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Upregulation of the long non-coding RNA FAM83H-AS1 in gastric cancer and its clinical significance

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ABSTRACT

Objectives: To explore the expression level and to investigate the clinical associations of the long non-coding RNA (lncRNA) FAM83H-AS1 in gastric cancer.
Methods: The expression level of FAM83H-AS1 were explored by quantitative reverse transcription PCR (qRT-PCR). The Cox regression models as well as log-rank test were utilized to investigate whether FAM83H-AS1 expression could be used as a prognosis predictor. The value of FAM83H-AS1 as a diagnostic biomarker was evaluated by receiver operating curves (ROC).
Results: Aberrantly upregulation of FAM83H-AS1 was identified in gastric cancer in comparison with that in normal tissues. We also found that upregulated FAM83H-AS1 was a risk factor relating to OS and DFS. The area under curve (AUC) was 0.8603 and 0.6778 for gastric cancer and lymph node metastasis, respectively.
Conclusion: Our results indicated that FAM83H-AS1 may function as an oncogene in gastric cancer and could be used as a prognosis predictor or diagnostic biomarker in gastric cancer.

1. Introduction

Gastric cancer (GC) represents currently one of the top ranking malignant diseases and the majority health burden worldwide [1–3]. Despite remarkable efforts has been made to classify the GC molecular subtypes and to improve the therapeutic techniques of GC, the 5-year prognosis of GC patients remains lower than 30% [4,5]. In order to improve the overall survival of GC, identification of new mechanisms associated with GC pathogenesis and novel prognosis predictors are of vital importance.

Recent large scale sequencing studies have revealed that the majority of human genomes are comprised of transcripts without protein-coding potential [6,7], of which long non-coding RNAs (lncRNAs) are defined as genes with more than 200 nucleotides in length [8,9]. A growing body of evidence demonstrated that lncRNAs are of vital importance in a wide range of biological processes [10]such as embryonic development [11], chromosome inactivation [12] and cell proliferation [10,13]. Moreover, roles of lncRNAs in malignant transformation have been reported [10,13–16]. For instance, MALAT1 was found to be overexpressed in lung cancer and to predict poor prognosis [17]. Microarray assays indicated that BCAR4 acts as scaffold for transcription factors and promotes metastasis of breast cancer [18]. Loss-of-function models indicated that BCAR4 may be used as therapeutic target in tumor treatment [18]. Zhang et al found that aberrantly overexpressed lncRNA Sox2ot predicted poorer overall survival of gastric malignancies [19]. A growing volume of literature has showed that the dysregulated expression of lncRNAs may be novel prognosis predictor and therapeutic target for cancer patients.

Upregulation of the lncRNA FAM83H-AS1 was recently reported to predicts poor prognosis and to promote malignant phenotypes of bladder cancer [20] and pancreatic ductal adenocarcinoma cancer [21]. However, roles of FAM83H-AS1 in GC remains unsolved. In this study, we investigated the expression level of FAM83H-AS1 of paired GC and non-tumorous samples and identified FAM83H-AS1 as a novel oncogenic risk factor for survival prediction of GC.

2. Materials and methods

2.1. Patients and tissue samples

A cohort containing 107 GC tissues and corresponding para-tumorous samples were collected at the First Affiliated Hospital of Anhui Medical University and none of them received any chemo- or radiotherapy prior to surgery. All the diagnosis was confirmed by pathological examination of surgical samples. The clinicopathological features were obtained from medical record. All patients provided informed

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consent, while ethnic approval (Quick-PJ20191006) was obtained from the ethics committee of our institute.

2.2. Cell lines

Gastric epithelial cell GES1 and cancer cell lines SGC7901, MKN45, MKN74, SNU216 stored in liquid nitrogen were used. All cells were cultured in RPMI-1640 or Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) containing 10% fetal bovine serum (FBS) (HyClone, USA). The cell culture incubator with 5% CO_2 at 37 ˚C were used.

