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ORIGINAL ARTICLE – GASTROINTESTINAL ONCOLOGY

Increased Expression of *DNAJC12* is Associated with Aggressive Phenotype of Gastric Cancer

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ABSTRACT

Background. Identification of gastric cancer-related molecules is necessary to elucidate the pathological mechanisms of this heterogeneous disease. The purpose of this study was to identify novel genes associated with aggressive phenotypes of gastric cancer.

Methods. Global expression profiling was conducted using tissues from four patients with metastatic gastric cancer to identify genes overexpressed in gastric cancer. Fifteen gastric cell lines and 262 pairs of surgically resected gastric tissues were subjected to mRNA expression analysis. The contribution of the candidate gene on gastric cancer cell proliferation, invasion, adhesion, and migration were evaluated using small interfering RNA.

Results. DnaJ heat shock protein family (Hsp40) member C12 (*DNAJC12*) was identified as a candidate gene by transcriptome analysis. In clinical samples, *DNAJC12* mRNA levels were higher in gastric cancer tissues compared with normal adjacent tissues. Patients with high *DNAJC12* expression showed significantly shorter overall survival in our cohort and in the extra-validation cohort analyzed by a published microarray dataset. High *DNAJC12* expression in gastric cancer tissues was significantly associated with lymphatic involvement, infiltrative growth type, lymph node metastasis, and advanced stage and was identified as an independent prognostic factor for overall survival in multivariable analysis. Increased expression of *DNAJC12* was found in 12 of 14 examined gastric cancer cell lines. Knockdown of *DNAJC12* expression significantly decreased the proliferation and invasion abilities of gastric cancer cells.

Conclusions. Our findings support *DNAJC12* as a candidate gene associated with aggressive phenotypes of gastric cancer.

Gastric cancer is a major global health problem and ranked as the second leading cause of cancer-related death in the world.[1] Although the development of treatment guidelines for gastric cancer is still under way, the establishment of effective therapy has been elusive because of the limited molecular targets for this disease.[2]

Gastric cancer is a heterogeneous disease both in terms of clinical and molecular features.[3,4] Biomarkers with an accurate predictive performance enable physicians to improve postoperative clinical care.[5,6] Thus, identification of gastric cancer-related molecules is critical to elucidate the underlying pathological mechanisms and will help identify novel biomarkers.

With the purpose to identify novel genes involved in gastric cancer, we conducted transcriptome analysis in gastric cancer samples using a next-generation sequencing platform and identified DnaJ heat shock protein family member C12 (*DNAJC12*) as an overexpressed gene. *DNAJC12* is a member of the heat shock proteins (HSPs), an evolutionary protein family that act as molecular chaperones.[7,8] To date, no studies have demonstrated a role for *DNAJC12* in the pathogenesis of gastric cancer. We conducted expression and functional analyses of *DNAJC12*

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to validate our global expression profiling data. To our knowledge, this is the first study that evaluated the potential role of *DNAJC12* in gastric cancer progression.

MATERIALS AND METHODS

Ethics

This study conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects and has been approved by the Institutional Review Board of Nagoya University, Japan (Approval Number 2014-0043). Written, informed consent for usage of clinical samples and data was obtained from all patients.

Transcriptome Analysis

Surgically resected gastric tissues from four patients with metastatic gastric cancer were subjected to transcriptome analysis. Global expression profiling was conducted using the HiSeq platform (Illumina, San Diego, CA) to compare the expression levels of 57,749 molecules in primary gastric cancer tissues to those of the corresponding noncancerous adjacent gastric mucosa.[9]

Quantitative Real-time Reverse-transcription Polymerase Chain Reaction

Total RNAs (10 μg per sample) were isolated from cells or tissues and used to generate complementary DNAs, which were amplified using specific PCR primers (Supplementary Table 1). Real-time detection of SYBR® Green fluorescence intensity was conducted using the ABI StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). The expression of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA was quantified in each sample for standardization. The quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) reactions in each sample were performed in triplicate. The expression level of each sample is presented as the value of the *DNAJC12* amplicon divided by that of *GAPDH*.[10]

Clinical Samples

Primary gastric cancer tissues and corresponding normal adjacent tissues were collected from 262 patients who underwent gastric resection for gastric cancer without neoadjuvant therapy at the Department of Gastroenterological Surgery, Nagoya University Hospital, between 2001 and 2014. Specimens were classified histologically using the 8th edition of the Union for International Cancer Control (UICC) classification.[11] Relevant clinicopathological parameters were acquired from medical records. Since 2006, adjuvant chemotherapy using S-1 (an oral fluorinated pyrimidine) is administered to all UICC stage II–III patients with gastric cancer, unless contraindicated by the patient's condition.[12,13]

Tissue samples were immediately frozen in liquid nitrogen and stored at − 80 °C. Tumor samples without necrotic areas (approximately 5 mm^2) were extracted by gross observation, and only samples confirmed to comprise more than 80% tumor components by hematoxylin and eosin staining were included in this study. Corresponding normal adjacent gastric mucosa samples > 5 cm from the edge of the tumors were obtained from the same patient.

