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Original contribution

FAM134B promotes esophageal squamous cell carcinoma in vitro and its correlations with clinicopathologic features☆,☆☆

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Summary Family with sequence similarity 134, member B (FAM134B) is an autophagy regulator of endoplasmic reticulum first discovered to be involved in the pathogenesis of esophageal squamous cell carcinoma (ESCC). The present study examined the functional behavior of FAM134B in cancer cells and the association of FAM134B expression with clinicopathologic factors in patients with ESCC. Expression at both the mRNA and protein levels was investigated using real-time polymerase chain reaction and immunohistochemistry. The results were correlated with the clinical and pathological features of the patients. In addition, in vitro functional assays were used to investigate the roles of FAM134B in ESCC cells in response to gene silencing with shRNA lentiviral particles. Overexpression of FAM134B mRNA and protein was present in 31.2% (n = 29/93) and 36.6% (n = 41/112), respectively, in tumors, whereas downregulation occurred in 39.8% (n = 37/93) and 63.4% (n = 71/112), respectively. Expression of FAM134B protein in ESCC correlated with histologic grade ($P = .002$) and pathologic stage ($P = .012$). In vitro suppression of FAM134B in ESCC induced significant reductions of cell proliferation and colony formation ($P < .05$). In addition, suppression of FAM134B caused reduction of wound healing, migration, and invasion capacities of ESCC. To conclude, FAM134B could play crucial roles in the initiation and progression of ESCC, and FAM134B protein expression has potential predictive value. Therefore, development of strategies targeting FAM134B could have therapeutic value in the management of patients with ESCC.

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1. Introduction

Esophageal squamous cell carcinoma (ESCC) is the predominant subtype of esophageal cancer in Asian countries and one of the most common malignancies worldwide [1,2]. This cancer encompasses complex molecular pathology because of its multifaceted genetic and epigenetic alterations [3-7]. These alterations include aberrant expression of key regulatory genes, thereby providing a selective force for cancer development.

We first identified *family with sequence similarity 134 member B* (*FAM134B*; also called *JK1* and *RETREG1*) in 2001 using comparative genomic hybridization analysis of ESCC [8-10]. Initial studies revealed that *FAM134B* acts as a transforming gene in ESCC and is overexpressed in ESCC cells [9]. Subsequent studies noted that FAM134B acts as a tumor suppressor and inhibits cancer growth in colorectal adenocarcinoma and breast carcinoma [11-15]. Pharmacologic upregulation of FAM134B resulted in reduced cancer cell growth and proliferation [13]. Furthermore, *FAM134B* mutations are common in patients with colorectal adenocarcinoma and ESCC with aggressive biological behavior [15,16]. In addition, FAM134B regulates endoplasmic reticulum turnover and is involved in viral pathogenesis, allergic rhinitis, vascular dementia, and neuronal disorders [11,17].

There has been no in-depth functional analysis, expression profiling, or clinical pathologic studies of FAM134B in patients with ESCC despite its initial identification in that cancer. Therefore, the current study examined FAM134B mRNA and protein expression in ESCC as well as their relations to the clinicopathologic features of this cancer. Moreover, the functional roles of FAM134B protein in ESCC cells followed by downregulation were analyzed.

2. Materials and methods

2.1. Patients, tissues, and clinical data

Griffith University granted ethics approval for this study (MED/19/08/HREC).

The patients chosen for this study underwent surgical resection for primary ESCC. For each patient, we created and sampled 2 tissue blocks from the cancer and 2 blocks from the non-neoplastic esophageal mucosa in proximal resection margin as controls. For each pair of tissues, we put one in liquid nitrogen to snap freeze and stored it at −80°C and fixed the other in formalin and then paraffin embedded it. The remaining portions of the specimens were fixed in 10% formalin. Then, we examined the

