Ann Surg Oncol (2015) 22:S1436–S1443
DOI 10.1245/s10434-015-4418-2

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

The *miR-506*-Induced Epithelial–Mesenchymal Transition is Involved in Poor Prognosis for Patients with Gastric Cancer

Shotaro Sakimura, MD[1,2], Keishi Sugimachi, MD, PhD[1], Junji Kurashige, MD, PhD[1], Masami Ueda, MD[1], Hidenari Hirata, MD[1], Sho Nambara, MD[1], Hisateru Komatsu, MD[1], Tomoko Saito, MD[1], Yuki Takano, MD[1], Ryutaro Uchi, MD[1], Etsuko Sakimura, MD[1], Yoshiaki Shinden, MD[1], Tomohiro Iguchi, MD, PhD[1], Hidetoshi Eguchi, MD, PhD[1], Yugo Oba, MD[2], Sumio Hoka, MD, PhD[2], and Koshi Mimori, MD, PhD[1]

[1]Department of Surgery, Kyushu University Beppu Hospital, Beppu, Japan; [2]Department of Anesthesiology and Critical Care Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

ABSTRACT

Background. MicroRNAs have roles in the regulation of the epithelial–mesenchymal transition (EMT). Findings have shown that *miR-506* inhibits the expression of *SNAI2* and that low expression of *miR-506* is associated with poor prognoses in ovarian and breast cancers. This study investigated the role of *miR-506* in survival and the EMT in patients with gastric cancer.

Methods. In this study, *miR-506* and *SNAI2* mRNA levels were measured in 141 cases of gastric cancer by quantitative reverse transcription polymerase chain reaction, and the protein expressions of SNAI2 and E-cadherin in 39 cases were validated by immunohistochemical analysis. Next, the associations between their expression levels and clinicopathologic factors were evaluated. In addition, cell proliferation, migration, and luciferase activity of the 3′ untranslated region (UTR) of *SNAI2* were analyzed using pre-*miR-506* precursor in two human gastric cancer cell lines.

Results. Low expression of *miR-506* was significantly correlated with poor overall survival in both the univariate analysis ($P = 0.016$) and the multivariate analysis ($P < 0.05$). Low *miR-506* expression was significantly correlated with high *SNAI2* expression ($P = 0.009$) and poorly differentiated type ($P = 0.015$). In vitro, *miR-506* suppressed *SNAI2* expression by binding to its 3′UTR, resulting in increased expression of *E-cadherin* ($P < 0.05$), verified by immunohistochemical analysis. Pre-*miR-506* transfected cells showed significantly suppressed cell proliferation and migration ($P < 0.05$) compared with the control cells.

Conclusions. The EMT was directly suppressed by *miR-506,* and its low expression was an independent prognostic factor in gastric cancer patients. The data indicated that *miR-506* may act as a tumor suppressor and could be a novel therapeutic agent.

Gastric cancer is the fifth most common malignant tumor in the world and the third leading cause of cancer death worldwide. The incidence of gastric cancer and the resulting mortality have decreased worldwide, especially in developed countries, primarily because of better living conditions and improvements in diagnosis and treatment. However, gastric cancer remains a challenge in East Asia, with high incidence and mortality rates persisting.[1,2] Gastric cancer is difficult to cure unless it is found at an early stage because few symptoms are manifested during the early stage, and the disease usually is advanced when the diagnosis is determined.

The epithelial–mesenchymal transition (EMT) is a process through which epithelial cells lose their cell polarity and cell–cell adhesion and gain migratory and invasive properties to become mesenchymal cells.[3] The EMT, reported to play important roles in the progression and metastasis of cancer, is associated with a poor prognosis.[4] E-cadherin is required for the maintenance of cell adhesion, and lack of E-cadherin expression is important for the

Electronic supplementary material The online version of this article (doi:10.1245/s10434-015-4418-2) contains supplementary material, which is available to authorized users.

First Received: 1 July 2014;
Published Online: 24 February 2015

K. Mimori, MD, PhD
e-mail: kmimori@beppu.kyushu-u.ac.jp

EMT. Downregulation of E-cadherin expression due to mutation, deletion, CpG hypermethylation, or SNAI-mediated transcriptional repression of the *CDH-1* gene, which encodes E-cadherin, leads to the EMT in gastric cancer.[5–7]

MicroRNAs (miRNAs) are small noncoding RNAs of 20–25 nucleotides that bind to the 3′ untranslated region (UTR) of multiple-target mRNAs, enhancing their degradation and inhibiting their translation. Reports show an association of MiRNAs with a variety of diseases, including cancer.[8,9] Recent studies have shown that miRNAs regulate not only proliferation, differentiation, and migration,[10] but also the EMT by suppressing EMT-related transcription factors in cancer cells.[11,12]

Peritoneal metastasis, the most frequent pattern of metastasis, has been shown to correlate with poor prognosis in advanced gastric cancer.[13,14] Some studies have shown that the EMT plays a crucial role in the formation of peritoneal metastases by gastric cancer cells.[15,16] Therefore, it is necessary to elucidate the epigenetic mechanisms of the EMT to improve early diagnosis and treatment of peritoneal metastases.

