC-3 showed strong repression, and relatively high expression was observed in PK-9. Results of Western blot analyses correlated well with those of qRT-PCR (data not shown). We selected four pancreatic cancer cell lines (PANC-1, PCI-35, PK45P and AsPC-1) with strong repression of NDRG2 for further analyses. These cells were treated with a demethylating agent, 5-aza-dC, and/or a histone deacetylase (HDAC) inhibitor, TSA; results are shown in Fig 2B. Although no increased *NDRG2* expression was observed after the 5-aza-dC treatment alone, TSA treatment significantly up-regulated *NDRG2* expression. These results suggest that histone modification is one of the main causes for the decreased *NDRG2* expression.

3.3. Promoter hypermethylation was not involved in the decreased NDRG2 expression

It has been reported that hypermethylation is one of the main cause of suppressed expression of *NDRG2* in glioblastoma [7], meningioma [8], hepatocellular carcinoma [9], colorectal cancer [13] and oral squamous-cell carcinoma [16]. We also analyzed other types of cancer cell lines using bisulfite modified sequencing analyses and found that colon cancer cell lines Clone A and LS174T showed methylated and unmethylated CpG islands, respectively (data not shown). Using these cell lines as controls, we studied the methylation status in pancreatic cancer. All the pancreatic cancer cell lines were unmethylated except for MIA PaCa-2, which was partially methylated (Fig. 3). We further analyzed MSP using paired resected normal and cancerous pancreatic tissues from 22 pancreatic cancer patients. As expected, all of the specimens showed the unmethylated pattern, although one was partially methylated (data not shown). These results suggest that the transcriptional repression of *NDRG2* does not mainly depend on hypermethylation.

4. Discussion

Pancreatic cancer is a highly malignant gastrointestinal tumor. Only surgery with adjuvant chemotherapy can achieve a long-term perspective in patients with localized tumors. However, even under optimal treatment conditions, the 5-year survival rate do not exceed 25% [32]. To improve that situation, investigation of new therapeutic agents for pancreatic cancer treatment is essential.

Recently, an accumulation of evidence has indicated that the *NDRG2* gene downregulated in various cancers. In pancreatic cancer, however, little evidence has been reported [10], and no study on an association with prognosis has been reported to date. We demonstrated that NDRG2 expression was significantly reduced and found a significant association between poor prognosis and suppressed expression in pancreatic cancer. As there were no differences, including chemotherapeutic status, between the NDRG2 positive and negative groups, the expression of NDRG2 is likely to be an independent prognostic factor in pancreatic cancer.

We found that histone modification is one of the main mechanisms for downregulating the *NDRG2* expression in pancreatic cancer, and no such mechanisms have previously been reported to control *NDRG2* expression. Histone modification has emerged as a critical component of an epigenetic indexing system demarcating transcriptionally active chromatin domains. In general, while increased histone acetylation is associated with open and active chromatin and increased transcription, deacetylated histones are associated with condensed chromatin and transcriptional repression [33]. Histone deacethylases (HDACs) remove acethyl groups from histones, thereby inducing chromatin condensation and transcriptional repression [34]. Eighteen HDACs have been identified in humans, and they are subdivided into four classes based on their homology to yeast HDACs, their subcellular localization and their enzymatic activities [35]. In pancreatic cancer, high HDAC I expression together with HIF1α were associated with poor prognosis in a series of 39 pancreatic carcinomas [36]. Class I- and class II-selective HDAC inhibitors both synergize in inducing growth arrest and death of pancreatic cells [37]. Other research has also increased our understanding of HDAC function in pancreatic cancer [38]. At least 12 different HDAC inhibitors are undergoing clinical trials as monotherapies or in combination with other adjuvant therapies such as retinoic acid, paclitaxel, gemcitabine, or radiation in patients with various hematologic and solid tumors of the lung, breast, kidney, or bladder as well as with melanoma, glioblastoma, leukemia, lymphomas, and multiple myeloma [39,40]. In pancreatic cancer, promising results have been shown using suberoylanilindehydroxamic acid (SAHA), butyrate, and some other HDAC inhibitors in experimental studies [38,41]. TSA induced G2 arrest and apoptosis in human pancreatic cancer cell lines with mutated TP53 by induction of CDKN1A [42]. Synergistic enhancement of the cytotoxicity of TSA with proteasome inhibitor has also been reported [43]. Our present results that *NDRG2* expression is suppressed mainly by histone-mediated mechanisms and that suppression of *NDRG2* correlates with poor prognosis may provide some valuable clues for the clinical management of patients with pancreatic cancer utilizing a HDAC inhibitor.