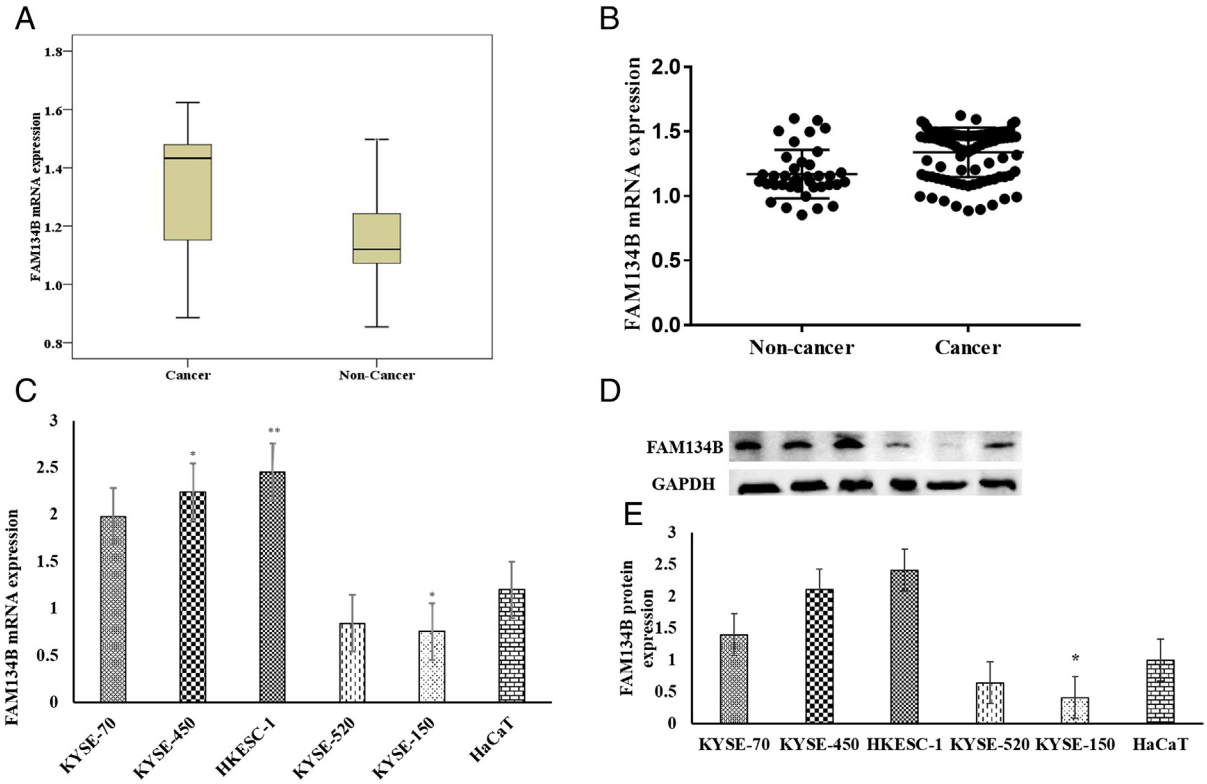

Fig. 1 Alteration of FAM134B mRNA and protein expression in ESCC. A, Expression of FAM134B mRNA in cancer and non-neoplastic tissue samples. Expression in cancer samples is significantly higher than in noncancer tissues ($P = .047$). B, Distribution of FAM134B mRNA in cancer and noncancer samples of the esophagus showing similar findings. C, Variations in expression of FAM134B in ESCC and non-neoplastic (HaCaT) cells. D, Expression of FAM134B protein in ESCC and HaCaT cells. E, Bar graph represents protein band intensities of FAM134B product in ESCC and HaCaT cells followed by GAPDH normalization. *$P < .05$ and **$P < .01$ compared with HaCaT cells.

specimen and harvested additional blocks for microscopic examination by our standard pathology protocol. For all the selected samples, we cut the sections using a microtome (Leica Biosystems, Buffalo Grove, IL) and stained them with hematoxylin and eosin for histologic confirmation of the diagnosis by an anatomic pathologist (A. K. L.) before further investigations.

The histologic grade of the selected ESCCs was assessed using the World Health Organization classification of digestive tract tumors [18]. We applied the criteria from the eighth edition of the American Joint Committee on Cancer manual for pathologic staging [19].

2.2. Cell lines

We used 5 ESCC cell lines, KYSE-520, KYSE-450, KYSE-150, KYSE-70, and HKESC-1, with characteristics that have been described in the literature [20-22].

2.3. Extraction of mRNA and cDNA conversion

From each frozen esophageal tissue, we cut seven 7-μm sections. Total RNA extraction from the tissues and the cultured cells was performed as previously reported [23,24]. Then, first-strand cDNA was generated using DyNAmo cDNA Synthesis Kits (Qiagen, Germantown Road Germantown, MD, USA) as described [25,26].

2.4. Quantitative real-time polymerase chain reaction

FAM134B mRNA expression changes in ESCC tissues samples were examined using the quantitative real-time polymerase chain reaction QuantStudio 6 Flex Real-Time PCR System (ThermoFisher Scientific, Waltham, MA) according to the previously published protocol [27]. A fold change of >2 was considered high FAM134B expression; 0.5 to 2, no change; and< 0.5, low expression.