Evaluation of the Clinical Significance of DNAJC12 Expression

DNAJC12 mRNA levels were determined in 262 matched pairs of resected gastric tissues. For external validation of our data, a freely available integrated dataset comprised of 1065 patients from three major cancer research centers (Berlin, Bethesda and Melbourne datasets) was accessed at http://kmplot.com/analysis/.[14]

Cell Lines

Fourteen gastric cancer cell lines (AGS, GCIY, IM95, KATOIII, MKN1, MKN7, MKN45, MKN74, N87, NUGC2, NUGC3, NUGC4, OCUM1, and SC-6-JCK) were obtained from the American Type Culture Collection (ATCC; Manassas, VA) or the Japanese Collection of Research Bioresources Cell Bank (JCRB; Osaka, Japan). The cell lines were cultured in RPMI-1640 (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum at 37 °C in 5% CO_2. The nontumorigenic epithelial cell line FHs74 (ATCC) was used as a control.

Small, Interfering RNA-Mediated Knockdown of DNAJC12

MKN1 and AGS cells were plated in a 24-well plate (5×10^4 cells/ml). Cells were transiently transfected the next day with 30 nM of small, interfering RNA (siRNA) specific for *DNAJC12* (Supplementary Table 1) or a control siRNA (siControl) combined with LipoTrust EX Oligo (Hokkaido System Science, Sapporo, Japan). After transfection, cells were cultured in serum-free Dulbecco's modified Eagle's medium (Sigma-Aldrich) for 72 h and used in functional assays. To evaluate knockdown effect of *DNAJC12* expression, qRT-PCR analysis and Western blot analysis using a rabbit anti-DNAJC12 polyclonal antibody

(12338-1-AP, ProteinTech Inc., Chicago, IL) diluted 1:1000 were performed as previously described.[15,16]

Cell Proliferation, Invasion, Adhesion, and Migration Assays

Cell proliferation was evaluated using the Cell Counting Kit-8 (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) as previously described.[17] Cell invasion was determined using BioCoat Matrigel invasion chambers (BD Biosciences, Bedford, MA) according to the manufacturer's protocol.[18] The CytoSelect 48-Well Cell Adhesion Assay (Cell Biolabs, Inc., San Diego, CA) was used to determine adherence of the cells to the extracellular matrix components fibronectin, collagen I, collagen IV, laminin I, and fibrinogen[19] Cell migration was evaluated using wound-healing assays, as previously described. The width of the wound was measured at 100-mm intervals (20 measurements per well, 40× magnification).[16]

Statistical Analysis

Relative levels of mRNA expression (*DNAJC12/GAPDH*) between gastric cancer and adjacent normal tissues were analyzed using the Mann–Whitney *U* test. The χ^2 test was used to analyze the significance of the association between the expression and clinicopathological parameters. Goodness of fit was assessed by calculating the area under the curve (AUC) of the receiver-operating characteristic (ROC) curve, and the optimal cutoff value was determined using the Youden index.[15] Disease-specific and disease-free survival rates were calculated using the Kaplan–Meier method, and the difference in survival curves was analyzed using the log-rank test. We performed multivariate regression analysis to detect prognostic factors using the Cox proportional hazards model, and variables with $P < 0.05$ were entered into the final model. All statistical analyses were performed using JMP 10 software (SAS Institute Inc., Cary, NC). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Identification of DNAJC12 as a Candidate Gastric Cancer-Related Gene

We first performed transcriptome analysis on gastric tissues compared with corresponding noncancerous adjacent gastric mucosa from four patients with metastatic gastric cancer. The transcriptome analysis results identified 25 candidate genes that were both (1) overexpressed in gastric cancer compared with the corresponding normal tissues and (2) expressed at comparable expression levels in primary gastric cancer and metastatic tissues (Table 1).

We conducted a literature review on the functions of the identified genes and selected *DNAJC12* for subsequent analyses for the following reasons.

1. Among the 24 identified candidates, *DNAJC12* was one of the 12 most highly expressed genes in primary gastric cancer tissues (approximately 18-fold higher compared with the corresponding noncancerous adjacent gastric mucosa).
2. *DNAJC12* encodes a trafficking protein that may be involved in growth factor signaling and transportation of anticancer drugs through the membrane.
3. Published data on the oncological roles of *DNAJC12* in gastric cancer are lacking.
4. The oligonucleotide sequence information of *DNAJC12* is available (http://www.ncbi.nlm.nih.gov/).