Recent studies have shown that *miR-506* controls the EMT by inhibiting the expression of SNAI2 and PRRX1 and that aberrant low expression of *miR-506* is associated with a poor prognosis in ovarian and breast cancers.[17,18] However, the importance of *miR-506* expression as a prognostic factor for the EMT and peritoneal metastasis has not been studied in gastric cancer to date. Therefore, the current study investigated the role of *miR-506* in the survival of Japanese patients with gastric cancer and analyzed the function of *miR-506* in the EMT in gastric cancer cell lines.

MATERIALS AND METHODS

Patients

This study enrolled 141 patients with gastric cancer who underwent gastrectomy at Kyushu University Beppu Hospital and affiliated hospitals between 1989 and 2009. Written informed consent was obtained from all the patients in accordance with the guidelines approved by the Institutional Research Board. This study was conducted under the supervision of the ethical board of Kyushu University and affiliated hospitals. Detailed information is described in the Supplementary Material.

Cell Lines

The human gastric cancer cell lines MKN7 and MKN45 were obtained from the Riken Bioresource Center (Tsukuba, Japan) and maintained in RPMI 1640 medium containing 10 % fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured at 37 °C in a humidified atmosphere containing 5 % carbon dioxide (CO_2).

Transfection with miR-506 Precursor (pre-miR-506)

Cells were transfected with either pre-*miR-506* or pre-*miR*-negative control (Ambion, Austin, TX, USA) using Lipofectamine RNAiMAX (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

Preparation of RNA for Reverse-Transcription Polymerase Chain Reaction

Total RNA was isolated using a modified acid-guanidine-phenol–chloroform procedure, as described previously.[19] Complementary DNA (cDNA) was synthesized from 8 μg total RNA using random hexamer primers and Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen Life Technologies).

Evaluation of Gene and miRNA Expression in Clinical Samples

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed in a LightCycler 480 instrument (Roche Applied Science, Basel, Switzerland) using a LightCycler 480 Probes Master kit (Roche Applied Science). The detailed protocol and the primer sequences used in this procedure are described in the Supplementary Material.

Construction of Reporter Plasmids and Evaluation of Luciferase Reporter Activity

To construct a luciferase reporter plasmid, most of the length of the *SNAI2* 3′UTR, which contained the *miR-506* binding sites, was subcloned into the pmirGlo Dual-luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA) located 5′ to the firefly luciferase. Nucleotide sequences of the constructed plasmids were confirmed by DNA-sequencing analysis. Detailed information is provided in the Supplementary Material.

Immunoblotting Analysis

Total cellular protein was extracted from MKN7 and MKN45 cells 48 h after transfection with pre-*miR-506*. Total protein (40 μg) was extracted from MKN cells and electroblotted as previously described.[20] Detailed information is provided in the Supplementary Material.

Immunohistochemical Analysis

Levels of E-cadherin and SNAI2 protein expression were measured by immunohistochemical analysis in 39 pathologic tissue samples available from 141 cases analyzed by RT-PCR. Formalin-fixed, paraffin-embedded tissue sections corresponding to the samples used for mRNA expression analysis were analyzed. Detailed information is provided in the Supplementary Material.

Cell Proliferation and Cell Migration Analysis

Cell proliferation was evaluated by MTT assay using a Cell Proliferation Kit 1 (Roche Applied Science) according to the manufacturer's instructions. Migration assays were conducted using the BD Falcon HTS Fluoro Block Insert (BD Biosciences, San Jose, CA, USA). Detailed information is provided in the Supplementary Material.

Statistical Analysis

Continuous variables are expressed as means ± standard deviations. Data were analyzed using JMP 9 software (JMP, Cary, NC, USA). Overall survival rates were calculated according to the Kaplan–Meier method, and the log-rank test was applied to compare the survival curves. Multivariate analysis for the survival was performed on the basis of the Cox proportional hazards model. The relationship between groups was analyzed using the Chi square test and Fisher's test. Continuous variables between two groups were analyzed using Student's *t* test after experiments had been repeated at least three times. A probability level of 0.05 was chosen for statistical significance.

