Overexpression of ZDHHC14 promotes migration and invasion of scirrhous type gastric cancer

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Abstract. Scirrhous type gastric cancer is highly aggressive and has a poorer prognosis than many other types of gastric carcinoma, due to its characteristic rapid cancer cell infiltration and proliferation, extensive stromal fibrosis, and frequent peritoneal dissemination. The aim of the present study was to identify novel prognostic markers or therapeutic targets for scirrhous type gastric cancer. We reviewed a list of genes with upregulated expression in scirrhous type gastric cancer and compared their expression with that in normal stomach from our previous *Escherichia coli (E. coli)* ampicillin secretion-trap (CAST) analysis. We focused on the ZDHHC14 gene, which encodes zinc finger, DHHC-type containing 14 protein. qRT-PCR analysis of ZDHHC14 in 41 gastric cancer cases revealed that compared to mRNA levels in normal non-neoplastic gastric mucosa, ZDHHC14 mRNA was overexpressed in 27% of gastric cancer tissue samples. The overexpression of ZDHHC14 was significantly associated with depth of tumor invasion, undifferentiated histology and scirrhous pattern. The invasiveness of ZDHHC14-knockdown HSC-44PE and 44As3 gastric cancer cells was decreased in comparison with that of the negative control siRNA-transfected cells, together with downregulation of MMP-17 mRNA. Integrins α5 and β1 were also downregulated in ZDHHC14-knockdown 44As3 cells. Forced expression of ZDHHC14 activated gastric cancer cell migration and invasion *in vitro*. These results indicate that ZDHHC14 is involved in tumor progression in patients with scirrhous type gastric cancer.

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Introduction

Gastric cancer (GC) remains one of the leading causes of cancer-related mortality worldwide, although therapeutic outcomes for early GC have recently improved (1). Generally, GC can be classified into two histological types: differentiated type and undifferentiated type, according to the Japanese classification of gastric carcinoma (2). Among the undifferentiated type GCs, lesions in scirrhous carcinomas of the stomach display rigid thickening of the gastric wall, leading to a leather bottle-like appearance. Histopathologically, scirrhous cancer cells do not form glands but cause diffuse infiltration. Due to these pathological features, early clinical diagnosis of scirrhous GC remains difficult (3) and it has a poorer prognosis than other types of GC. This reflects the rapid proliferation of these cancer cells, accompanied by progressive invasion and a high frequency of metastasis to the peritoneum (4), all of which distinguish it from differentiated types of GC (5). In fact, there is currently no reliable therapeutic target for this type of GC, thus the need for elucidation remains.

Comprehensive gene expression analysis is a powerful tool in the identification of potential therapeutic targets for GC and to better understand the development of GC at the molecular level. We previously performed several large-scale gene expression studies using array-based hybridization (6) and serial analysis of gene expression (SAGE) (7,8) enabling identification of several genes including regenerating islet-derived family, member 4 (REG4, which encodes REGIV) (9); olfactomedin 4 (OLFM4) (10); palate, lung and nasal epithelium carcinoma-associated protein (PLUNC) (11); and GJB6 (encoding connexin 30) (12). Our recent study of *Escherichia coli (E. coli)* ampicillin secretion trap (CAST) analysis on two scirrhous type GC tissues identified several candidate cancer-specific genes. Among them, transmembrane 9 superfamily member 3 (TM9SF3) expression was associated with tumor progression and was involved in the cancer cell invasion process. Moreover, TM9SF3-positive status in GC significantly correlated with depth of invasion and tumor stage, demonstrated by immunohistochemical analysis (unpublished data).

We analyzed and verified several candidate genes from previous CAST libraries. Among these genes, in the present study, we focused on the zinc finger DHHC domain-containing protein 14 (ZDHHC14) gene expression, due to its frequent overexpression in GC tissue in comparison to expression in corresponding non-neoplastic mucosa, and we analyzed expression levels by qRT-PCR. ZDHHC14 has been reported to be one of the genes overexpressed in acute biphenotypic leukemia through chromosomal translocation (13). However, to our knowledge, the detailed function and expression profiles of the ZDHHC14 gene in the majority of human cancers has not yet been elucidated.

This is the first study of ZDHHC14 expression in human GC; we examined the relationship between ZDHHC14 expression and clinicopathologic characteristics, including tumor stage, TNM grading and histological type. Here, we also clarified the biological significance of ZDHHC14 mRNA expression, via qRT-PCR analysis of surgically resected GC tissues. Furthermore, using an siRNA knockdown system and forced expression vector, the biological role of ZDHHC14 gene in cancer cell adhesion, migration and invasion was examined in GC cell lines.

Materials and methods

Tissue samples. In total, 164 primary tumor samples were collected from patients diagnosed with GC. For quantitative reverse transcription-PCR (RT-PCR) analysis, 41 GC samples and corresponding non-neoplastic mucosa samples were obtained during surgery at the Hiroshima University Hospital, Japan. Informed consent was obtained from all study subjects. The remaining 123 GC samples used for mRNA analysis were obtained from patients who underwent surgery at the Gastroenterological Center, Yokohama City University Medical Center, and at the Department of Surgery, Yokohama City University, Japan, from January 2002 to July 2007. Informed consent was obtained from each participant, and ethics approval was granted by the committee of Yokohama City University Medical Center. Noncancerous samples were purchased from Clontech (Palo Alto, CA, USA). The 164 cases were histologically classified as differentiated type (papillary adenocarcinoma or tubular adenocarcinoma) and undifferentiated type (poorly differentiated adenocarcinoma, signet ring cell carcinoma or mucinous adenocarcinoma), according to the Japanese Classification of Gastric Carcinomas (2). Tumor staging was assessed according to the International Union Against Cancer TNM classification of malignant tumors.