Expression Levels of DNAJC12 mRNA in Surgically Resected Gastric Tissues

We next evaluated *DNAJC12* mRNA levels in primary gastric cancer tissues and corresponding normal adjacent tissues from 262 gastric cancer patients. The patient population included 189 males and 73 females aged from 26 to 96 years (66.6 ± 11.2 years, mean ± standard deviation). Pathologically, 161 and 101 patients were diagnosed with undifferentiated and differentiated gastric cancer, respectively. Based on the 8th edition of the UICC classification, 20, 69, 109, and 64 patients were in stages I, II, III, and IV, respectively, and 198 patients in stages I–III underwent R0 resection.

In 168 (64%) of the 262 gastric cancer patients, *DNAJC12* mRNA expression was increased in gastric cancer tissues compared with the corresponding adjacent normal tissues. The mean expression level of *DNAJC12* mRNA was higher in gastric cancer tissues compared with that of normal adjacent tissues ($P < 0.001$; Fig. 1a).

Prognostic Implications of DNAJC12 mRNA Expression Levels

The optimal cutoff value of *DNAJC12* mRNA levels in gastric cancer tissues for detecting and predicting mortality of recurrence was determined by ROC curve analysis. The AUC value of *DNAJC12* levels was 0.606 for detection of cancer-related mortality within 3 years after surgery, and the optimal cutoff value was 0.00925 (sensitivity 57%, specificity 62%) (Supplementary Fig. 1a). We categorized patients into high (above the cutoff value) or low *DNAJC12* (below the cutoff value) groups and found that the overall survival rate of the high *DNAJC12* group was

TABLE 1 List of genes overexpressed in primary cancerous tissues from patients with metastatic gastric cancer

Function	Symbol	GC/normal		Name	Meta/GC	
		Log_2	*P*		Log_2	*P*
Trafficking protein	DNAJC12	4.15	<0.0001	DnaJ heat shock protein family member C12	− 1.16	0.1038
	RBP4	4.25	<0.0001	Retinol binding protein 4	1.51	0.0515
	SYT7	4.29	<0.0001	Synaptotagmin 7	0.30	0.6281
Transcription factor	FNDC1	4.50	<0.0001	Fibronectin type III domain containing 1	− 0.89	0.1592
	GNG4	4.84	<0.0001	G protein subunit gamma 4	0.29	0.7296
	ELF5	5.00	0.0001	E74 like ETS transcription factor 5	− 0.85	0.3319
	HOXC10	6.49	0.0001	Homeobox C10	1.68	0.0752
Cell membrane receptor	GRB7	3.98	<0.0001	Growth factor receptor bound protein 7	− 0.03	0.9716
	UTS2R	4.50	<0.0001	Urotensin 2 receptor	0.50	0.5675
	TNFRSF11B	4.57	<0.0001	TNF receptor superfamily member 11b	0.53	0.4265
Cell-surface glycoprotein	MELTF	3.27	<0.0001	Melanotransferrin	− 0.19	0.7380
Cellular adhesin	COMP	3.15	0.0003	Cartilage oligomeric matrix protein	0.91	0.1072
	CLDN1	3.27	<0.0001	Claudin 1	0.71	0.1568
	THBS2	3.76	<0.0001	thrombospondin 2	0.20	0.7759
	THBS4	4.01	<0.0001	Thrombospondin 4	0.95	0.2787
Growth factor	INHBA	3.76	<0.0001	Inhibin beta A subunit	− 0.37	0.5028
Mediator of neural transmission	VSNL1	4.04	<0.0001	Visinin like 1	1.09	0.1528
	CPLX2	4.36	0.0007	Complexin 2	1.88	0.2436
	NPY	4.86	<0.0001	Neuropeptide Y	0.09	0.9008
Metabolic enzyme	PADI2	3.01	<0.0001	Peptidyl arginine deiminase 2	− 1.29	0.0758
	KLK10	3.26	0.0003	Kallikrein related peptidase 10	− 0.76	0.2984
	AKR1C4	3.28	0.0009	Aldo–keto reductase family 1 member C4	0.59	0.4064
	PLA2G2A	3.70	<0.0001	Phospholipase A2 group IIA	− 0.43	0.4529
Regulator of cell cycle	CDC25B	3.17	0.0006	Cell division cycle 25B	− 0.66	0.3947
	CCNE1	3.41	<0.0001	Cyclin E1	− 1.06	0.0709

GC primary gastric cancer tissue, *Normal* corresponding adjacent normal gastric tissue, *Meta* hepatic metastasis tissue

significantly lower than that of the low *DNAJC12* group (5-year survival rates, 46% and 71%, respectively, $P < 0.001$; Fig. 1b). A consistent result was observed in the extra-validation cohort analyzed by an integrated microarray dataset comprised of 1065 patients from three major cancer research centers (Berlin, Bethesda and Melbourne datasets) (Fig. 1b).