RESULTS

Clinicopathologic Significance of miR-506 Expression in Gastric Cancer

Expression of *miR-506* was examined in 141 tumors by qRT-PCR to investigate the clinical significance of *miR-506* in gastric cancer. Cases were subdivided into two groups [a low-expression group ($n = 85$) and a high-expression group ($n = 56$)] according to the level of *miR-506* expression. These groups were classified using the minimum *P* value approach, which is a comprehensive method for finding the optimal risk separation cutoff point in continuous measurements.[21] Clinicopathologic factors then were analyzed between the two groups (Supplementary Table 1). The group with low *miR-506* expression contained significantly more poorly differentiated grades than the group with high *miR-506* expression ($P = 0.015$). The patients with low *miR-506* expression divided by the median value of *miR-506* also tended to have a lower survival rate than those with high expression ($P = 0.051$, data not shown).

With regard to overall survival, the patients with low *miR-506* expression had a significantly lower survival rate than those with high expression ($P = 0.0160$; Fig. 1a). We also performed subgroup analysis of the patients without peritoneum metastasis ($n = 118$). The patients with low *miR-506* expression also had a significantly lower survival rate in this subgroup ($P = 0.0096$; Fig. 1b). Uni- and multivariate analyses for overall survival showed that *miR-*

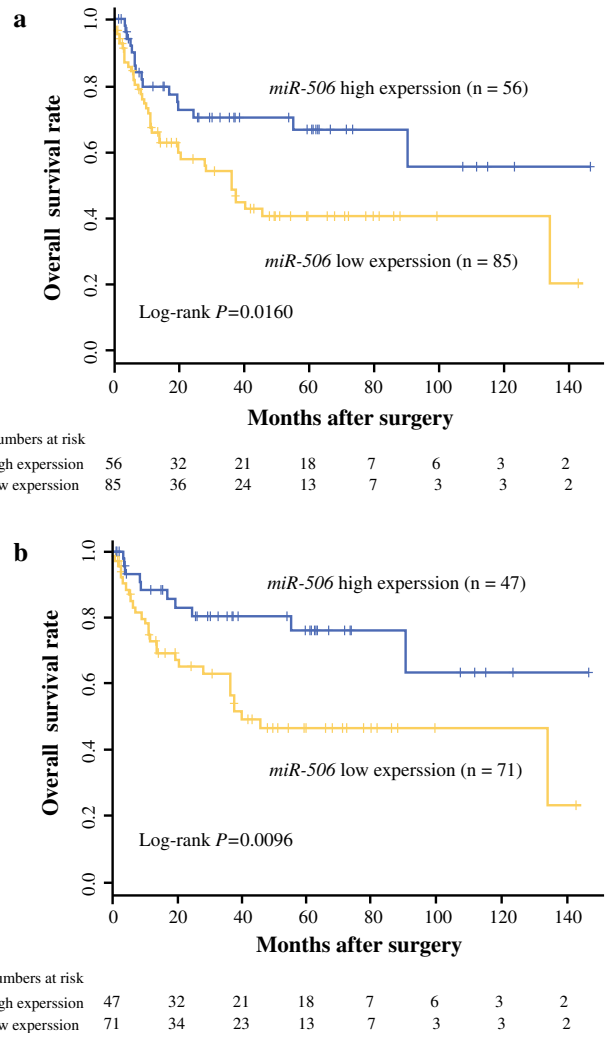

FIG. 1 The low expression of *miR-506* was significantly correlated with poor overall survival. Kaplan–Meier overall survival curves according to *miR-506* expression levels are shown. **a** The overall survival of patients with low *miR-506* expression ($n = 85$) was significantly lower than that of patients with high expression ($n = 56$; $P = 0.0160$, log-rank test). **b** The overall survival of patients with low *miR-506* expression ($n = 71$) was significantly lower than that of patients with high expression ($n = 47$) among the patients without peritoneum metastasis ($P = 0.0096$, log-rank test)

TABLE 1 Uni- and multivariate analyses of clinicopathologic features for overall survival (Cox proportional regression model)

Features	Univariate analysis			Multivariate analysis		
	HR	95 % CI	*P* Value	HR	95 % CI	*P* Value
Age (>70/≤70)	0.626	0.345–1.089	0.099	1.830	1.031–3.384	0.039
Gender (male/female)	1.491	0.844–2.781	0.174	1.964	1.084–3.743	0.025
Histologic grade (well & moderately/poorly & nondifferentiated)[a]	1.647	0.959–2.892	0.071	1.295	0.718–2.375	0.392
Depth of the tumor (T1, 2/T3, 4)	4.957	2.389–12.040	<0.001	2.013	0.742–6.277	0.177
Lymph node metastasis (absent/present)	5.363	2.581–13.038	<0.001	3.078	1.270–8.564	0.011
Venous invasion (absent/present)	3.164	1.859–5.420	<0.001	1.159	0.606–2.215	0.654
Peritoneum metastasis (absent/present)	4.933	2.635–8.928	<0.001	3.281	1.632–6.530	0.001
Stages 1, 2/3, 4	5.429	2.948–10.780	<0.001	–	–	–
miR-506 expression (low/high)	2.017	1.149–3.713	0.014	1.899	1.053–3.588	0.033