2.5. TMA and immunohistochemical staining

We used a few tissue microarray (TMA) blocks containing formalin-fixed paraffin-embedded tissues from patients with ESCC [28,29]. The donor tissues in these TMA blocks were

Table 1 Association of *FAM134B* mRNA expression with clinicopathologic factors of patients with ESCC

Characteristic	Number	Expression (%)			*P*
		High	Low	No change	
Total patients	93	29 (31.2)	37 (39.8)	27 (29.0)	–
Sex					.228
Male	79 (84.9)	27 (34.2)	29 (36.7)	23 (29.1)	
Female	14 (15.1)	2 (14.3)	8 (57.1)	4 (28.6)	
Age (y)					.654
<60	33 (35.5)	12 (36.4)	13 (39.4)	8 (24.2)	
≥60	60 (64.5)	17 (28.3)	24 (4.0)	19 (31.7)	
Site					.171
Upper	10 (1.8)	4 (4.0)	4 (4.0)	2 (2.0)	
Middle	52 (55.9)	12 (23.0)	20 (38.5)	20 (38.5)	
Lower	31 (33.3)	13 (41.9)	13 (41.9)	5 (16.2)	
Size (cm)					.098
<6	62 (66.7)	19 (3.6)	21 (33.9)	22 (35.5)	
≥6	31 (33.3)	10 (32.3)	16 (51.6)	5 (16.1)	
Differentiation					.326
Well or moderate	76 (81.7)	26 (34.2)	28 (36.8)	22 (29.0)	
Poor	17 (18.3)	3 (17.6)	9 (52.9)	5 (29.4)	
T stage					.887
1, 2	9 (9.7)	3 (33.3)	4 (44.5)	2 (22.2)	
3, 4	84 (9.3)	26 (31.0)	33 (39.3)	25 (29.8)	
Lymph node metastasis					.824
Present	70 (75.3)	23 (32.8)	27 (38.6)	20 (28.6)	
Absent	23 (24.7)	6 (26.1)	10 (43.5)	7 (3.4)	
Distant metastasis					.820
Present	9 (9.7)	2 (22.2)	4 (44.5)	3 (33.3)	
Absent	84 (9.3)	27 (32.1)	33 (39.3)	24 (28.6)	
Pathologic stage					.979
I, II	27 (29.0)	8 (29.6)	11 (4.7)	8 (29.6)	
III, IV	66 (71.0)	21 (31.8)	26 (39.4)	19 (28.8)	

Table 2 Association of FAM134B protein expression with clinicopathologic features of ESCC

Characteristic	Number (%)	Expression, n (%)		*P*
		High	Low	
Total patients	112	41 (36.6)	71 (63.4)	–
Sex				.592
Male	90 (8.4)	33 (36.6)	57 (63.4)	
Female	22 (19.6)	8 (36.4)	14 (63.6)	
Age (y)				.155
<60	41 (36.6)	18 (43.9)	23 (56.1)	
≥60	71 (63.4)	23 (32.4)	48 (67.6)	
Site				.927
Upper	18 (16.1)	6 (33.3)	12 (66.7)	
Middle	55 (49.1)	21 (38.2)	34 (61.8)	
Lower	39 (34.8)	14 (35.9)	25 (64.1)	
Size (cm)				.224
<6	68 (6.7)	22 (32.4)	46 (67.6)	
≥6	44 (39.3)	19 (43.2)	25 (56.8)	
Differentiation				.002 [a]
Well or moderate	95 (84.8)	29 (3.5)	66 (69.5)	
Poor	17 (15.2)	12 (7.6)	5 (29.4)	
T stage				.139
1, 2	6 (5.4)	4 (66.7)	2 (33.3)	
3, 4	106 (94.6)	37 (34.9)	69 (65.1)	
Lymph node metastasis				.128
Present	77 (68.8)	25 (32.5)	52 (67.5)	
Absent	35 (32.2)	16 (45.7)	19 (54.3)	
Distant metastasis				.291
Present	9 (8.0)	2 (22.2)	7 (77.8)	
Absent	103 (92.0)	39 (37.8)	64 (62.2)	
Pathologic stage				.012 [a]
I, II	26 (23.2)	14 (53.8)	12 (46.2)	
III, IV	86 (76.8)	27 (31.4)	59 (68.6)	

[a] Statistically significant difference.

collected during the same period as the tissue blocks used in the mRNA expression study.

Immunohistochemical staining was performed to detect the expression of FAM134B protein using anti-FAM134B antibody from Santa Cruz Biotechnology (Santa Cruz, CA) as previously described [30]. Nuclear staining was considered positive staining. The FAM134B protein expression in tissues was classified into four categories; ie, no or negligible staining (0), weakly positive staining with less than 10% of tumor cells positive (+), moderate intensity with 10% to 50% of cells stained (++), and strong staining intensity in >50% cells (+++). Overall, cases with scores of "0" or "+" were grouped as having "low"