We next examined the potential correlations between *DNAJC12* expression and patient clinicopathological characteristics (Supplementary Table 2). High *DNAJC12* expression was significantly associated with differentiation, lymphatic involvement, infiltrative growth type, lymph node metastasis, and UICC Stage. Univariate analysis of overall survival showed that carcinoembryonic antigen (CEA) > 5 ng/ml, carbohydrate antigen (CA) 19–9 > 37 IU/ml, tumor size (≥ 50 mm), macroscopic type (Borrmann type 4/5), pT4, undifferentiated tumor, lymphatic involvement, vessel invasion, invasive growth, lymph node metastasis, positive peritoneal lavage cytology, and high *DNAJC12* mRNA expression in gastric cancer tissues were significant prognostic factors of adverse outcomes (Supplementary Table 3). Multivariable analysis identified high *DNAJC12* mRNA expression as one of the independent prognostic factors (hazard ratio 1.7; 95% confidence interval [CI] 1.07–2.72; $P = 0.024$).

Subgroup Analysis in Patients with Stage I–III Gastric Cancer

We next performed subgroup analysis in the patients with Stage I–III gastric cancer to evaluate predictive value of *DNAJC12* expression for disease recurrences after curative gastrectomy. Of the 198 patients with Stage I–III gastric cancer, the overall survival rate was slightly lower in the high *DNAJC12* group ($n = 86$) compared with the low *DNAJC12* group ($n = 112$), but the difference was not significant (5-year survival rates, 67% and 81%, respectively, $P = 0.106$; Fig. 2a). In contrast, the disease-free

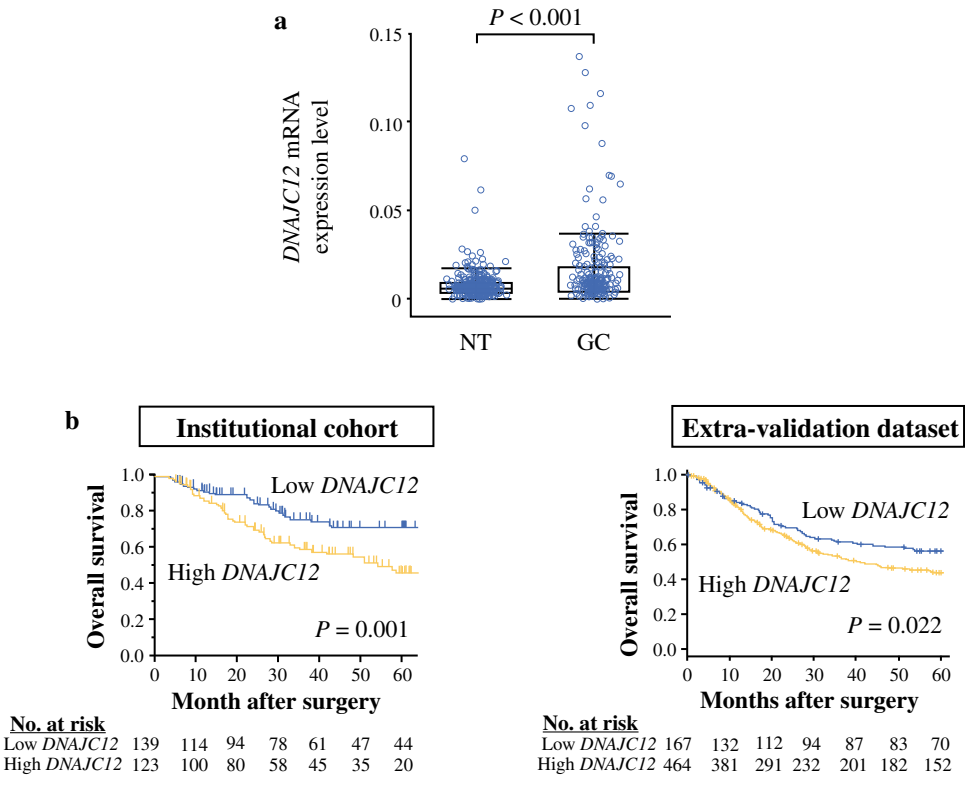

FIG. 1 Expression analyses of *DNAJC12* in clinical samples from 262 gastric cancer patients. **a** Mean *DNAJC12* mRNA levels in gastric cancer (GC) tissues compared with the corresponding adjacent normal tissues (NT). **b** Overall survival time in the current dataset of 262 clinical samples (left) and extra-validation dataset described in the Methods (right), according to high and low *DNAJC12* expression