Quantitative RT-PCR and western blot analysis. Quantitative RT-PCR was performed with an ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) as previously described (14). We calculated the ratio of target gene mRNA expression levels between GC tissue (T) and corresponding non-neoplastic mucosa (N). T/N ratios >2-fold were considered to represent overexpression. β-actin (ACTB gene) was used as housekeeping internal control. Western blot analysis was performed as previously described (15). Rabbit polyclonal anti-ZDHHC14 (83260; Abcam) antibody was used for western blotting.

RNA interference (RNAi). For knockdown of endogenous ZDHHC14, RNAi was performed. siRNA oligonucleotides for ZDHHC14 and a negative control were purchased from Invitrogen (Carlsbad, CA, USA). Three independent oligonucleotides were used for ZDHC14 siRNA as follows: ZDHHC14 siRNA1 sequence, 5'-CAAGCCTGATCGACAGAAGAGG GTA-3'; ZDHHC14 siRNA2 sequence, 5'-GACCAGTGCATT CAGAGCACCAAAT-3' and ZDHHC14 siRNA3 sequence, 5'-AAGATGAGCACATGGGCCACCAGTT-3'. Transfection was performed using Lipofectamine RNAiMAX Reagent (Invitrogen) according to the manufacturer's protocol. Briefly, 100 pmol siRNA and 12 μl Lipofectamine RNAiMAX were mixed in 1 ml RPMI medium (10 nmol/l final siRNA concentration). After a 20-min incubation, the mixture was added to the cells and these were plated on dishes for each assay. In all experiments, the cells analyzed were incubated for 48 h after transfection.

Cell lines and in vitro invasion assays. Three cell lines derived from human GC were used. The TMK-1 cell line was established in our laboratory from a poorly differentiated adenocarcinoma (16). HSC-44PE and 44As3 cell lines were established by one of the authors (K.Y., National Cancer Center Hospital East) (3,17). All cell lines were maintained in RPMI-1640 (Nissui Pharmaceutical Co, Ltd, Tokyo, Japan) containing 10% fetal bovine serum (Biowhittaker, Walkersville, MD, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37˚C. The HSC-44PE cells were seeded at a density of 2,000 cells/well in 96-well plates. Cell growth was monitored at day 0, 1, 2 and 4 for the MTT assay, as mentioned elsewhere (18). Modified Boyden chamber assays were carried out to examine invasiveness. Cells were suspended at 200,000 cells/well in RPMI-1640 medium plus 1% serum then plated into the upper chamber of a Matrigel-coated Transwell insert (8 μm pore diameter; Chemicon, Temecula, CA, USA). Medium containing 10% serum was added in the bottom chamber using 24-well plate format. On day 1 and 2, non-invading cells in the upper chamber were removed by clean cotton swab and the cells attached on the lower surface of the insert were stained with Cell Stain (Chemicon). The invading cells were counted by light microscope on day 1 and 2.

Cell migration and adhesion assay. To evaluate cell motility, a wound-healing assay was performed. Negative control and siRNA treated or overexpressing cells were seeded after cell number adjustment performed via automated cell counter (TC10; Bio-Rad). Cells grown to subconfluence were scraped with the sharp edge of the pipette tip, creating a cell-free area. Cells migrating into the scraped area were observed and images were captured every 6 h (19) after mounting cells on fibronectin- and collagen-coated glass slides. The cell adhesion assay was performed by PBS wash at timepoints of 5, 15 and 30 min, and 1, 2 and 3 h post-seeding, in order to examine the remaining cells attached to the glass surface in both control cells and RNAi treated or overexpressed cells. Images were captured using an inverted microscope (Eclipse TE300; Nikon, Japan).

Forced-expression vector construction and generation of stable cell line. For constitutive expression of ZDHHC14 gene,

cDNA was PCR-amplified and subcloned into pcDNA^{TM}3.1(+) vector (V790-20; Invitrogen) in-frame with *Eco*RI and *Xho*I restriction enzymes for directional cloning. PCR primers for cDNA amplification were also designed to include *Eco*RI and *Xho*I sequence. Both transient and stable transfections were carried out with the FuGENE6 Transfection Reagent (Roche Diagnostics). The pcDNA^{TM}3.1(+) vector contains the neomycin resistance gene for selection of stable cell lines. Complete selection of vector construct containing ZDHHC14 or empty vector was performed using G418/neomycin (Geneticin).

Statistical analysis. Correlations between clinicopathologic parameters and ZDHHC14 expression were analyzed by Chi-square test. A P-value of <0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed using JMP software (version 9.0.2; SAS Institute, Carey, NC, USA).