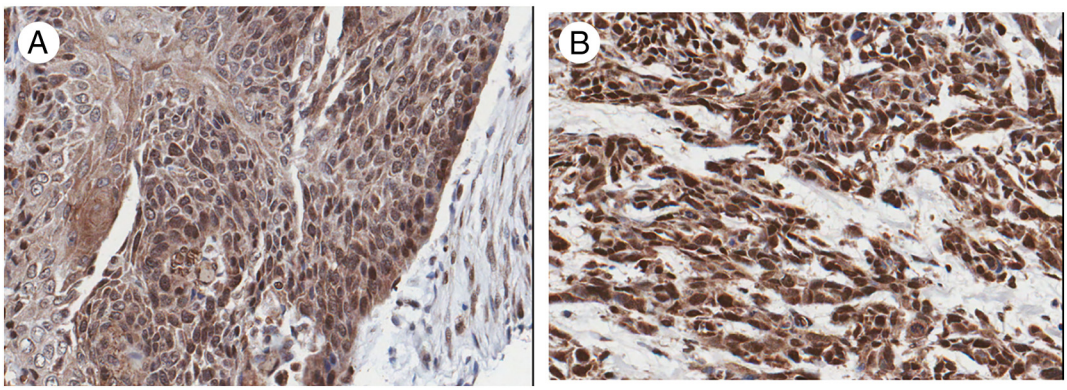

Fig. 2 Expression of FAB134B protein in squamous cell carcinoma of the esophagus. A, Well-differentiated (low-grade) lesions showing low expression. B, Poorly differentiated (high-grade) ESCC tissues exhibiting high expression (hematoxylin and eosin; original magnification ×20).

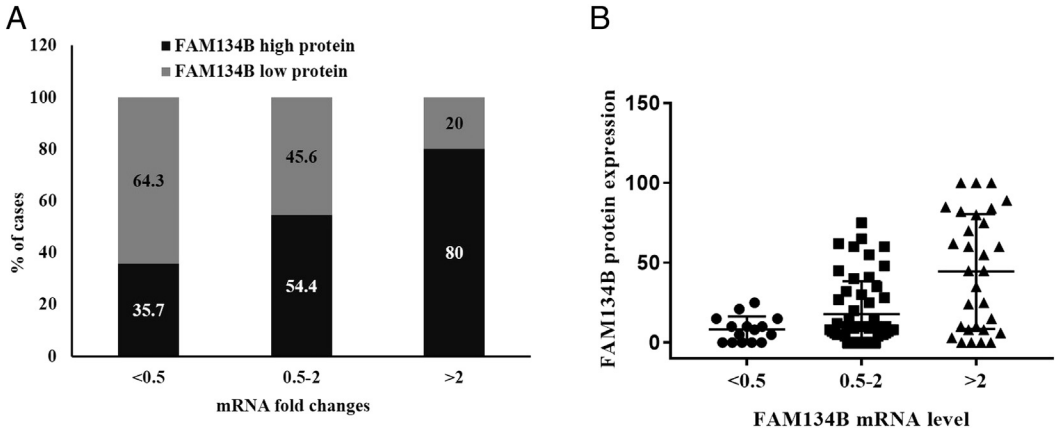

Fig. 3 Association of FAM134B mRNA and protein expression in ESCC. A, The majority of patients (80%) with ESCC expressing higher mRNA showed high FAM134B protein expression, and approximately 64% of patients with ESCC having lower mRNA expression displayed low FAM134B protein. B, Distribution of FAM134B protein in patients with ESCC having mRNA fold changes of <0.5, 0.5-2.0, and >2. Higher fold changes of FAM134B mRNA were associated with high protein expression; and lower fold changes, with low expression.

expression, whereas those with a score of "++" or "+++" were considered to have high expression.

2.6. Western blot analysis

Western blotting was used to detect and quantify FAM134B protein in ESCC cells and non-cancer HaCaT cells as previously described [15]. Total proteins were extracted from cells with lysis buffer (Bio-Rad, Gladesville, NSW, Australia). Next, total protein (30 μg) was separated by 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad) and transferred to nitrocellulose membranes (Bio-Rad). The membranes were then developed to detect protein bands according to the published protocol [15]. Expression of FAM134B protein in these cell lines was quantified and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with Image J 1.48 software (Santa Cruz Biotechnology Inc, Dallas, TX, USA).

2.7. Suppression of *FAM134B* in esophageal cancer cells using shRNA lentiviral vector

The ESCC cell lines KYSE-450 and KYSE-150 were cultured at approximately 2×10^4 cells/cm^2 in 24-well plates. After 24 hours of growth, cells were transfected with *FAM134B* shRNA lentiviral particles (KYSE-450$^{FAM134B-}$ and KYSE-150$^{FAM134B-}$) and scramble-control shRNA lentiviral particles (KYSE-450^{Scr+} and KYSE-150^{Scr+}). Subsequently, stable FAM134B knockout cells were obtained according to the established protocol [15].

2.8. Cell proliferation assay

The cell proliferation assay was performed to examine the effect of FAM134B suppression on the growth of ESCC cells (KYSE-450 and KYSE-150) as previously described [27]. For this experiment, Cell Counting Kit–8 (Sigma-Aldrich, St Louis, MO) was used. The FAM134B-suppressed (KYSE-450$^{FAM134B-}$ and KYSE-150$^{FAM134B-}$) and control (KYSE-450^{Scr+} and KYSE-150^{Scr+}) cells were first seeded on a flat-bottom 96-well plate at 1×10^4 cells/well. Proliferation was determined on days 0 to 3 with Cell Counting Kit–8 following the manufacturer's instructions.