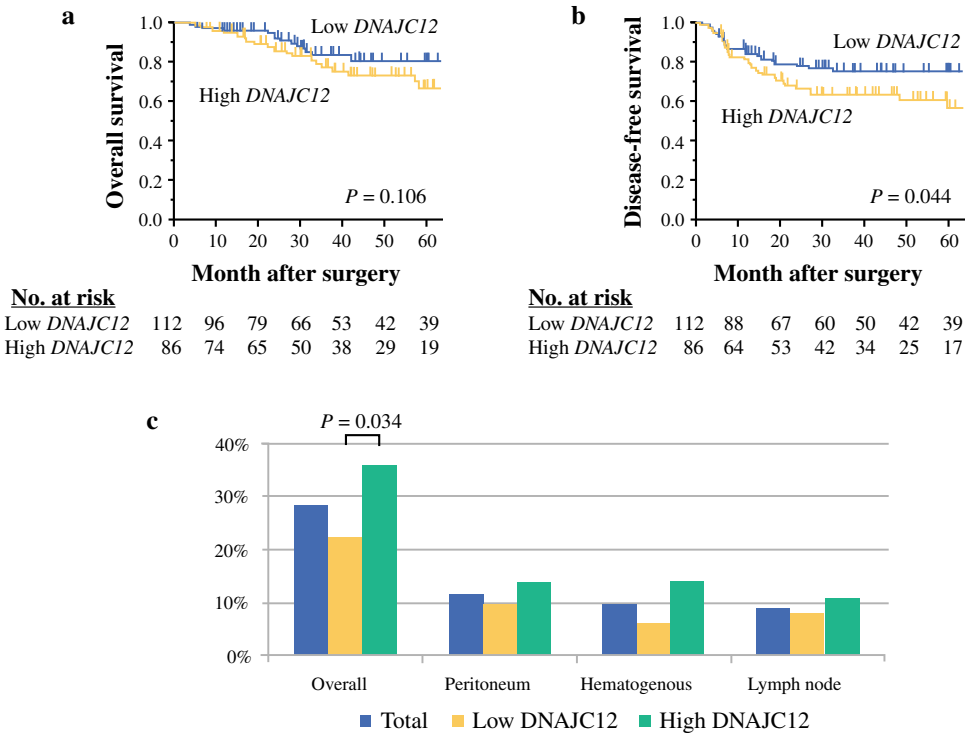

FIG. 2 Subgroup analysis focusing on 198 patients with Stage I–III gastric cancer. Overall (**a**) and disease-free (**b**) survival of patients according to high (n = 86) or low (n = 112) *DNAJC12* expression. **c** Frequencies of the sites of initial recurrence after curative gastrectomy in patients according to *DNAJC12* expression

survival rate was significantly lower in the high *DNAJC12* group than in the low *DNAJC12* group (3-year, disease-free survival rates, 63% and 77%, respectively, P = 0.044; Fig. 2b). The overall incidence of recurrence was significantly higher in the high *DNAJC12* group than that in the low *DNAJC12* group (P = 0.034); however, no significant difference was observed in the first incidence of

peritoneal, hematogenous or lymph node recurrence (Fig. 2c).

Analyses of DNAJC12 mRNA Expression in Gastric Cancer Cell Lines

To characterize *DNAJC12* in gastric cancer, we next evaluated levels of *DNAJC12* mRNA in 14 gastric cancer cell lines compared with control epithelial cells. *DNAJC12* mRNA levels were more than twofold higher in 12 gastric cell lines (AGS, MKN1, N87, IM95, MKN7, NKN45, GCIY, KATO-III, NUGC2, NUGC4, SC-6-JCK, and OCUM1) compared with control FHs74 cells (Supplementary Fig. 1b). *DNAJC12* mRNA levels did not differ according to the extent of differentiation of the gastric cancer cells. We selected AGS and MKN1 cells for subsequent analyses, because these cells exhibited high levels of *DNAJC12* mRNA expression (the third and fourth highest), and these cell lines are easy to use in functional analyses.

Effect of DNAJC12 Knockdown on Biological Activities of Gastric Cancer Cells

To evaluate the function of *DNAJC12* in gastric cancer cells, we knocked down *DNAJC12* in MKN1 and AGS cells using siRNA. After confirming knockdown efficacy of the *DNAJC12* siRNA by qRT-PCR analysis (Fig. 3a) and Western blotting analysis (Fig. 3b), we evaluated cell proliferation of MKN1 and AGS cells with *DNAJC12*