2.3. Quantitative real-time PCR (qRT-PCR)

Total RNA from enrolled samples or cells were extracted by TRIzol reagent (Invitrogen, USA), while complementary DNA was obtained in assistance with the Primer-Script™ RT-PCR kit (TaKaRa, Cat. No. RR036Q, Dalian, China). The SYBR Mix (Qiagen, Cat. No. 204145) was used for qRT-PCR on ABI 7900 system (Biosystems, USA). Relative expression levels were calculated with GAPDH as the internal control and all the assays were conducted in triplicate. The primers used in our study were as follows:

FAM83H-AS1 sense, 5′-TCCTCAAGCAAAGCACTC-3'
FAM83H-AS1 antisense, 5′-TACGGCAGAAAGAACCAA-3'
GAPDH sense, 5′-GGAGCGAGATCCCTCCAAAAT-3'
GAPDH antisense, 5′-GGCTGTTGTCATACTTCTCATGG-3'

2.4. Cell transfection and MTS, transwell and spheres formation assays

The cell transfection and proliferation assays were conducted according to previously reported [22]. Briefly, the cells were transfected with siRNA targeting FAM83H-AS1 or negative control (NC) using lipofectamine2000 (Invitrogen, USA). Two days after transfection, the cells were resuspended and incubated in the 96-well plate at 500 cells per well while the left cells were used for RNA extraction. The MTS (Promega, Cat. No. G1111) were added into cells before measurement of absorbance of each 96-well plate.

For spheres formation assays, the abovementioned cells were seeded in the ultralow attachment 24-well plates (Corning) at a density of 200 cells/well and the spheres were photographed and recorded 3–5 days thereafter. All assays were conducted in triplicate.

2.5. Statistical analysis

The software used in our study conducting statistical analysis including GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA) and SPSS package (IBM, USA). Student's t-test, chi-square test or Fisher's exact test was selected as appropriate to explore the associations between FAM83H-AS1 and clinicopathological indices. Kaplan-Meier and Cox regression model were used for univariate and multivariate survival analysis, respectively. The ROC was generated with the GraphPad Prism 5.0. Two-sided P values were calculated and less than 0.05 was regarded as significant.

3. Results

3.1. Overexpression of FAM83H-AS1 in human GC tissues

As shown in Fig. 1A, FAM83H-AS1 in GC tissues was aberrantly overexpressed than that of paired non-tumorous tissues ($P < 0.0001$). The median fold change of cancer tissues relative to non-tumorous ones for FAM83H-AS1 was 2.37 (Fig. 1A). Moreover, overexpression of FAM83H-AS1 was also observed in cancer cell lines (SGC7901, MKN45, MKN74, SNU216) compared with that in the immortalized gastric epithelial cells GES1.

3.2. Expression of FAM83H-AS1 in online database

To validate our results in GC patients, we further mined expression of FAM83H-AS1 in TCGA stomach adenocarcinoma data (TCGA-STAD) project via online tools (http://ibl.mdanderson.org/tanric/) and found upregulated FAM83H-AS1 in tumor samples (n = 285) compared with normal ones (n = 33) (Fig. 2A, $P < 0.0001$), which is consistent with that in our cohorts. However, based on TCGA-STAD data, non-significant association of FAM83H-AS1 with overall survival of gastric cancer (Fig. 2B, $P = 0.3624$) was observed.