2.9. Wound healing assay

To determine the effect of *FAM134B* knockout on the capacity of cells to migrate, a wound healing assay was performed [23]. Cells were cultured as a monolayer until 70%–80% confluent, and then gaps were created using a 200-μL pipette tip. Finally, wound areas of all cell types were measured on different days and compared with Image J 1.48 software as previously described [22].

2.10. Invasion assay

To investigate the effect of *FAM134B* knockout on in vitro cell penetration/invasion, a barrier-coated cell invasion assay was used. For this experiment, a CultreCoat 96-well basement membrane extract–coated invasion assay (Trevigen, Gaithersburg, MD) kit, consisting of basement membrane components, was used as previously published [23].

2.11. Colony formation assay

To determine the effect of FAM134B suppression on clone formation capacity of ESCC cells, equal numbers of cancer cells of each type were seeded in 6-well plates with complete growth medium. After 16 days, when microscopic clones were noted in every plate, cell growth was stopped. The colony formation capacity of the cells was calculated as previously described [31].

2.12. Statistical analysis

Comparisons between variable groups were analyzed using the χ^2 test, likelihood ratio, and Fisher exact test. All clinical, pathologic, and follow-up information and mRNA expression changes were computerized. Statistical analysis was then performed using the Statistical Package for the Social Sciences for Windows (version 25.0; IBM SPSS, New York, NY). Survival analysis was tested using the Kaplan-Meier method. All the in vitro experiments were done in triplicate. The significance level of the tests was taken as $P < .05$.

3. Results

3.1. Aberrant expression of FAM134B mRNA in ESCC tissues and cells

The expression of FAM134B mRNA in 93 ESCC and matched non-neoplastic tissues samples is presented in Fig. 1A and B. The difference of the FAM134B mRNA expression ratio between cancer and noncancer tissue samples (1.349 ± 0.042 versus 1.169 ± 0.015) was statistically significant ($P = .047$). Among the ESCC samples, 31.2% (n = 29) showed high FAM134B mRNA expression, and 39.8% (n = 37) had low expression (Table 1). The remaining 29% (n = 27) of the cancer samples showed no significant changes in FAM134B mRNA expression. Expression in ESCC showed no correlation with clinical or pathological features (Table 1).

Aberrant FAM134B expression at the mRNA and protein levels was noted in the ESCC cell lines used compared with non-neoplastic HaCaT cells after normalizing to GAPDH (Fig. 1C-E). Among the cancer cells, KYSE-450, KYSE-70, and HKESC-1 showed higher expression of FAM134B mRNA and protein, whereas KYSE-520 and KYSE-150 exhibited reduced expression compared with that of HaCaT cells.

3.2. Expression of FAM134B protein in ESCC associated with clinicopathologic factors

On immunostaining, 36.6% (n = 41) of the 112 ESCCs showed high FAM134B protein expression, the remainder showing low expression (Table 2). The expression of FAM134B protein in non-neoplastic esophageal mucosa, dysplastic squamous mucosa, low-grade (grade 1 or 2; well or moderately differentiated) squamous cell carcinoma, and high-grade (grade 3; poorly differentiated) squamous cell carcinoma is presented in Fig. 2. Poorly differentiated ESCC often had high expression of FAM134B protein, whereas well/moderately differentiated ESCC had low expression ($P = .002$). Most of the patients with early-stage ESCC (I or II) exhibited high expression of FAM134B protein compared with those with tumors of advanced stages (III or IV; 53.8% versus 31.4%; $P = .012$; Table 2).