The present study indicates that *NDRG2* is likely to be a tumor suppressor gene, reinforcing the data previously reported. In addition, we have demonstrated that inactivation of *NDRG2* associates with poor prognosis in pancreatic cancer. Furthermore, we conclude that epigenetic silencing, such as histone modification, might be the major cause of the frequent loss of NDRG2 expression. Further studies elucidating NDRG2 function will provide a unique and powerful tool for developing novel and useful applications for diagnosis and treatment of patients with pancreatic cancer.

Acknowledgments

We are grateful to Dr. Barbara Lee Smith Pierce (University of Maryland University College) for editorial work in the preparation of this manuscript, to Naomi Kanai, Emiko Kondo, Emiko Shibuya, Midori Chiba, and Keiko Inabe for excellent technical assistance, and to Biomedical Research Core (Tohoku University School of Medicine) for technical support. This work was supported in part by Grants-in-Aid (Grant #17015003, 22591512, and 23590452) and by the Academic Frontier Project for Private Universities: matching fund subsidy 2006–2010 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Grant-in-Aid for Cancer Research (Grant #18–19) from the Ministry of Health, Labour and Welfare of Japan, Pancreas Research Foundation of Japan, and Gonryo Medical Foundation.

References

[1] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, CA Cancer J. Clin. 62 (2012) (2012) 10–29.

[2] M. Ducreux, V. Boige, D. Goere, E. Deutsch, P. Ezra, D. Elias, D. Malka, The multidisciplinary management of gastrointestinal cancer. Pancreatic cancer: from pathogenesis to cure, Best Pract. Res. Clin. Gastroenterol. 21 (2007) 997–1014.

[3] S. Boulkroun, M. Fay, M.C. Zennaro, B. Escoubet, F. Jaisser, M. Blot-Chabaud, N. Farman, N. Courtois-Coutry, Characterization of rat NDRG2 (N-Myc downstream regulated gene 2), a novel early mineralocorticoid-specific induced gene, J. Biol. Chem. 277 (2002) 31506–31515.

[4] Y. Deng, L. Yao, L. Chau, S.S. Ng, Y. Peng, X. Liu, W.S. Au, J. Wang, F. Li, S. Ji, H. Han, X. Nie, Q. Li, H.F. Kung, S.Y. Leung, M.C. Lin, N-Myc downstream-regulated gene 2 (NDRG2) inhibits glioblastoma cell proliferation, Int. J. Cancer 106 (2003) 342–347.