3.3. Correlations between FAM83H-AS1 and clinicopathological indices

Based on the median expression level, patients were separated as low expression arm (n = 54) and high expression arm (n = 53). Associations between clinicopathological indices and FAM83H-AS1 expression were shown in Table 1. Overexpressed FAM83H-AS1 was not significantly related to gender, age, tumor size, tumor differentiation, lymph node metastasis or TNM stage (all $P > 0.05$) (Table 1). We then used Kaplan-Meier analysis to compare the survival between patients with high or low expressed FAM83H-AS1. Our data showed that FAM83H-AS1 overexpression was significantly associated with poorer OS and DFS ($P = 0.0178$ and $P = 0.0304$, respectively) (Fig. 3A and B). Additionally, univariate analysis demonstrated that advanced TNM stage ($P < 0.001$), presence of lymph node metastasis ($P = 0.036$) and overexpressed FAM83H-AS1 ($P = 0.020$) predicted OS (Table 2). Further multivariate analysis was conducted using Cox's regression model. As shown in Table 2, only TNM stage ($P = 0.002$) and upregulated FAM83H-AS1 ($P = 0.020$) were identified as independent factors (Table 2).

3.4. Oncogenic roles of FAM83H-AS1 in vitro

Moreover, we used small interfering RNA (siRNA) to validated the oncogenic roles of FAM83H-AS1. MKN74 and MKN45 cells was transfected with the indicated siRNAs and qRT-PCR assays confirmed efficient knockdown (Fig. 4A). Further MTS and spheres formation assays revealed that FAM83H-AS1 promoted cell proliferation and anchorage-independent growth (Fig. 4B and 4C), which further suggested oncogenic roles of FAM83H-AS1 in GC.

3.5. Diagnostic evaluation of FAM83H-AS1 in GC

In order to investigate whether upregulated FAM83H-AS1 could predict GC and lymph node metastasis, ROC curves were generated. Our results showed that FAM83H-AS1 may be a promising biomarker for GC, as the area under the ROC curve (AUC) was 0.8603 (95%CI: 0.8118–0.9089; $P < 0.0001$) (Fig. 5A). Meanwhile, we found that FAM83H-AS1 might serve as a diagnostic biomarker for lymph node metastasis as shown in Fig. 5B (AUC = 0.6778; 95%CI: 0.5543–0.8013; $P < 0.0070$).

4. Discussion

GC often initiates without any obvious clinical symptoms and most patients are diagnosed at advanced stage [2], leading to a poor prognosis of GC patients [23,24]. Despite recent advances in targeted therapy of advanced GC, the prognosis still remains relatively poor worldwide [25,26]. Identification of novel biomarkers for prognostic prediction is helpful in selecting therapeutic strategies of cancer [27]. Tissue specific nature and differentiated conservatism of lncRNAs indicated that they might serve as diagnosis biomarkers [28,29]. Moreover, plenty of studies indicated that many lncRNAs exert important functions in GC progression [10,13,19,30,31]. However, the majority of lncRNAs including FAM83H-AS1 in GC has been elusive.

Mountainous evidence have revealed the tumor suppressive and/or

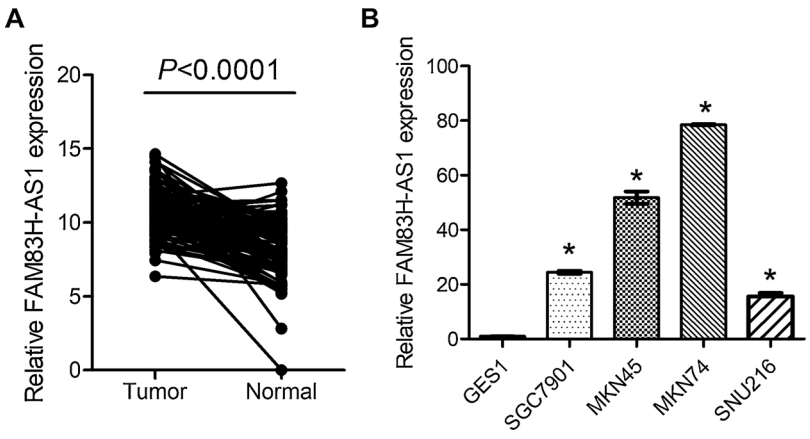

Fig. 1. FAM83H-AS1 is upregulated in GC. (A) Expression level of FAM83H-AS1 in enrolled samples. Results were presented as normalized △cycle threshold (△Ct) of Ct(FAM83H-AS1) relative to that of Ct(GAPDH). (B) Expression level of FAM83H-AS1 in cell lines. *$P < 0.05$ compared with GES1 cells.