Staging was classified by the Union for International Cancer Control (UICC), 7th edition

HR hazard ratio, *CI* confidence interval

[a] Well (well-differentiated adenocarcinoma, moderately (moderately differentiated adenocarcinoma), poorly (poorly differentiated adenocarcinoma, nondifferentiated (nondifferentiated adenocarcinoma)

506 expression was an independent and significant prognostic factor (relative risk 1.78; 95 % confidence interval, 1.00–3.30; $P = 0.049$; Table 1).

Regulation of SNAI2 Expression by miR-506 in Gastric Cancer

We next explored the potential target genes of *miR-506* in gastric cancer. Using TargetScan, an online tool available at http://www.targetscan.org/vert_50/, we identified a potential *miR-506* binding site in the 3′UTR of the transcript encoding SNAI2 (Supplementary Fig. 1). A luciferase reporter assay was conducted for direct investigation of binding and repression between *miR-506* and *SNAI2*. Transient cotransfection of MKN7 and MKN45 cells with the reporter plasmid and pre-*miR-506* significantly reduced luciferase activity compared with the negative control cells ($P < 0.05$; Fig. 2a). These data indicated that *SNAI2* mRNA is a direct functional target of *miR-506* in gastric cancer.

Endogenous miR-506 expression then was measured in three gastric cancer cell lines. Cell lines with high endogenous miR-506 expression (MKN-1 and NUGC-3) showed significantly lower SNAI2 expression than cells with low endogenous miR-506 expression (MKN-7) (Supplementary Fig. 2). Next, we sought to confirm that *miR-506* mediated the expression of *SNAI2* in two gastric cancer cell lines (MKN7 and MKN45) using pre-*miR-506*. We confirmed that *miR-506* expression in cells transfected with pre-*miR-506* was significantly higher than in cells transfected with pre-*miR*-negative control using qRT-PCR ($P < 0.05$; Supplementary Fig. 3). Moreover, *SNAI2* expression was significantly suppressed in MKN7 cells exposed to pre-*miR-506* ($P < 0.05$; Fig. 2b). In MKN45 cells transfected with pre-*miR-506*, the expression level of *SNAI2* tended to be suppressed, but this difference was not statistically significant due to the low basal expression of this target (Fig. 2b).

To investigate the function of *miR-506* in the EMT, we examined the expression level of the epithelial marker, *E-cadherin,* after overexpression of *miR-506* in gastric cancer cells. As shown in Fig. 2c, MKN7 cells transfected with pre-*miR-506* exhibited significantly increased expression of E-cadherin mRNA and protein.

Moreover, in clinical samples, the group with high *miR-506* mRNA expression exhibited significantly lower *SNAI2* expression than the group with low *miR-506* expression ($P = 0.009$; Fig. 2d). We explored the protein expression of SNAI2 in 39 gastric cancer patients. Immunohistochemical analysis showed that SNAI2 protein expression inversely correlated with *miR-506* mRNA expression, and the correlation was statistically significant ($P = 0.006$, Supplementary Table 2). Expression of SNAI2 was inversely correlated with E-cadherin expression in identical lesions of resected gastric cancer samples, as shown in Fig. 3. These data directly demonstrated that *miR-506* controlled the expression of *SNAI2* through binding to its 3′UTR and induced the EMT in gastric cancer.

Suppression of Cell Proliferation and Migration in Gastric Cancer by miR-506

Next, we evaluated the role of *miR-506* in determining the malignant potential of gastric cancer. Proliferation assays were conducted with gastric cancer cells transfected with pre-*miR-506* and with negative control cells. The