[5] X. Qu, Y. Zhai, H. Wei, C. Zhang, G. Xing, Y. Yu, F. He, Characterization and expression of three novel differentiation-related genes belong to the human NDRG gene family, Mol. Cell. Biochem. 229 (2002) 35–44.
[6] V. Melotte, X. Qu, M. Ongenaert, W. van Criekinge, A.P. de Bruine, H.S. Baldwin, M. van Engeland, The N-myc downstream regulated gene (NDRG) family: Diverse functions, multiple applications, FASEB J. 24 (2010) 4153–4166.
[7] M. Tepel, P. Roerig, M. Wolter, D.H. Gutmann, A. Perry, G. Reifenberger, M.J. Riemenschneider, Frequent promoter hypermethylation and transcriptional downregulation of the NDRG2 gene at 14q11.2 in primary glioblastoma, Int. J. Cancer 123 (2008) 2080–2086.
[8] E.A. Lusis, M.A. Watson, M.R. Chicoine, M. Lyman, P. Roerig, G. Reifenberger, D.H. Gutmann, A. Perry, Integrative genomic analysis identifies NDRG2 as a candidate tumor suppressor gene frequently inactivated in clinically aggressive meningioma, Cancer Res. 65 (2005) 7121–7126.
[9] D.C. Lee, Y.K. Kang, W.H. Kim, Y.J. Jang, D.J. Kim, I.Y. Park, B.H. Sohn, H.A. Sohn, H.G. Lee, J.S. Lim, J.W. Kim, E.Y. Song, D.M. Kim, M.N. Lee, G.T. Oh, S.J. Kim, K.C. Park, H.S. Yoo, J.Y. Choi, Y.I. Yeom, Functional and clinical evidence for NDRG2 as a candidate suppressor of liver cancer metastasis, Cancer Res. 68 (2008) 4210–4220.
[10] X.L. Hu, X.P. Liu, S.X. Lin, Y.C. Deng, N. Liu, X. Li, L.B. Yao, NDRG2 expression and mutation in human liver and pancreatic cancers, World J. Gastroenterol. 10 (2004) 3518–3521.
[11] H. Shi, N. Li, S. Li, C. Chen, W. Wang, C. Xu, J. Zhang, H. Jin, H. Zhang, H. Zhao, W. Song, Q. Feng, X. Feng, X. Shen, L. Yao, Q. Zhao, Expression of NDRG2 in esophageal squamous cell carcinoma, Cancer Sci. 101 (2010) 1292–1299.
[12] S.C. Choi, S.R. Yoon, Y.P. Park, E.Y. Song, J.W. Kim, W.H. Kim, Y. Yang, J.S. Lim, H.G. Lee, Expression of NDRG2 is related to tumor progression and survival of gastric cancer patients through Fas-mediated cell death, Exp. Mol. Med. 39 (2007) 705–714.
[13] A. Piepoli, R. Cotugno, G. Merla, A. Gentile, B. Augello, M. Quitadamo, A. Merla, A. Panza, M. Carella, R. Maglietta, A. D'Addabbo, N. Ancona, S. Fusilli, F. Perri, A. Andriulli, Promoter methylation correlates with reduced NDRG2 expression in advanced colon tumour, BMC Med. Genomics 2 (2009) 11.
[14] H. Shi, H. Jin, D. Chu, W. Wang, J. Zhang, C. Chen, C. Xu, D. Fan, L. Yao, Suppression of N-myc downstream-regulated gene 2 is associated with induction of Myc in colorectal cancer and correlates closely with differentiation, Biol. Pharm. Bull. 32 (2009) 968–975.
[15] H. Zhao, J. Zhang, J. Lu, X. He, C. Chen, X. Li, L. Gong, G. Bao, Q. Fu, S. Chen, W. Lin, H. Shi, J. Ma, X. Liu, Q. Ma, L. Yao, Reduced expression of N-Myc downstream-regulated gene 2 in human thyroid cancer, BMC Cancer 8 (2008) 303.
[16] H. Furuta, Y. Kondo, S. Nakahata, M. Hamasaki, S. Sakoda, K. Morishita, NDRG2 is a candidate tumor-suppressor for oral squamous-cell carcinoma, Biochem. Biophys. Res. Commun. 391 (2010) 1785–1791.
[17] L. Gao, G.J. Wu, X.W. Liu, R. Zhang, L. Yu, G. Zhang, F. Liu, C.G. Yu, J.L. Yuan, H. Wang, L.B. Yao, Suppression of invasion and metastasis of prostate cancer cells by overexpression of NDRG2 gene, Cancer Lett. 310 (2011) 94–100.