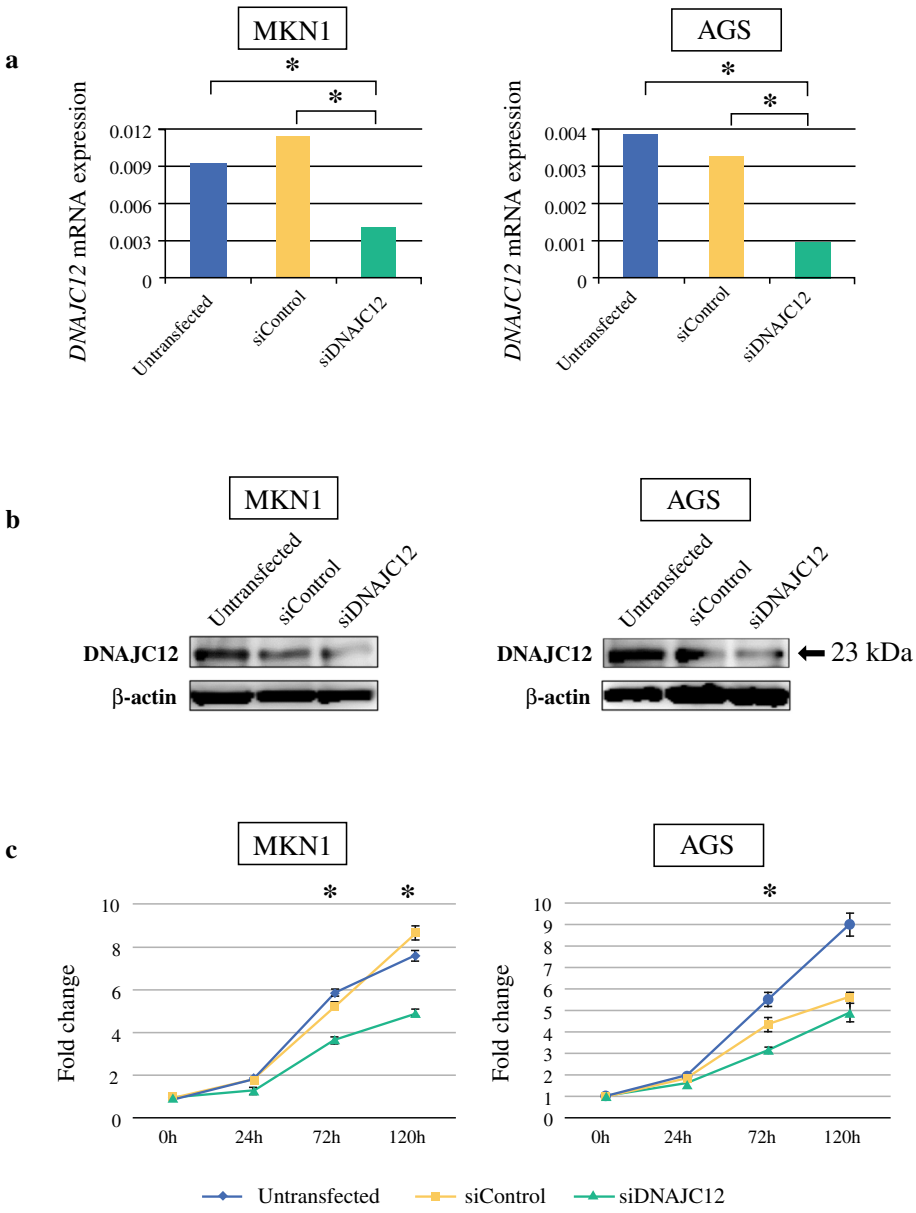

FIG. 3 **a** Confirmation of siRNA-mediated knockdown of *DNAJC12* in MKN1 and AGS cells was determined using qRT-PCR assay. **b** Confirmation of siRNA-mediated knockdown of DNAJC12 protein in MKN1 and AGS cells was determined using western blotting analysis. **c** Cell proliferation of gastric cancer cells with siRNA-mediated knockdown of *DNAJC12*. MKN1 and AGS cells were transfected with *DNAJC12* siRNA, control siRNA, or untreated and cell viability was evaluated at the indicated time points. $^*P < 0.05$. Error bars indicate standard deviation