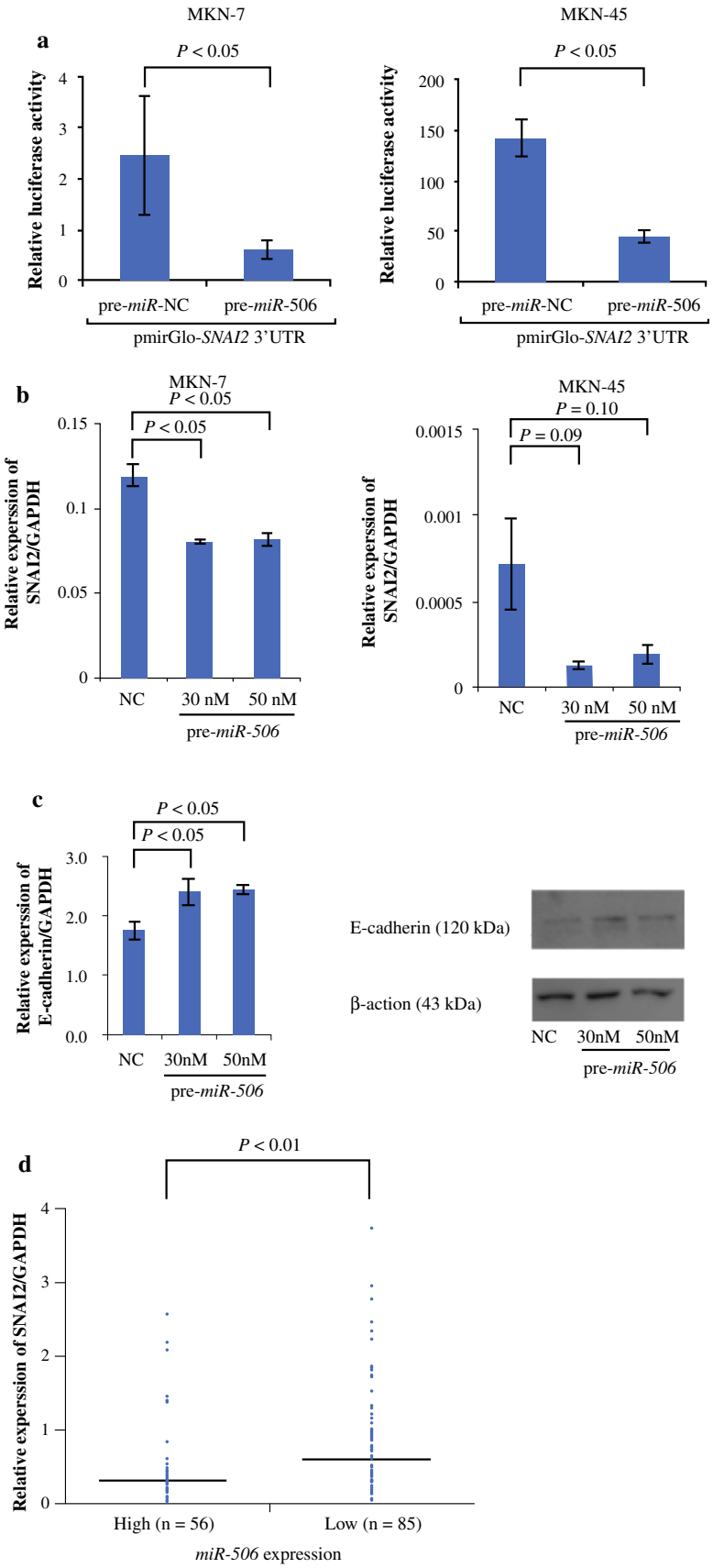

FIG. 2 In gastric cancer cell lines and samples from patients with gastric cancer, *miR-506* suppressed the expression of *SNAI2* and subsequently increased the expression of *E-cadherin*. **a** Luciferase assays demonstrated that *miR-506* repressed its target in MKN7 cells (*left*) and MKN45 cells (*right*) ($P < 0.05$). Relative luciferase level = [(sample Luc/sample Renilla)/(control Luc/control Renilla)]. *Luc,* raw firefly luciferase activity; *Renilla,* internal transfection control Renilla activity. The error bar represents the standard deviation (SD) from three replicates. **b** Expression of *SNAI2/GAPDH* as measured by quantitative real-time polymerase chain reaction (PCR) analysis in MKN7 cells (*left*) and MKN45 cells (*right*) after transfection with pre-*miR-506*. **c** Expression of E-cadherin transcripts and protein was measured by quantitative real-time PCR analysis and Western blot analysis, respectively, in MKN7 cells transfected with pre-*miR-506*. Protein expression was normalized to the expression of β-actin. **d** The group with high *miR-506* expression exhibited significantly lower *SNAI2* expression than the group with low expression ($P = 0.009$). Error bars represent the SD from three replicates. NC, pre-*miR* negative control; 30 nmol, pre-*miR-506* 30 nmol; 50 nmol, pre-*miR-506* 50 nmol