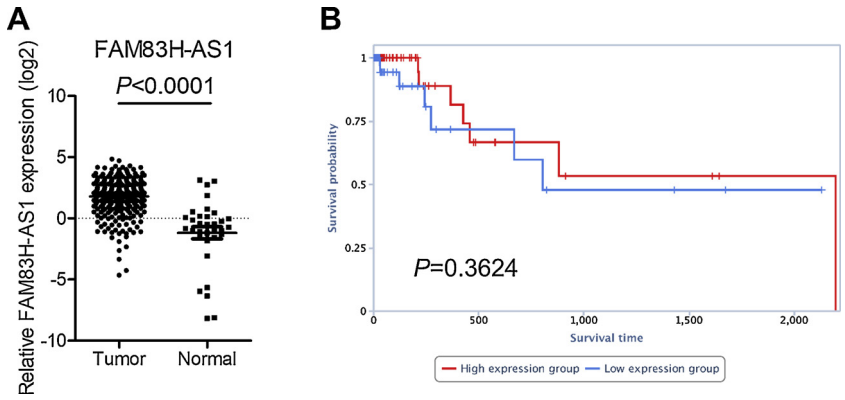

Fig. 2. FAM83H-AS1 is upregulated in the TCGA database. Expression level (A) and survival analysis (B) of FAM83H-AS1 obtained from TCGA database via online TANRIC tool (http://ibl.mdanderson.org/tanric/).

Table 1
The correlation between clinicopathological parameters and FAM83H-AS1 expression.

	FAM83H-AS1 expression		*P*
	Low, n(%)	High, n(%)	
Age			
<60	28 (46.7)	32 (53.3)	0.438
≥60	26 (55.3)	21 (44.7)	
Gender			
Male	38 (52.8)	34 (47.2)	0.541
Female	16 (45.7)	19 (54.3)	
Tumor size			
<5 cm	36 (57.1)	27 (42.9)	0.118
≥5 cm	18 (40.9)	26 (59.1)	
Tumor location			
Upper/middle	23 (46.9)	26 (53.1)	0.563
Lower/others	31 (53.4)	27 (46.6)	
Differentiation status			
Well or Moderate	18 (50.0)	18 (50.0)	1.000
Poor	36 (50.7)	35 (49.3)	
T stage			
T1-T2	7 (43.8)	9 (56.3)	0.598
T3-T4	47 (51.6)	44 (48.4)	
N stage			
N0-N1	22 (45.8)	26 (54.2)	0.440
N2-N3	32 (54.2)	27 (45.8)	
Lymph node metastasis			
Present	37 (45.1)	45 (54.9)	0.067
Absent	17 (68.0)	8 (32.0)	
TNM stage			0.838
I-II	17 (48.6)	18 (51.4)	
III-IV	37 (51.4)	35 (48.6)	

*$P < 0.05$.

oncogenic roles of lncRNAs in a number of human malignancies [8,9,17] including GC [13,19,30]. Dysregulated lncRNAs is found in various human tumors and acts as prognostic biomarkers [32–34]. For example, P. Luo et al [33] showed downregulation of the lncRNA SRA1 in hepatocellular carcinoma. LncRNA FEZF1-AS1 promotes aggressive phenotype of GC via interaction with p21 via LSD1-mediated H3K4me2 demethylation [13]. The lncRNA FAM83H-AS1 has been demonstrated to be upregulated in lung cancer and to promote tumor progression through MET/EGFR pathway [35].