Results

Evaluation of ZDHHC14 mRNA level in GC tissue and corresponding non-neoplastic mucosa. Our previous CAST analysis showed that ZDHHC14 was expressed in the scirrhous type GC tissue CAST library but not in the normal stomach tissue CAST library (unpublished data). To confirm whether the ZDHHC14 gene is cancer-specific, quantitative RT-PCR for ZDHHC14 was performed in 41 GC tissues and their corresponding non-neoplastic gastric mucosa. A ratio of target gene mRNA expression levels between GC tissue (T) and corresponding non-neoplastic mucosa (N) (T/N ratios) of >2-fold was considered to represent overexpression. ZDHHC14 mRNA was overexpressed in 11/41 GC cases (26.8%) (Fig. 1A).

Relationship between high ZDHHC14 expression and clinicopathologic parameters. To analyze the relationship between clinicopathologic parameters and ZDHHC14 expression in GC, we performed qRT-PCR analysis of 123 surgically resected GC tissues (Table I). ZDHHC14 expression was overexpressed in 62/123 (50%) of GCs. ZDHHC14 highly-expressed GC cases showed a significant correlation with the depth of invasion (P=0.0275), and undifferentiated histological subtype (P=0.0169). There was a tendency towards an association between later pathological stage and high ZDHHC14 expression (data not shown). Grouping scirrhous type GC cases and non-scirrhous type GC cases separately revealed a strong correlation between scirrhous type GC and high expression of ZDHHC14 (P=0.0247). There was no significant association between ZDHHC14 expression and other parameters (age, gender, nodal or stage).

Effect of ZDHHC14 downregulation on cell adhesion, motility and invasive activity. To analyze the biological significance of ZDHHC14 protein in GC, we performed siRNA knockdown on this gene, using scirrhous type GC cell lines. Gene silencing in HSC-44PE and 44As3 scirrhous type GC cell lines was confirmed by western blot analysis (Fig. 1B). Very similar western blot results were obtained from both cell lines; therefore Fig. 1C (measuring GC invasiveness) displays results obtained using the 44As3 cell line.

Table I. Relationship between high ZDHHC14 expression and clinicopathologic parameters in 123 GC cases, evaluated by qRT-PCR.

	ZDHHC14 expression (%)		
	High (n=62)	Low (n=61)	P-value
Age (years)			
≤65 (n=35)	13 (37)	22	NS
>65 (n=88)	49 (56)	39	
Gender			
Male (n=87)	42 (48)	45	NS
Female (n=36)	20 (56)	16	
T grade			
T1/T2 (n=39)	14 (36)	25	0.0275
T3/T4 (n=84)	48 (57)	36	
N grade			
N0 (n=50)	23 (46)	27	NS
N1/N2/N3 (n=73)	39 (53)	34	
Stage			
Stage I/II (n=65)	28 (43)	37	NS
Stage III/IV (n=58)	34 (59)	24	
Histology			
Differentiated (n=64)	28 (44)	36	0.0169
Undifferentiated (n=59)	34 (58)	25	
Type			
Scirrhous (n=19)	14 (74)	5	0.0247
Non-scirrhous (n=104)	48 (46)	56	

P-values were calculated by Chi-square test. Histology was determined according to the Japanese Classification of Gastric Carcinomas. NS, not significant.

To determine the possible role of ZDHHC14 in the invasiveness of GC cells, a Transwell invasion assay was performed in the 44As3 GC cell line. Invasion ability was significantly abrogated in ZDHHC14 knockdown GC cells compared to negative control siRNA-transfected GC cells (Fig. 1C). We also investigated the possible proliferative effect of ZDHHC14. We performed an MTT assay 2 days after negative control siRNA and ZDHHC14-siRNA transfection in 44As3 cells; there was no significant difference in proliferation rate between control and knockdown cells (data not shown). Furthermore, we observed that the knockdown cells were abnormally loosely attached to the 96-well plate, leading to our closer analysis of the effect of ZDHHC14 on cancer cell adhesion and migration. We investigated cell adhesion via an assay using fibronectin- and collagen-coated culture slides. In the adhesion assay, ZDHHC14-knockdown cells showed significantly impaired attachment to the culture glass compared to negative control cells (Fig. 1D). This effect was more significant on fibronectin-coated culture glass. Next, we performed a cell migration assay, which showed significantly