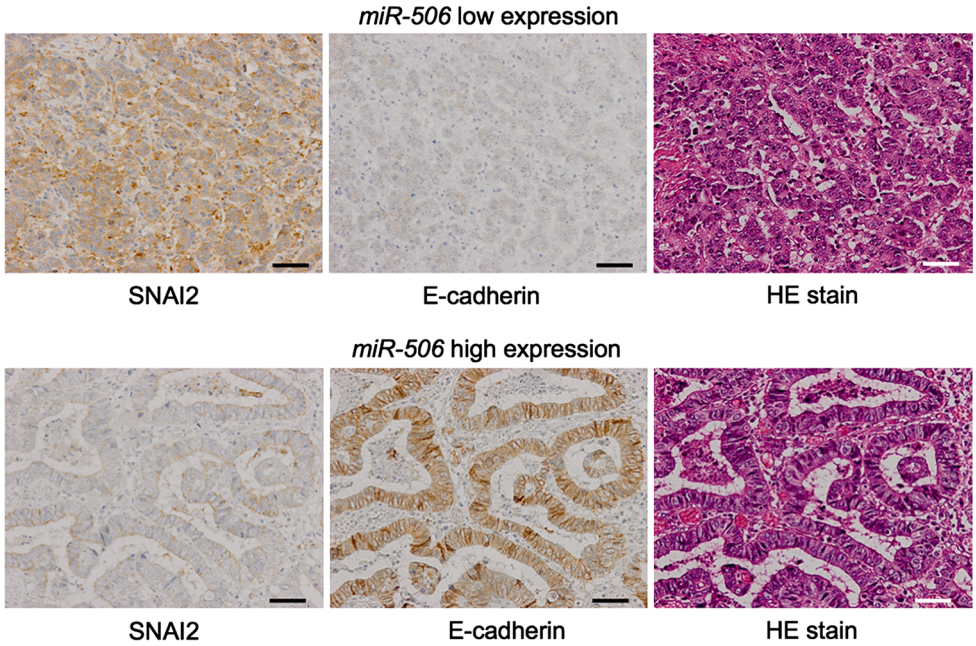

FIG. 3 Immunohistochemical analysis of clinical samples. Clinical samples showed an inverse correlation between the expression of SNAI2 and the expression of E-cadherin, as examined by immunohistochemical staining. *Scale bar*: 50 μm

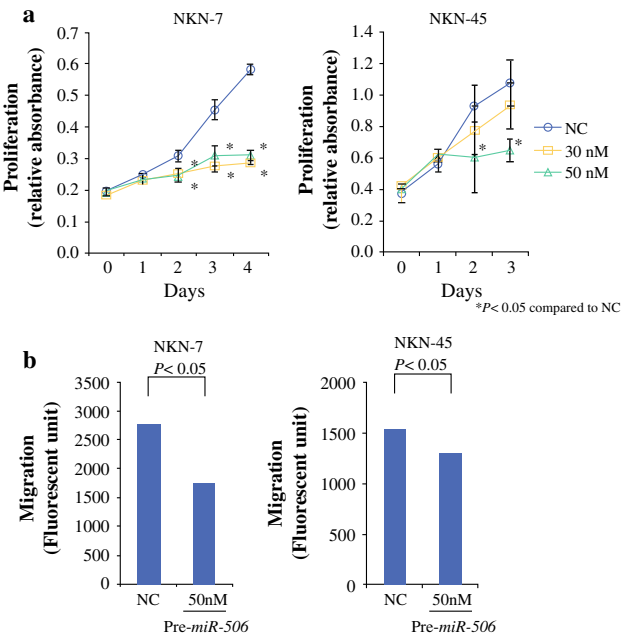

FIG. 4 Overexpression of *miR-506* suppressed the proliferation and migration of gastric cancer cells. As shown, *miR-506* significantly suppressed the **a** proliferation and **b** migratory capacity of gastric cancer cells compared with control cells ($P < 0.05$). Error bars represent the standard deviation from three replicates. NC, pre-*miR* negative control; 30 nmol, pre-*miR-506* 30 nmol; 50 nmol, pre-*miR-506* 50 nmol

findings showed that *miR-506* significantly suppressed the proliferation of both gastric cancer cell lines ($P < 0.05$; Fig. 4a). The expression of *miR-506* also significantly inhibited the migratory capacity of the cells compared with that of control cells ($P < 0.05$; Fig. 4b). These data demonstrated that impaired expression of *miR-506* promoted the malignant potential of gastric cancer.

DISCUSSION

In this study, the expression of *miR-506* was inversely correlated with the expression of *SNAI2* in clinical samples, and gastric cancer cell lines with *miR-506* overexpression exhibited decreased expression of *SNAI2* and increased expression of *E-cadherin*. Moreover, the study also provided direct evidence that *miR-506* suppresses *SNAI2* and that overexpression of *miR-506* significantly suppresses cell migration. This is the first report to show that *miR-506* controls the EMT by inhibiting *SNAI2* in gastric cancer.