Z. Baratieh et al. found that FAM83H-AS1 did not show consistently differential expression in gastric cancer with that of PlncRNA-1 and TUG1 [36]. However, only 32 paired tissues were included for statistical analysis. Our work enrolled 107 paired GC tissues and normal gastric samples. Amplification of the sample size may explain the different results of our study. Moreover, overexpressed FAM83H-AS1 was identified as independent prognosis predictor for OS. ROC curves showed promising diagnostic value of FAM83H-AS1 expression in lymph node metastasis and differentiation between cancer and non-cancer tissues. Transient loss-of-function assays confirmed the oncogenic roles of FAM83H-AS1 in GC. However, there are still some limitations in our study, such as, the mechanisms of FAM83H-AS1 was not discussed in our research.

5. Conclusion

In conclusion, FAM83H-AS1 was found to be overexpressed in GC and predicted poor prognosis. However, the underlying mechanism warrants further investigation.

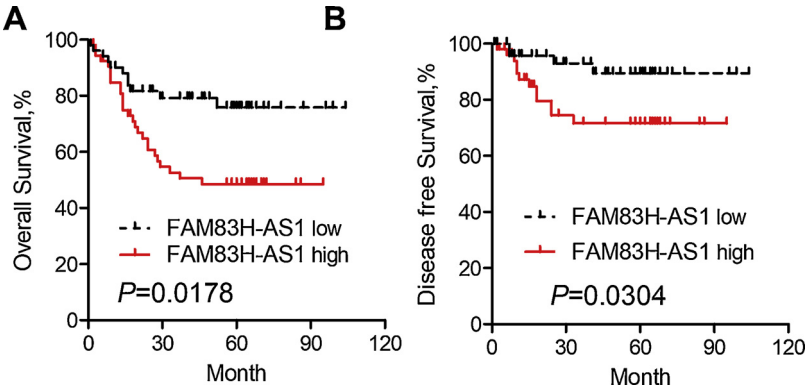

Fig. 3. FAM83H-AS1 predicts prognosis of GC patients. Kaplan–Meier OS (A) and DFS (B) curves of enrolled patients.

Table 2
Univariate and multivariate analyses of various potential prognostic factors in GC patients.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (< 60/≥60)	1.08(0.57-2.05)	0.806	–	–
Gender (male/female)	1.32(0.69-2.53)	0.407	–	–
Tumor size (≥5 cm/ < 5 cm)	0.82(0.43-1.58)	0.553	–	–
Tumor location (upper, middle/lower, others)	0.65(0.34-1.23)	0.183	–	–
Differentiation (poor/well, moderate)	1.38(0.68-2.77)	0.373	–	–
T stage (T3-4/T1-2)	2.49(0.77-8.11)	0.129	–	–
N stage (N2-3/N0-1)	1.49(0.77-2.85)	0.233	–	–
Lymph node metastasis (Present/absent)	3.03(1.07-8.54)	0.036*	1.09(0.36-3.34)	0.882
TNM stage (III-IV/I-II)	6.10(2.16-17.2)	0.000*	5.94(1.94-18.2)	0.002*
FAM83H-AS1(High/low)	2.26(1.14-4.47)	0.020*	2.27(1.14-4.51)	0.020*

HR: hazard ratio; CI: confidence interval; *$P < 0.05$.