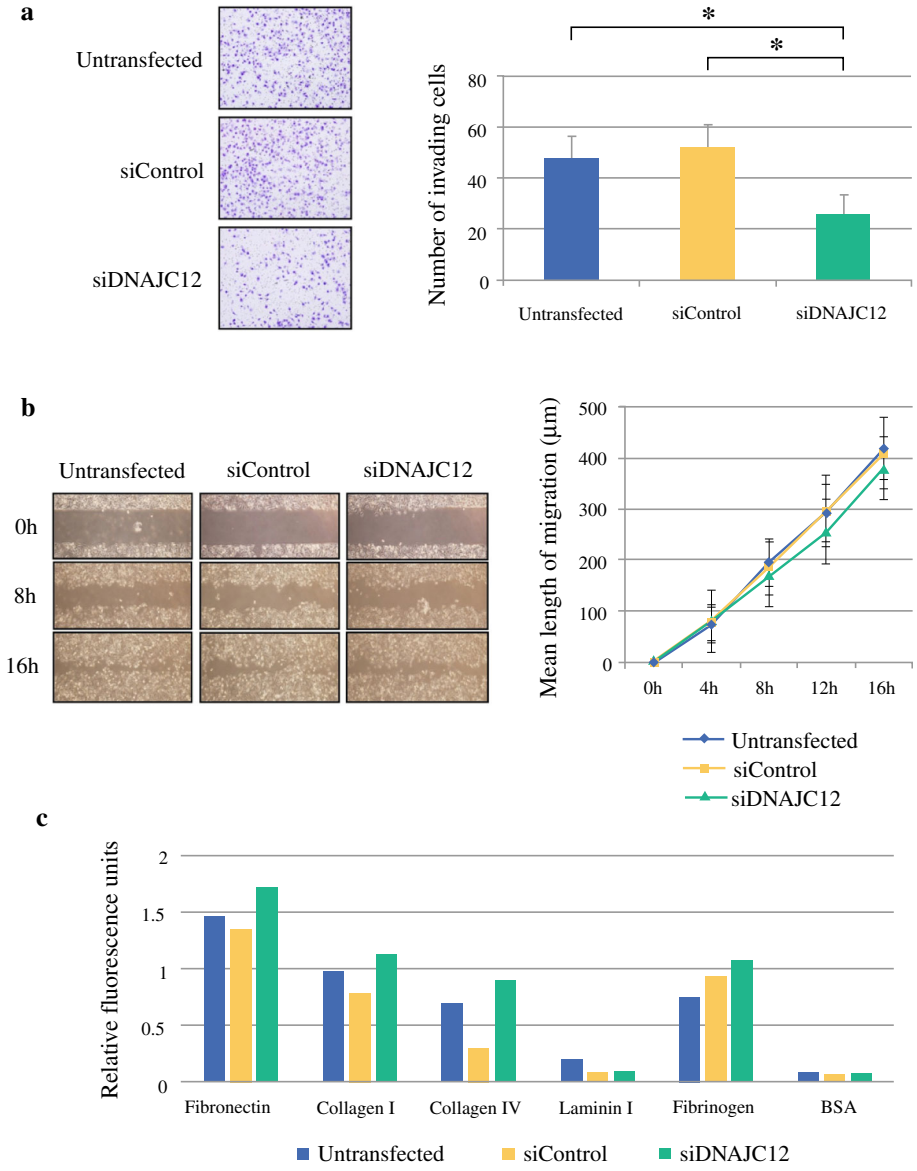

FIG. 4 Effects of siRNA-mediated knockdown of *DNAJC12*. **a** Cell invasion assay. MKN1 cells were transfected with *DNAJC12* siRNA, control siRNA or untreated and cell invasion was evaluated as described in Methods. Top panels show representative images of stained MKN1 cells (× 200 magnification). The bottom graph shows the mean numbers of invading cells in eight randomly selected fields. $^{*}P < 0.05$. Error bars indicate standard deviation. **b** Wound-healing assays in MKN1 cells transfected as indicated. Top panels show representative images from assays at the indicated times. Bottom graph shows mean length of wound healing assay at the indicated times. Error bars indicate standard deviation. **c** Cell adhesion assay. Adhesion to four components of the extracellular matrix (fibronectin, collagen I, collagen IV, and fibrinogen) was not decreased by knockdown of *DNAJC12* expression

knockdown and cell invasiveness, adhesion, and migration of MKN1 cells with *DNAJC12* knockdown. Inhibition of *DNAJC12* expression significantly decreased the proliferation of MKN1 cells at 72 h and 120 h after transfection (Fig. 3c). Similarly, the proliferation of AGS cells was decreased by knockdown of *DNAJC12* expression at 72 h after transfection (Fig. 3c). Furthermore, the invasiveness of MKN1 cells was reduced by inhibiting *DNAJC12* expression compared with the control cells (Fig. 4a). However, the migration ability of MKN1 cells was slightly reduced by inhibiting *DNAJC12* expression (Fig. 4b). In addition, knockdown of *DNAJC12* expression in MKN1 cells had little influence on the adhesive capacity to four components of the extracellular matrix (fibronectin, collagen I, collagen IV, and fibrinogen; Fig. 4c).

DISCUSSION

In the present study, we sought to identify a novel gene associated with aggressive phenotypes of gastric cancer. For this purpose, we conducted a transcriptome analysis and identified DNAJC12 as a candidate biomarker. High *DNAJC12* expression in primary gastric cancer tissues was associated with a shorter survival time and increased recurrence rate. Furthermore, knockdown of *DNAJC12* expression led to attenuated proliferation and invasion abilities of MKN1 cells.