Previous studies have shown that the EMT plays an important role in peritoneal metastasis.[15,16] In this study, however, we did not observe a relationship between the expression of *miR-506* and the incidence of peritoneal metastasis. The data indicated that although reduced expression of *miR-506* induces the EMT, other factors besides epigenetics are essential to the formation of peritoneal metastasis.[22,23]

Previous studies have shown that low expression of *miR-506* is associated with a poor prognosis in serous ovarian and breast cancers.[17,18] Consistent with this, our study initially showed that low expression of *miR-506* was an independent prognostic factor in gastric cancer patients. The low expression of *miR-506* was significantly correlated with poorer differentiation and more invasive properties. However, no significant relationships were found between the expression of *miR-506* and lymph node metastasis or

peritoneal metastasis. The group with low *miR-506* expression contained more undifferentiated histopathologic grades, which are characterized by more malignant, mesenchymal-like cells with low *E-cadherin* expression.[24,25] This indicates that *miR-506* is essential to maintain the differentiation of cancer cells and that loss of *miR-506* expression could lead to a poor prognosis.

In addition to its functions in the EMT, recent findings have shown that *miR-506* acts as a tumor suppressor. In lung cancer, ectopic expression of *miR-506* suppresses cell viability to induce the accumulation of reactive oxygen species (ROS),[26] and *miR-506* inhibits tumor growth by targeting the hedgehog pathway transcription factor *Gli3* and the *CDK4/6-FOXM1* axis in cervical cancer and ovarian cancer.[27,28] In our study, overexpression of *miR-506* by transfection with pre-*miR-506* significantly suppressed cell proliferation in gastric cancer cell lines, suggesting that *miR-506* may act as a tumor-suppressor miRNA to prevent gastric cancer progression.

Many miRNAs have been shown to control the EMT in various cancers.[29] For example, the *miR-200* family, *miR-30a*, and others have been shown to control the EMT by repressing the expression of EMT-related target proteins, such as *ZEB* or *Vimentin*.[12,30] It is possible that *miR-506* indirectly controls the EMT by pathways other than the *miR-506-SNAI2* axis. However, only one practical axis exists between *miR-506* and *SNAI2* because other EMT-related genes, such as *SNAI1*, *ZEB1*, and *ZEB2*, do not have binding sites for *miR-506* in their 3′UTRs.

Few studies have described the mechanisms through which *miR-506* expression is controlled in highly malignant cancers. One study showed that nuclear factor-κB (NF-κB) plays an important role in the EMT.[31] Additionally, NF-κB has been shown to suppress the expression of *miR-506* by binding to its promoter region in breast cancer.[18] Interestingly, genomic sequences upstream of *miR-506* may be putative p53-response elements. Consistent with this, p53 has been shown to promote the expression of *miR-506* in lung cancer.[26] In the current study, miR-506 was associated with proliferation and migration ability but was not correlated with invation and *p53* expression (data not shown). Therefore, further study is warranted to investigate another mechanism, such as amplification, deletion, or methylation, that controls the expression of *miR-506*.

In conclusion, this study showed that *miR-506* directly controlled the EMT by regulating *SNAI2* and was an independent prognostic factor in Japanese gastric cancer patients. Moreover, our data supported the conclusion that *miR-506* may act as a tumor suppressor in the context of gastric cancer. Because miRNAs have recently been recognized as potential therapeutic agents or targets in various diseases and because systemic delivery of synthetic miRNA has been shown to inhibit the growth of tumors,[32,33] we hypothesize that *miR-506* could be a potential therapeutic molecule in the treatment of gastric cancer. Further studies investigating the potential applications and significance of *miR-506* are ongoing.

ACKNOWLEDGMENT We thank K. Oda, M. Kasagi, S. Kohno, T. Kawano, and M. Aoyagi for their technical assistance. This work was supported in part by Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research (Grant numbers 21591644, 21791295, 21791297, 215921014, and 21679006).