[18] S.P. Song, S.B. Zhang, R. Liu, L. Yao, Y.Q. Hao, M.M. Liao, Y.D. Zhang, Z.H. Li, NDRG2 down-regulation and CD24 up-regulation promote tumor aggravation and poor survival in patients with gallbladder carcinoma, Med. Oncol. 29 (2012) 1879–1885.
[19] M.P. Tschan, D. Shan, J. Laedrach, M. Eyholzer, E.O. Leibundgut, G.M. Baerlocher, A. Tobler, D. Stroka, M.F. Fey, NDRG1/2 expression is inhibited in primary acute myeloid leukemia, Leuk. Res. 34 (2010) 393–398.
[20] S.J. Li, W.Y. Wang, B. Li, B. Chen, B. Zhang, X. Wang, C.S. Chen, Q.C. Zhao, H. Shi, L. Yao, Expression of NDRG2 in human lung cancer and its correlation with prognosis, Med. Oncol. 30 (2013) 421.
[21] N. Liu, L. Wang, X. Li, Q. Yang, X. Liu, J. Zhang, Y. Wu, S. Ji, Y. Zhang, A. Yang, H. Han, L. Yao, N-Myc downstream-regulated gene 2 is involved in p53-mediated apoptosis, Nucleic Acids Res. 36 (2008) 5335–5349.
[22] A. Kim, M.J. Kim, Y. Yang, J.W. Kim, Y.I. Yeom, J.S. Lim, Suppression of NF-kappaB activity by NDRG2 expression attenuates the invasive potential of highly malignant tumor cells, Carcinogenesis 30 (2009) 927–936.
[23] N. Liu, L. Wang, X. Liu, Q. Yang, J. Zhang, W. Zhang, Y. Wu, L. Shen, Y. Zhang, A. Yang, H. Han, L. Yao, Promoter methylation, mutation, and genomic deletion are involved in the decreased NDRG2 expression levels in several cancer cell lines, Biochem. Biophys. Res. Commun. 358 (2007) 164–169.
[24] J. Zhang, F. Li, X. Liu, L. Shen, J. Liu, J. Su, W. Zhang, Y. Deng, L. Wang, N. Liu, W. Han, S. Ji, A. Yang, H. Han, L. Yao, The repression of human differentiation-related gene NDRG2 expression by Myc via Miz-1-dependent interaction with the NDRG2 core promoter, J. Biol. Chem. 281 (2006) 39159–39168.
[25] International Union Against Cancer (UICC), TNM Classification of Malignant Tumor, sixth ed., Wiley-Liss, New York, 2002.
[26] T. Furukawa, M. Sunamura, F. Motoi, S. Matsuno, A. Horii, Potential tumor suppressive pathway involving DUSP6/MKP-3 in pancreatic cancer, Am. J. Pathol. 162 (2003) 1807–1815.
[27] H. Sasaki, K. Miura, A. Horii, N. Kaneko, W. Fujibuchi, L. Kiseleva, Z. Gu, Y. Murata, H. Karasawa, T. Mizoi, T. Kobayashi, M. Kinouchi, S. Ohnuma, N. Yazaki, M. Unno, I. Sasaki, Orthotopic implantation mouse model and cDNA microarray analysis indicates several genes potentially involved in lymph node metastasis of colorectal cancer, Cancer Sci. 99 (2008) 711–719.
[28] T. Tabata, N. Tsukamoto, A.A. Fooladi, S. Yamanaka, T. Furukawa, M. Ishida, D. Sato, Z. Gu, H. Nagase, S. Egawa, M. Sunamura, A. Horii, RNA interference targeting against S100A4 suppresses cell growth and motility and induces apoptosis in human pancreatic cancer cells, Biochem. Biophys. Res. Commun. 390 (2009) 475–480.
[29] K. Ogawa, K. Shiga, S. Saijo, T. Ogawa, N. Kimura, A. Horii, A novel G106D alteration of the SDHD gene in a pedigree with familial paraganglioma, Am. J. Med. Genet. A. 140 (2006) 2441–2446.