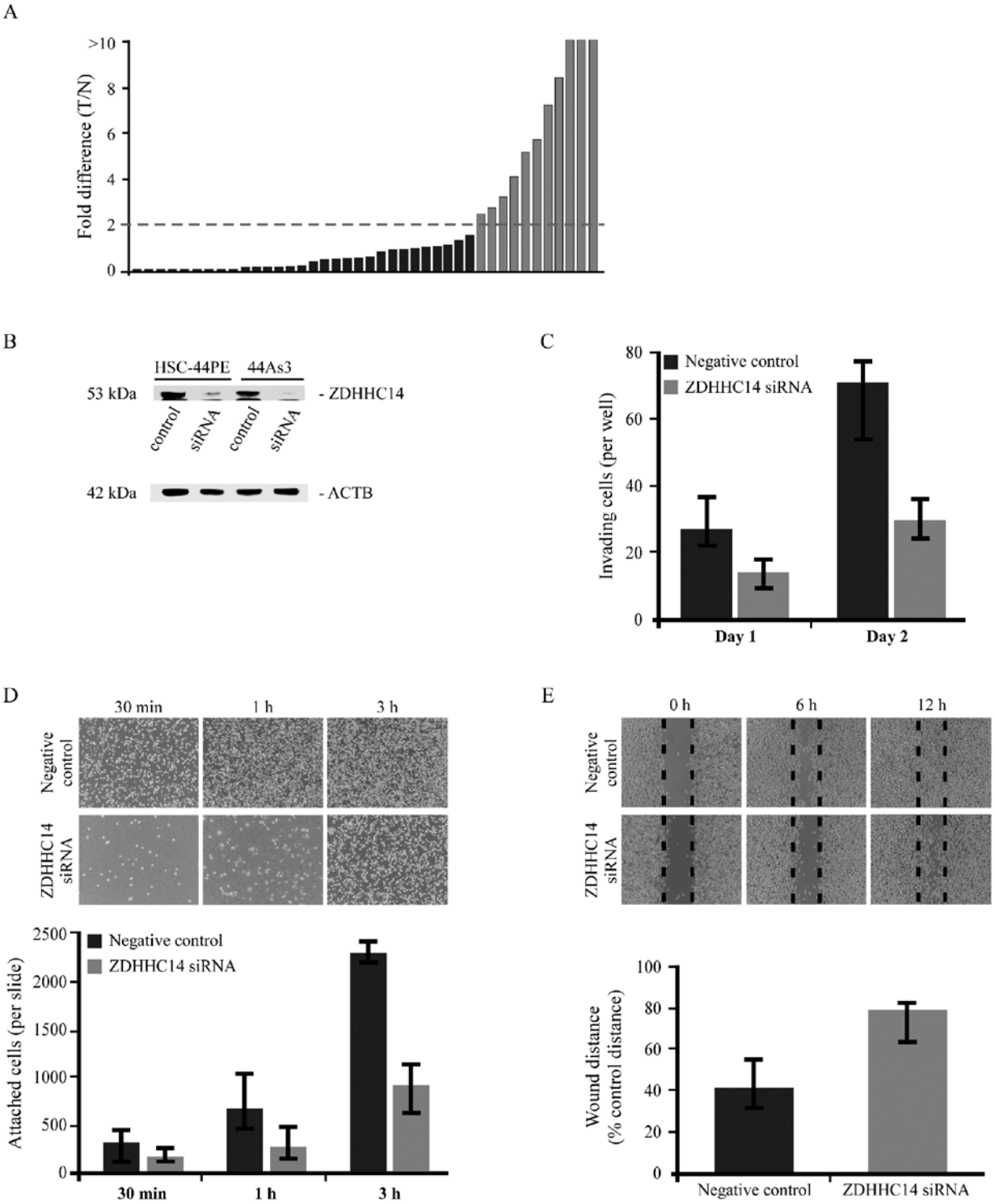

Figure 1. Quantitative RT-PCR analysis of ZDHHC14 and effects of ZDHHC14 RNAi knockdown on HSC-44PE and 44As3 cells. (A) T/N ratio of ZDHHC14 mRNA level between GC tissue (T) and corresponding non-neoplastic mucosa (N) in 41 gastric cancer (GC) cases. (B) Western blot analysis of ZDHHC14 in HSC-44PE and 44As3 GC cells transfected with ZDHHC14 siRNA and negative control siRNA. (C) Effect of ZDHHC14 knockdown on cell invasion in 44As3 GC cells. The cells transfected with ZDHHC14 siRNA or negative control siRNA were incubated in Boyden chambers. After 1 and 2 days, invading cells were counted. (D) ZDHHC14-siRNA transfected 44As3 cells incubated on fibronectin-coated culture glass slides displayed fewer adherent cells than siRNA negative control-transfected cells. Bar graph shows the numbers of attached cells. (E) Effect of ZDHHC14-siRNA knockdown on cell migration of 44As3 cells. By wound healing assay, migration activity was evaluated by wound contraction percentage and closure time at 6 h after scratching. Bar graph shows the distance between wound edges. Bars and error bars show mean and SD, respectively, of three different experiments.

impaired motility in ZDHHC14-knockdown cells compared to negative control cells. Following cell scraping, cells were examined from 0 to 12 h at intervals, until confluent (Fig. 1E).

Alteration of gene expression as a result of ZDHHC14 knockdown. Based on these results, we analyzed the alteration of gene expression in cancer invasion-associated genes and those