REFERENCES

1. IARC. GLOBOCAN 2012: Estimated cancer incidence, mortality and prevalence worldwide in 2012. Retrieved 10 January 2014 at http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.
2. Hohenberger P, Gretschel S. Gastic cancer. *Lancet.* 2003; 362:305–15.
3. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial–mesenchymal transitions in development and disease. *Cell.* 2009;139:871–90.
4. Thiery JP. Epithelial–mesenchymal transitions in tumour progression. *Nat Rev Cancer.* 2002;2:442–54.
5. Katoh M. Epithelial–mesenchymal transition in gastric cancer (review). *Int J Oncol.* 2005;27:1677–83.
6. Murai T, Yamada S, Fuchs BC, et al. Epithelial-to-mesenchymal transition predicts prognosis in clinical gastric cancer. *J Surg Oncol.* 2014;109:684–9.
7. Kaurah P, MacMillan A, Boyd N, et al. Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. *J Am Med Assoc.* 2007;297:2360–72.
8. Bhayani MK, Calin GA, Lai SY. Functional relevance of miRNA sequences in human disease. *Mutat Res.* 2012;731:14–9.
9. Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. *Development.* 2005;132: 4653–62.
10. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer.* 2006;6:857–66.
11. Bouyssou JM, Manier S, Huynh D, Issa S, Roccaro AM, Ghobrial IM. Regulation of microRNAs in cancer metastasis. *Biochim Biophys Acta.* 2014;1845:255–65.
12. Song F, Yang D, Liu B, et al. Integrated microRNA network analyses identify a poor prognosis subtype of gastric cancer characterized by the miR-200 family. *Clin Cancer Res.* 2014;20:878–89.
13. Duarte I, Llanos O. Patterns of metastases in intestinal and diffuse types of carcinoma of the stomach. *Hum Pathol.* 1981; 12:237–42.
14. Maehara Y, Moriguchi S, Kakeji Y, et al. Pertinent risk factors and gastric carcinoma with synchronous peritoneal dissemination or liver metastasis. *Surgery.* 1991;110:820–3.
15. Okugawa Y, Inoue Y, Tanaka K, et al. Smad interacting protein 1 (SIP1) is associated with peritoneal carcinomatosis in intestinal type gastric cancer. *Clin Exp Metastasis.* 2013;30:417–29.
16. Okugawa Y, Toiyama Y, Tanaka K, et al. Clinical significance of zinc finger E-box binding homeobox 1 (ZEB1) in human gastric cancer. *J Surg Oncol.* 2012; 106:280–5.
17. Yang D, Sun Y, Hu L, et al. Integrated analyses identify a master microRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. *Cancer Cell.* 2013;23:186–99.
18. Arora H, Qureshi R, Park WY. miR-506 regulates epithelial mesenchymal transition in breast cancer cell lines. *PloS One.* 2013;8:e64273.

19. Mori M, Mimori K, Yoshikawa Y, et al. Analysis of the gene-expression profile regarding the progression of human gastric carcinoma. *Surgery.* 2002;131:S39–47.
20. Ieta K, Ojima E, Tanaka F, et al. Identification of overexpressed genes in hepatocellular carcinoma, with special reference to ubiquitin-conjugating enzyme E2C gene expression. *Int J Cancer.* 2007;121:33–8.
21. Mizuno H, Kitada K, Nakai K, Sarai A. PrognoScan: a new database for meta-analysis of the prognostic value of genes. *BMC Med Genomics.* 2009;2:18.
22. Akagawa S, Ohuchida K, Torata N, et al. Peritoneal myofibroblasts at metastatic foci promote dissemination of pancreatic cancer. *Int J Oncol.* 2014;45:113–20.
23. Miyake S, Kitajima Y, Nakamura J, et al. HIF-1alpha is a crucial factor in the development of peritoneal dissemination via natural metastatic routes in scirrhous gastric cancer. *Int J Oncol.* 2013;43:1431–40.
24. Berx G, Van Roy F. The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression. *Breast Cancer Res.* 2001;3:289–93.
25. Paredes J, Figueiredo J, Albergaria A, et al. Epithelial E- and P-cadherins: role and clinical significance in cancer. *Biochim Biophys Acta.* 2012;1826:297–311.
26. Yin M, Ren X, Zhang X, et al. Selective killing of lung cancer cells by miRNA-506 molecule through inhibiting NF-kappaB p65 to evoke reactive oxygen species generation and p53 activation. *Oncogene.* 2014. doi: 10.1038/onc.2013.597.
27. Wen SY, Lin Y, Yu YQ, et al. miR-506 acts as a tumor suppressor by directly targeting the hedgehog pathway transcription factor Gli3 in human cervical cancer. *Oncogene.* 2014. doi:10.1038/onc.2014.9.
28. Liu G, Sun Y, Ji P, et al. MiR-506 suppresses proliferation and induces senescence by directly targeting the CDK4/6-FOXM1 axis in ovarian cancer. *J Pathol.* 2014;233:308–18.
29. Guo F, Parker Kerrigan BC, Yang D, et al. Posttranscriptional regulatory network of epithelial-to-mesenchymal and mesenchymal-to-epithelial transitions. *J Hematol Oncol.* 2014;7:19.
30. Liu Z, Chen L, Zhang X, et al. RUNX3 regulates vimentin expression via miR-30a during epithelial–mesenchymal transition in gastric cancer cells. *J Cell Mol Med.* 2014;18:610–23.
31. Maier HJ, Schmidt-Strassburger U, Huber MA, Wiedemann EM, Beug H, Wirth T. NF-kappaB promotes epithelial–mesenchymal transition, migration, and invasion of pancreatic carcinoma cells. *Cancer Lett.* 2010;295:214–28.
32. Takeshita F, Patrawala L, Osaki M, et al. Systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. *Mol Ther.* 2010;18:181–7.
33. Lanford RE, Hildebrandt-Eriksen ES, Petri A, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science.* 2010; 327:198–201.