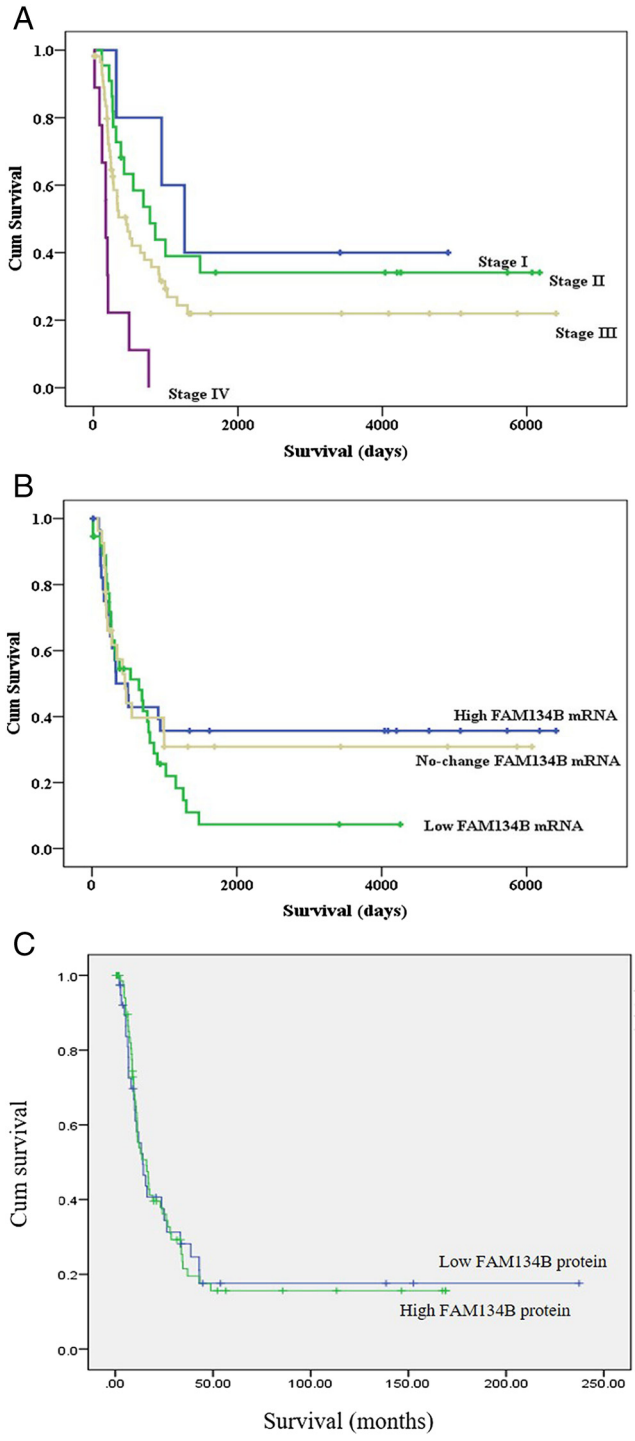

Fig. 4 Relation of FAM134B expression and survival rates of patients with ESCC. A, Survival rate of patients with ESCC correlated with pathological stage of cancer ($P = .001$). B, Patients with high FAM134B mRNA expression had better survival rates than those with no change or low FAM134B mRNA expressions (83 months versus 71 months versus 28 months, respectively). C, Patients with high or low expression of FAM134B protein did not show a significant difference in survival rates.