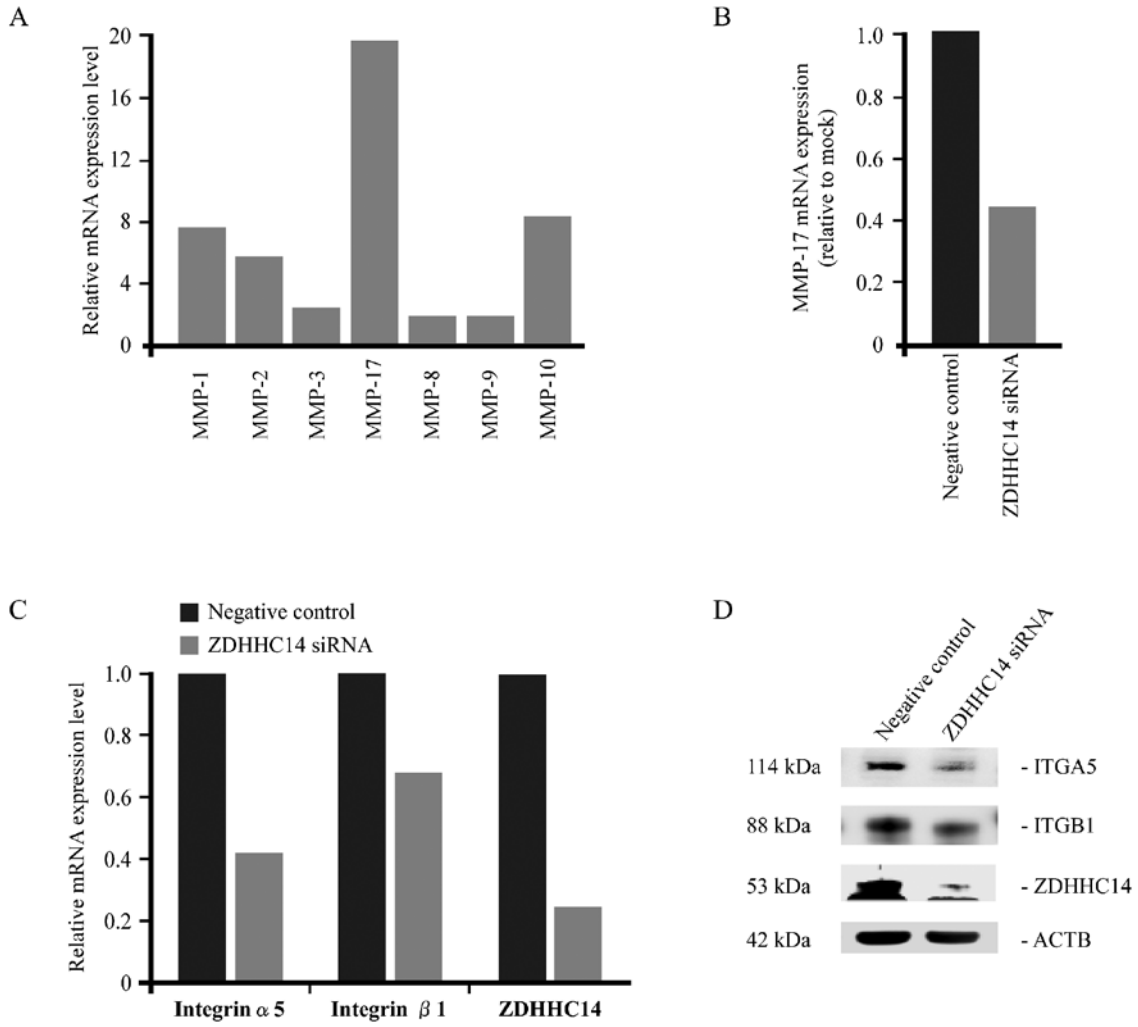

Figure 2. Quantitative RT-PCR analyses of invasion-related proteins and cell adhesion-related proteins. (A) mRNA levels of MMP subunits in 44As3 scirrhous gastric cancer cell line. (B) MMP17 (MT4-MMP) showed downregulation of mRNA in ZDHHC14 siRNA-transfected cells in comparison to negative control cells. (C) mRNA expression of integrin α5 and β1 subunits was reduced following ZDHHC14 RNAi treatment. (D) Western blot analysis of 44As3 cells, confirming changes in integrin α5 and β1 protein expression in siRNA-treated cells.

related with cell attachment after ZDHHC14-siRNA treatment. In semi-quantitative RT-PCR analysis, MT4-MMP (MMP-17) showed the highest expression among all other MMPs after normalization (Fig. 2A). Significant downregulation of MMP-17 expression was observed in ZDHHC14-siRNA transfected 44As3 cells, compared with negative control (mock) transfection (Fig. 2B). We also analyzed the expression of several subunits of integrin α and β by qRT-PCR (Fig. 2C) and by western blot analysis (Fig. 2D); these analyses both showed a downregulation of integrin α5 and integrin β1 expression post-ZDHHC14-siRNA treatment.

Forced expression of ZDHHC14 promotes GC cell migration and invasion. TMK-1 cells were selected for forced-expression experiments from our screening of 8 different GC cell lines, due to their low ZDHHC14 protein expression (data not shown). The TMK-1 GC cell line was stably transfected with pcDNA3.1-ZDHHC14. For the transfection experiments, clones were selected in G418 antibiotic and examined for ZDHHC14 expression by western blotting (Fig. 3A). TMK-1 cells demonstrating stable expression were designated as TMK-1-ZDHHC14-1, TMK-1-ZDHHC14-2 and TMK-1-ZDHHC14-3. Endogenous and exogenous levels of ZDHHC14 protein were also investigated by western blotting with anti-ZDHHC14 antibody to demonstrate the relative overexpression of ZDHHC14. As shown in Fig. 3A, TMK-1-ZDHHC14-1, TMK-1-ZDHHC14-2 and TMK-1-ZDHHC14-3 expressed ZDHHC14 protein at significantly higher levels than TMK-1 cells transfected with empty vector (TMK-1-pcDNA3.1). Next, Boyden chamber invasion assays were performed. ZDHHC14-transfected TMK-1 cells were more invasive than cells transfected with control vector (Fig. 3B). To determine the effect of ZDHHC14 overexpression on cell adhesion and migration *in vitro*, we performed cell adhesion and migration assays. In the adhesion assay, ZDHHC14-overexpressed cells showed significant attachment to culture glass slides compared to empty vector-transfected cells (Fig. 3C). In the cell migration assay, ZDHHC14-overexpressed cells migrated significantly more rapidly compared to empty vector-transfected control cells after cell scraping (Fig. 3D).