[30] N. Kaneko, K. Miura, Z. Gu, H. Karasawa, S. Ohnuma, H. Sasaki, N. Tsukamoto, S. Yokoyama, A. Yamamura, H. Nagase, C. Shibata, I. Sasaki, A. Horii, SiRNA-mediated knockdown against CDCA1 and KNTC2, both frequently overexpressed in colorectal and gastric cancers, suppresses cell proliferation and induces apoptosis, Biochem. Biophys. Res. Commun. 390 (2009) 1235–1240.
[31] M. Sato, Y. Mori, A. Sakurada, S. Fujimura, A. Horii, The H-cadherin (CDH13) gene is inactivated in human lung cancer, Hum. Genet. 103 (1998) 96–101.
[32] J.P. Neoptolemos, D.D. Stocken, C. Bassi, P. Ghaneh, D. Cunningham, D. Goldstein, R. Padbury, M.J. Moore, S. Gallinger, C. Mariette, M.N. Wente, J.R. Izbicki, H. Friess, M.M. Lerch, C. Dervenis, A. Olah, G. Butturini, R. Doi, P.A. Lind, D. Smith, J.W. Valle, D.H. Palmer, J.A. Buckels, J. Thompson, C.J. McKay, C.L. Rawcliffe, M.W. Buchler, European study group for pancreatic, ADJUVANT chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial, JAMA 304 (2010) 1073–1081.
[33] K.N. Bhalla, Epigenetic and chromatin modifiers as targeted therapy of hematologic malignancies, J. Clin. Oncol. 23 (2005) 3971–3993.
[34] M. Haberland, R.L. Montgomery, E.N. Olson, The many roles of histone deacetylases in development and physiology: implications for disease and therapy, Nat. Rev. Genet. 10 (2009) 32–42.
[35] S. Thiagalingam, K.H. Cheng, H.J. Lee, N. Mineva, A. Thiagalingam, J.F. Ponte, Histone deacetylases: unique players in shaping the epigenetic histone code, Ann. N.Y. Acad. Sci. 983 (2003) 84–100.
[36] K. Miyake, T. Yoshizumi, S. Imura, K. Sugimoto, E. Batmunkh, H. Kanemura, Y. Morine, M. Shimada, Expression of hypoxia-inducible factor-1alpha, histone deacetylase 1, and metastasis-associated protein 1 in pancreatic carcinoma: correlation with poor prognosis with possible regulation, Pancreas 36 (2008) e1–e9.
[37] G. Wang, J. He, J. Zhao, W. Yun, C. Xie, J.W. Taub, A. Azmi, R.M. Mohammad, Y. Dong, W. Kong, Y. Guo, Y. Ge, Class I and Class II histone deacetylases are potential therapeutic targets for treating pancreatic cancer, PLoS ONE 7 (2012) e52095.
[38] G. Schneider, O.H. Kramer, P. Fritsche, S. Schuler, R.M. Schmid, D. Saur, Targeting histone deacetylases in pancreatic ductal adenocarcinoma, J. Cell Mol. Med. 14 (2010) 1255–1263.
[39] M. Dokmanovic, C. Clarke, P.A. Marks, Histone deacetylase inhibitors: overview and perspectives, Mol. Cancer Res. 5 (2007) 981–989.
[40] M. New, H. Olzscha, N.B. La Thangue, HDAC inhibitor-based therapies: can we interpret the code?, Mol Oncol. 6 (2012) 637–656.
[41] D.I. Dovzhanskiy, S.M. Arnold, T. Hackert, I. Oehme, O. Witt, K. Felix, N. Giese, J. Werner, Experimental in vivo and in vitro treatment with a new histone deacetylase inhibitor belinostat inhibits the growth of pancreatic cancer, BMC Cancer 12 (2012) 226.
[42] M. Donadelli, C. Costanzo, L. Faggioli, M.T. Scupoli, P.S. Moore, C. Bassi, A. Scarpa, M. Palmieri, Trichostatin A, an inhibitor of histone deacetylases, strongly suppresses growth of pancreatic adenocarcinoma cells, Mol. Carcinog. 38 (2003) 59–69.
[43] J. Bai, A. Demirjian, J. Sui, W. Marasco, M.P. Callery, Histone deacetylase inhibitor trichostatin A and proteasome inhibitor PS-341 synergistically induce apoptosis in pancreatic cancer cells, Biochem. Biophys. Res. Commun. 348 (2006) 1245–1253.