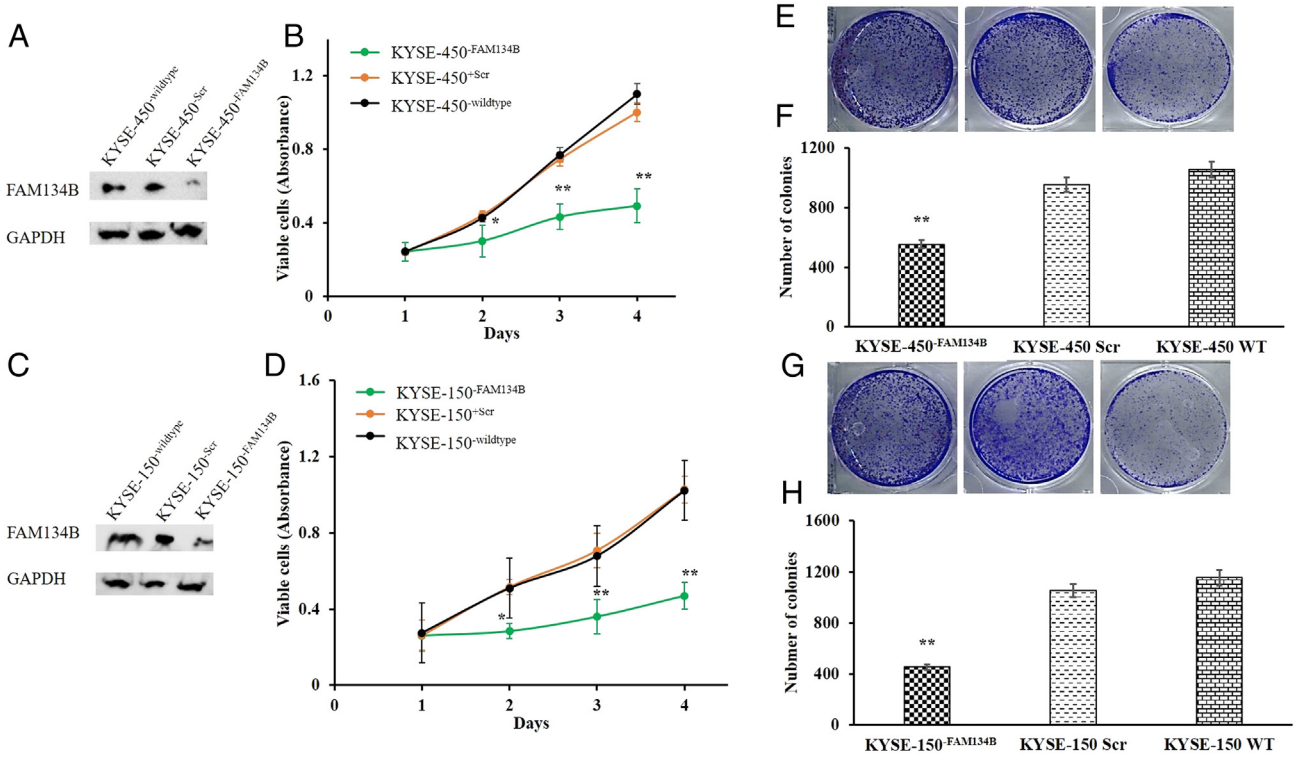

Fig. 5 Suppression of FAM134B reduced cancer cell growth and proliferation. A, KYSE-450 cells treated with FAM134B shRNA showed significant reduction of FAM134B protein compared with control cells. B, KYSE-450$^{FAM134B-}$ cells showed significant reduction in cell proliferation compared with control KYSE-450^{Scr+} and nontransfected KYSE-450$^{wild\text{-}type}$ cells. C, KYSE-150 cells treated with FAM134B shRNA showed significant reduction of FAM134B protein compared with controls. D, KYSE-150$^{FAM134B-}$ cells showed remarkable reduction in cell proliferation relative to KYSE-450^{Scr+} and KYSE-450$^{wild\text{-}type}$ cells. Significant reduction of colony formation capacity of KYSE-450$^{FAM134B-}$ (E and F) and KYSE-150$^{FAM134B-}$ (G and H) cells was noted in comparison with the control cells. *P < .05 and **P < .01 compared with that of scramble control and wild-type cells.

3.3. Correlation between FAM134B mRNA and protein expression in ESCC

A statistically significant positive correlation of FAM134B mRNA and protein expression was noted (r = 0.38; P = .01; Fisher exact test). As shown in Fig. 3, 80% (n = 24/30) of the cancer samples with high FAM134B mRNA expression (fold change >2) had overexpression of FAM134B protein, whereas FAM134B mRNA downregulation (fold change <0.5) induced lower protein expression in 64% (n = 9/14; Fig. 3A). Fig. 3B shows the distribution of FAM134B protein expression among the patients with ESCC having different fold changes of the mRNA expression.

3.4. *FAM134B* expression and patients' survival

The median overall follow-up time of the patients with ESCC was 60 months. The survival rates correlated with the pathologic stage of the cancer (P = .001; Fig. 4A). Patients with high FAM134B mRNA had better survival rates than patients with no change or low FAM134B mRNA (83 months versus 71 months versus 28 months, respectively) compared with those with lower FAM134B expression (Fig. 4B). In addition, patients with low FAM134B protein expression had a slightly better survival time (58 months versus 40 months) than those having high FAM134B protein expression (Fig. 4C). Nevertheless, the difference between these groups did not reach statistical significance.

3.5. Suppression of FAM134B induction reduced cancer cell proliferation

KYSE-450$^{FAM134B-}$ and KYSE-150$^{FAM134B-}$ cells showed significant reduction of FAM134B protein concentration compared with the control cells (Fig. 5A and C). KYSE-450$^{FAM134B-}$ cells showed significant reduction in proliferation on days 1 to 3 compared with KYSE-450^{Scr+} and KYSE-450$^{wild\text{-}type}$ cells (Fig. 5B). A similar result was observed in KYSE-150$^{FAM134B-}$ cells (Fig. 5D). In addition, a significant reduction of the colony-formation capacity was observed in KYSE-450$^{FAM134B-}$ and KYSE-150$^{FAM134B-}$ cells in comparison with KYSE-450^{Scr+} and KYSE-150^{Scr+} as well as in KYSE-450$^{wild\text{-}type}$ and KYSE-150$^{wild\text{-}type}$ cells (Fig. 5E-H).