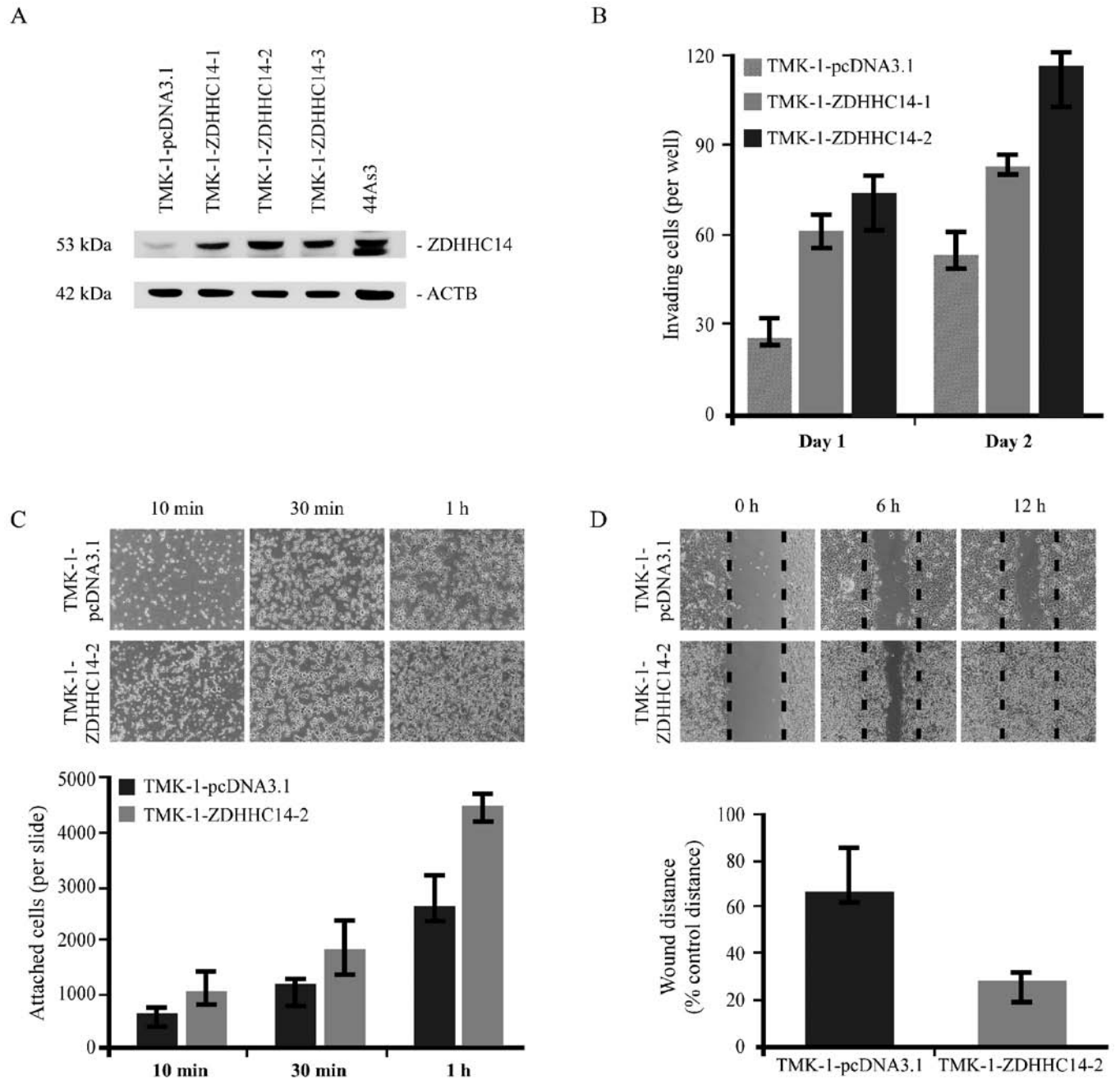

Figure 3. Effects of ZDHHC14 overexpression in TMK-1 cells. (A) Western blot analysis of ZDHHC14 in TMK-1 cell line stably transfected with pcDNA3.1-ZDHHC14 or pcDNA3.1. (B) Effect of ZDHHC14 overexpression on cell invasion. TMK-1 cells transfected with pcDNA3.1-ZDHHC14 or pcDNA3.1 were incubated in Boyden chambers. After 1 and 2 days, invading cells were counted. (C) pcDNA3.1-ZDHHC14 stably transfected TMK-1 cells displayed more adherent cells on fibronectin-coated culture glass slide than empty vector control cells. (D) Effect of ZDHHC14 overexpression on cell migration of TMK-1 cells. By wound healing assay, migration activity was evaluated by wound contraction percentage and closure time at 6 h after scratching. The bar graph shows the distance between wound edges. Bars and error bars show mean and SD, respectively, of three different experiments.