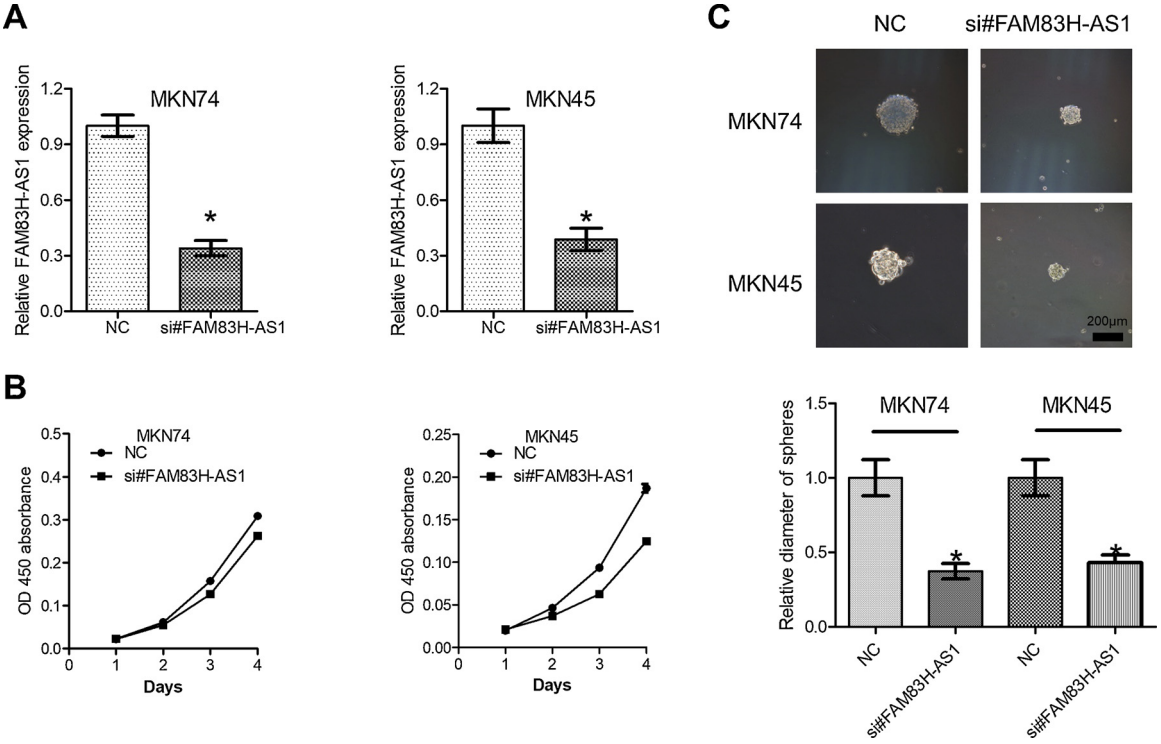

Fig. 4. Oncogenic roles of FAM83H-AS1 in GC. (A) Knockdown efficiency of siRNA targeting FAM83H-AS1 in MKN74 and MKN45 cells. (B) OD450 value of MKN74 and MKN45 cells after transfection with siRNA targeting FAM83H-AS1. (C) Sphere formation analysis of MKN74 and MKN45 cells after transfection with siRNA targeting FAM83H-AS1.

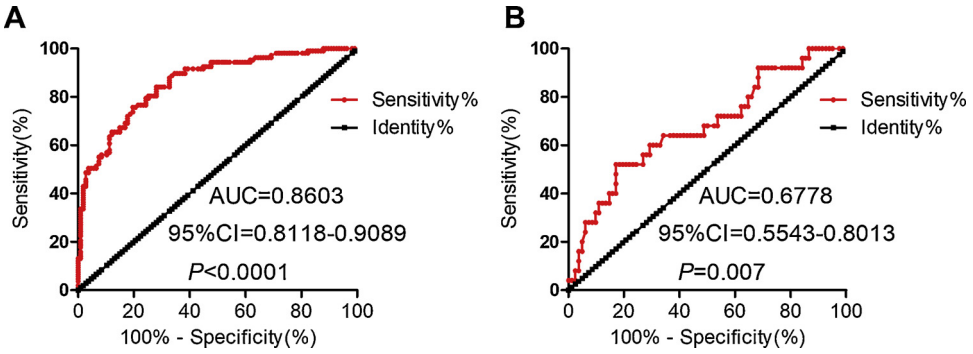

Fig. 5. Diagnostic value of FAM83H-AS1 in GC. ROC curve analysis of FAM83H-AS1 as a diagnostic marker for gastric cancer(A) and lymph node metastasis(B), respectively.

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