DNAJC12 belongs to HSP family, which constitute a large family of proteins that often are classified based on their molecular weight into HSP10, HSP40, HSP60, HSP70, HSP90, etc.[7,20,21] HSPs are produced by cells

during exposure to stressful conditions and during wound healing or tissue remodeling.[22,23] Selected HSPs perform chaperone function and play crucial roles in folding/unfolding of proteins, assembly of multiprotein complexes, transport/sorting of proteins into correct subcellular compartments, cell-cycle control and signaling, and protection of cells against stress/apoptosis.[24,25] Additionally, HSPs have been implicated in antigen presentation with the role of chaperoning and transferring antigenic peptides to the class I and class II molecules of the major histocompatibility complexes.[23,26] With respect to roles in malignancies, HSPs can exhibit antiapoptotic properties and function in various cancer-related processes, such as in tumor cell proliferation, invasion, metastases, and death.[21,24,25] Notably, several of these proteins are significantly elevated and robustly associated with therapeutic resistance and poor survival in various malignancies.[27,28]

DNAJC12 is ubiquitously expressed in various organs and is reported as responsible for hyperphenylalaninemia.[8,29] Several studies have demonstrated a relationship between *DNAJC12* expression and cancer. High levels of *DNAJC12* expression in breast cancer tissues were associated with estrogen receptor transactivation activity.[30] Moreover, *DNAJC12* overexpression acts as a negative predictive factor for the response to neoadjuvant concurrent chemoradiotherapy and is significantly associated with shorter survival in rectal cancer patients receiving neoadjuvant concurrent chemoradiotherapy followed by surgery.[31] In the current study, we found that tissue *DNAJC12* mRNA levels were higher in gastric cancer tissues compared with normal adjacent tissues, and elevated expression of *DNAJC12* was found in 12 (85%) of 14 gastric cancer cell lines, supporting our hypothesis that *DNAJC12* might play oncogenic roles in gastric cancer based on the transcriptome analysis. Correlations between increased tissue *DNAJC12* expression and shortened overall survival time demonstrated by our institutional dataset and the extra-validation cohort highlighted the significance of *DNAJC12* expression as a prognostic biomarker for gastric cancer.

Our functional analyses revealed that siRNA-mediated inhibition of *DNAJC12* expression attenuated proliferation and invasion abilities of gastric cancer cells. Because dysregulated proliferation and invasion are one of the major contributors to cancer-related growth, progression, and metastasis, our findings indicated that *DNAJC12* plays an important role in the malignant behavior of gastric cancer. In contrast, *DNAJC12* expression had little influence on adhesion and migration of gastric cancer cells. *DNAJC12* belongs to DnaJ heat shock protein family (HPS40), which acts as a cofactor of HSP70.[8,32] Interaction between HSP40 and HSP70 reportedly facilitates hydrolysis of adenosine triphosphate and performs diverse biological functions.[33,34] HSP70, consisted of 17 members, improves overall protein integrity and directly inhibits apoptosis by blocking the recruitment of procaspase-9.[35–37] In recent reports, it has been demonstrated that inhibition of HSP70 attenuated invasion activities of renal cell carcinoma cells and stimulation of HSP70 expression resulted in increased invasion of bladder cancer cells.[34,36] Our data on significant association of *DNAJC12* expression with proliferation and invasion activities of gastric cancer cells might be attributed to interference in oncological functions of HSP70. Further studies, including pathway analyses manly focusing on HSP70 families and apoptotic analyses, are needed to elucidate the underlying molecular mechanisms of *DNAJC12* contributing to these phenotypes.

Several limitations of the analysis should be acknowledged. First, with respect to utility of tissue *DNAJC12* expression as a biomarker of gastric cancer progression, the diagnostic impact was limited, because it demonstrated limited discriminatory ability (AUC value 0.606) in the present study. This study includes a relatively small number of samples and was retrospective in nature. Prospective, observational studies are needed to validate the cutoff and predictive performance of *DNAJC12* expression. Second, further information on biological functions of *DNAJC12* and accumulation of knowledge on interactions between HSPs in malignancies is mandatory. Third, the expression analysis using cell lines provided limited information because of lack of access to additional controls. Lastly, further experiments will be required to clarify the mechanisms of *DNAJC12* in gastric cancer, particularly apoptosis-related analysis to uncover association with HSP70.

This study showed that increased *DNAJC12* expression was associated with an aggressive phenotype of gastric cancer. *DNAJC12* may represent a promising predictor for patient prognosis and a potential target of molecular therapy in gastric cancer.

DISCLOSURES None.

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