Discussion

In the present study, we focused on the novel target gene ZDHHC14 among genes specifically upregulated in our previously reported scirrhous type GC CAST library, derived from surgically resected GC tissue samples (unpublished data). Our rationale for the in-depth analysis of ZDHHC14 was 2-fold: firstly, quantitative RT-PCR analysis revealed that ZDHHC14 was more frequently overexpressed in GC tissue than in non-neoplastic gastric mucosa. Secondly, the biological role of ZDHHC14 in the majority of human cancers has not been elucidated. We showed that ZDHHC14 expression was associated with undifferentiated type GC, scirrhous type GC variant and greater depth of invasion by qRT-PCR analysis on 123 human GC cases. Forced expression of ZDHHC14 in GC cell lines promoted cancer cell migration and cell attachment *in vitro*, and also stimulated cancer cell invasion. Furthermore, knockdown of ZDHHC14 by RNAi inhibited integrin α5 and β1 expression and exerted effects opposite to those observed in the stably-overexpressing GC cell line. Taken together, these

results suggest that ZDHHC14 constitutes a potential therapeutic target in GC.

ZDHHC14 encodes zinc finger DHHC domain-containing protein 14, also known as DHHC14 protein or NEW1CP. DHHC proteins are evolutionarily conserved genes that can be found in multiple eukaryotes (20). There are 23 human DHHC proteins (DHHC1-DHHC24) encoded by 23 human ZDHHC genes, named ZDHHC1-ZDHHC24 (ZDHHC10 is omitted) (21,22). Besides the DHHC domain, these proteins also contain four or more transmembrane domains. Although the role of DHHC14 remains to be characterized in mammals, its *Drosophila* homolog has recently been confirmed as DHHC palmitoyltransferase, which is involved in the regulation of Fat signaling (23). Protein palmitoylation is a classical and reversible lipid modification that has been shown to regulate protein stability and intracellular localization, in addition to protein-protein and protein-lipid interactions (24,25). To our knowledge, ZDHHC14 was the first palmitoyltransferase discovered to be upregulated in hematological cancer, through the chromosomal translocation in patients with acute biphenotypic leukemia. This suggests the potential regulatory role of ZDHHC14 in cellular differentiation of leukemogenesis (13). However, the biological function and role of ZDHHC14 gene in solid tumors is poorly understood.

Cell migration plays an important role in cancer cell invasion and is generally presumed to be regulated by cell adhesion molecules. For example, integrins bind extracellular matrix (ECM) and link the ECM to intracellular signaling pathways; the turnover of integrin focal contacts may promote cell migration (26,27). It has previously been reported that integrin subunits are the substrates of palmitoylation enzymes (28). Increasing evidence indicates that during progression from tumor growth to metastasis, specific integrin signals enable cancer cells to detach from neighboring cells, re-orientate their polarity during migration, and survive and proliferate in foreign microenvironments (29). In the present study, ZDHHC14 transient knockdown revealed alteration in both mRNA and protein expression of integrin α5 and β1 subunits. Integrin α5β1 regulates the function of integrin αvβ3 on endothelial cells during their migration *in vitro* or angiogenesis *in vivo* (30). However, the mechanism by which ZDHHC14 transcriptionally downregulates these integrin subunits remains unclear. On the other hand, ZDHHC14 knockdown affects MMP-17 expression. Reports that the stem region of MMP-17 contains two cysteine residues of unknown function suggest that these sites might play an important role in regulation of protease function since cysteine residues are known sites for a variety of post-translational modifications including palmitoylation, disulfide bond formation and prenylation (31). ZDHHC14 may therefore have a critical role in cell migration, although the precise molecular mechanism needs to be clarified. The data in the present study verified that ZDHHC14 is associated with cancer progression and invasion of cancer cells by mediating cell adhesion and migration activity. Results of our clinicopathologic parameter analyses in surgically resected GC tissues are consistent with the cell adhesion and migration data.

Collectively, ZDHHC14 is a promising therapeutic target for management of stomach cancer, particularly scirrhous type GC. Identification of the substrates associated with ZDHHC14 via use of strategies such as siRNA knockdown and overexpression in stable GC cell lines will provide further insights into GC pathobiology and open new therapeutic avenues for more effective treatment of GC. Subsequent studies examining the specific substrates of the ZDHHC14 and other novel candidate genes may further elucidate the molecular mechanisms of the genes contributing to GC and its progression.

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References

1. Yasui W: Future perspectives of gastric cancer treatment: from bench to bedside. Pathobiology 78: 293-294, 2011.
2. Japanese Gastric Cancer Association: Japanese classification of gastric carcinoma: 3rd English edition. Gastric Cancer 14: 101-112, 2011.
3. Yanagihara K, Takigahira M, Tanaka H, Komatsu T, Fukumoto H, Koizumi F, Nishio K, Ochiya T, Ino Y and Hirohashi S: Development and biological analysis of peritoneal metastasis mouse models for human scirrhous stomach cancer. Cancer Sci 96: 323-332, 2005.
4. Hippo Y, Yashiro M, Ishii M, Taniguchi H, Tsutsumi S, Hirakawa K, Kodama T and Aburatani H: Differential gene expression profiles of scirrhous gastric cancer cells with high metastatic potential to peritoneum or lymph nodes. Cancer Res 61: 889-895, 2001.
5. Jass JR, Sobin LH and Watanabe H: The World Health Organization's histologic classification of gastrointestinal tumors. A commentary on the second edition. Cancer 66: 2162-2167, 1990.
6. Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H and Brown EL: Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat Biotechnol 14:1675-1680, 1996.
7. Velculescu VE, Zhang L, Vogelstein B and Kinzler KW: Serial analysis of gene expression. Science 270: 484-487, 1995.
8. Oue N, Hamai Y, Mitani Y, Matsumura S, Oshimo Y, Aung PP, Kuraoka K, Nakayama H and Yasui W: Gene expression profile of gastric carcinoma: identification of genes and tags potentially involved in invasion, metastasis, and carcinogenesis by serial analysis of gene expression. Cancer Res 64: 2397-2405, 2004.
9. Mitani Y, Oue N, Matsumura S, Yoshida K, Noguchi T, Ito M, Tanaka S, Kuniyasu H, Kamata N and Yasui W: Reg IV is a serum biomarker for gastric cancer patients and predicts response to 5-fluorouracil-based chemotherapy. Oncogene 26: 4383-4393, 2007.
10. Oue N, Sentani K, Noguchi T, Ohara S, Sakamoto N, Hayashi T, Anami K, Motoshita J, Ito M, Tanaka S, Yoshida K and Yasui W: Serum olfactomedin 4 (GW112, hGC-1) in combination with Reg IV is a highly sensitive biomarker for gastric cancer patients. Int J Cancer 125: 2383-2392, 2009.
11. Sentani K, Oue N, Sakamoto N, Arihiro K, Aoyagi K, Sasaki H and Yasui W: Gene expression profiling with microarray and SAGE identifies PLUNC as a marker for hepatoid adenocarcinoma of the stomach. Mod Pathol 21: 464-475, 2008.
12. Sentani K, Oue N, Sakamoto N, Anami K, Naito Y, Aoyagi K, Sasaki H and Yasui W: Upregulation of connexin 30 in intestinal phenotype gastric cancer and its reduction during tumor progression. Pathobiology 77: 241-248, 2010.

13. Yu L, Reader JC, Chen C, Zhao XF, Ha JS, Lee C, York T, Gojo I, Baer MR and Ning Y: Activation of a novel palmitoyltransferase ZDHHC14 in acute biphenotypic leukemia and subsets of acute myeloid leukemia. Leukemia 25: 367-371, 2011.
14. Kondo T, Oue N, Yoshida K, Mitani Y, Naka K, Nakayama H and Yasui W: Expression of POT1 is associated with tumor stage and telomere length in gastric carcinoma. Cancer Res 64: 523-529, 2004.
15. Yasui W, Ayhan A, Kitadai Y, Nishimura K, Yokozaki H, Ito H and Tahara E: Increased expression of p34cdc2 and its kinase activity in human gastric and colonic carcinomas. Int J Cancer 53: 36-41, 1993.
16. Ochiai A, Yasui W and Tahara E: Growth-promoting effect of gastrin on human gastric carcinoma cell line TMK-1. Jpn J Cancer Res 76: 1064-1071, 1985.
17. Yanagihara K, Tanaka H, Takigahira M, Ino Y, Yamaguchi Y, Toge T, Sugano K and Hirohashi S: Establishment of two cell lines from human gastric scirrhous carcinoma that possess the potential to metastasize spontaneously in nude mice. Cancer Sci 95: 575-582, 2004.
18. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH and Boyd MR: Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 48: 589-601, 1988.
19. Sentani K, Oue N, Naito Y, Sakamoto N, Anami K, Oo HZ, Uraoka N, Aoyagi K, Sasaki H and Yasui W: Upregulation of HOXA10 in gastric cancer with the intestinal mucin phenotype: reduction during tumor progression and favorable prognosis. Carcinogenesis 33: 1081-1088, 2012.
20. Ohno Y, Kihara A, Sano T and Igarashi Y: Intracellular localization and tissue-specific distribution of human and yeast DHHC cysteine-rich domain-containing proteins. Biochim Biophys Acta 1761: 474-483, 2006.
21. Linder ME and Deschenes RJ: Model organisms lead the way to protein palmitoyltransferases. J Cell Sci 117: 521-526, 2004.
22. Mitchell DA, Vasudevan A, Linder ME and Deschenes RJ: Protein palmitoylation by a family of DHHC protein S-acyltransferases. J Lipid Res 47: 1118-1127, 2006.
23. Matakatsu H and Blair SS: The DHHC palmitoyltransferase approximated regulates Fat signaling and Dachs localization and activity. Curr Biol 18: 1390-1395, 2008.
24. Dunphy JT and Linder ME: Signalling functions of protein palmitoylation. Biochim Biophys Acta 1436: 245-261, 1998.
25. Resh MD: Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim Biophys Acta 1451: 1-16, 1999.
26. Hynes RO: Integrins: Versatility, modulation, and signaling in cell adhesion. Cell 69: 11-25, 1992.
27. Moissoglu K and Schwartz MA: Integrin signalling in directed cell migration. Biol Cell 98: 547-555, 2006.
28. Yang X, Kovalenko OV, Tang W, Claas C, Stipp CS and Hemler ME: Palmitoylation supports assembly and function of integrin-tetraspanin complexes. J Cell Biol 167: 1231-1240, 2004.
29. Guo W and Giancotti FG: Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 5: 816-826, 2004.
30. Dormond O and Rüegg C: Regulation of endothelial cell integrin function and angiogenesis by COX-2, cAMP and Protein Kinase A. Thromb Haemost 90: 577-585, 2003.
31. Sohail A, Sun Q, Zhao H, Bernardo MM, Cho JA and Fridman R: MT4-(MMP17) and MT6-MMP (MMP25), a unique set of membrane-anchored matrix metalloproteinases: properties and expression in cancer. Cancer Metastasis Rev 27: 289-302, 2008.