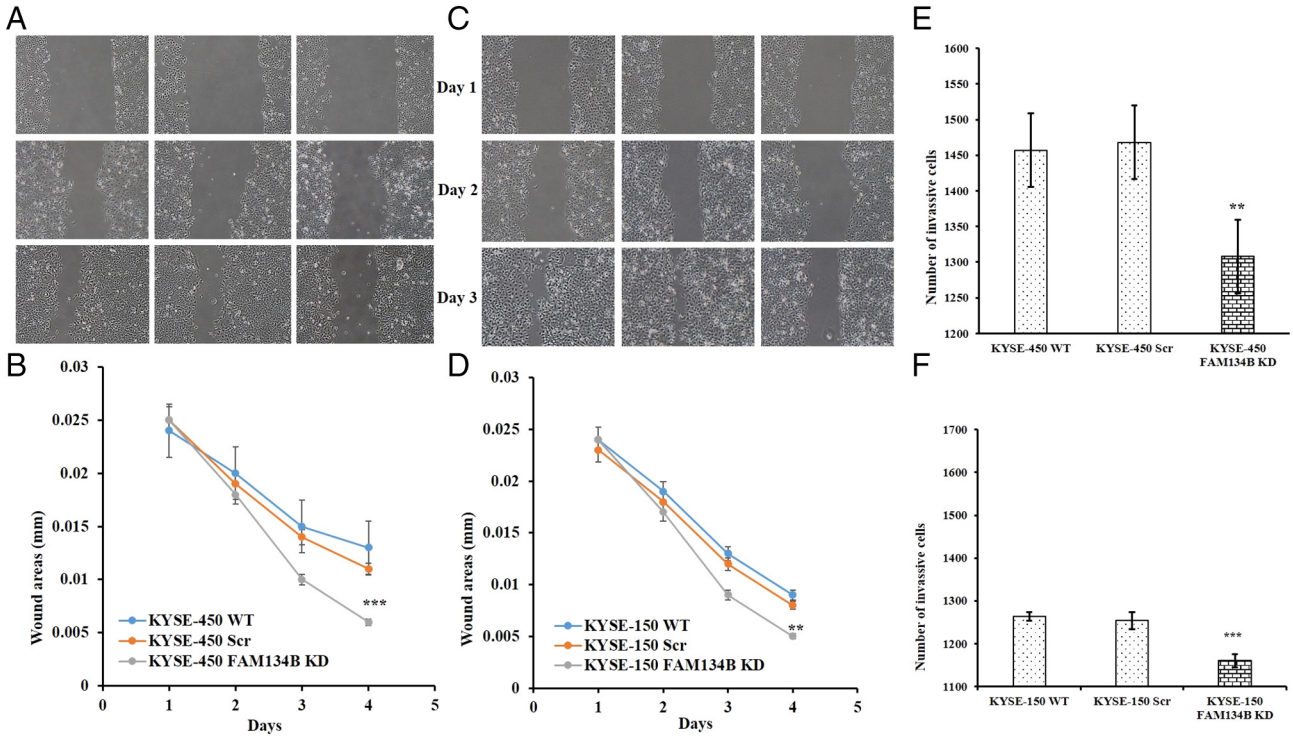

Fig. 6 Suppression of FAM134B inhibited ESCC cell migration and invasion. A, KYSE-450$^{FAM134B-}$ cells had a significantly lower cell migration potential than the control (KYSE-450^{Scr+}) and wild-type (KYSE-450$^{wild\text{-}type}$) cells. B, KYSE-150$^{FAM134B-}$ cells showed reduced wound healing compared with control cells. C, Significant difference in unhealed wound areas between controls and FAM134B-suppressed KYSE-450$^{FAM134B-}$ cells on day 3. D, In KYSE-150$^{FAM134B-}$ cells, the unhealed wounds were much larger than those of control cells. Similarly, both KYSE-450$^{FAM134B-}$ (E) and KYSE-150$^{FAM134B-}$ (F) cell lines showed reduced invasiveness in comparison with control cells. *$P < .01$ and **$P < .001$ compared with scramble control and wild-type cells.

3.6. Suppression of FAM134B inhibited ESCC cell migration and invasion properties

KYSE-450$^{FAM134B-}$ and KYSE-150$^{FAM134B-}$ cells had a significantly lower migration potential than KYSE-450^{Scr+}, KYSE-150^{Scr+}, KYSE-450$^{wild\text{-}type}$, and KYSE-150$^{wild\text{-}type}$ cells, as they healed the scratches slowly compared with their counterparts (Fig. 6A and C). A significant difference in unhealed wound areas was noted between controls and FAM134B-suppressed cells on day 3 in the case of both cell lines (Fig. 6B and D). Similarly, KYSE-450$^{FAM134B-}$ and KYSE-150$^{FAM134B-}$ cells showed reduced invasive properties (Fig. 6E and F). In the case of KYSE-450$^{FAM134B-}$ cells, invasive cells were 1308 ± 66, whereas the invasive KYSE-450^{Scr+} cells were 1468 ± 73. KYSE-150 cells revealed similar results (1160 ± 58 versus 1254 ± 63).

4. Discussion

This study for the first time reports insights into the functional roles and clinical significance of FAM134B in a large cohort of patients with ESCC. Aberrant expression of FAM134B in both mRNA and protein occurred in ESCC tissues and cells. There was a significant relation between aberrant protein expression and pathological factors, including histologic grade and pathologic stage. In addition, the investigators observed significant changes in cell proliferation, colony formation, migration, and invasive properties of ESCC cells after suppression of FAM134B. Thus, FAM134B could play a direct role in cancer pathogenesis through regulation of cellular proliferation, invasion, and migration, functioning as a tumor promoter in ESCC.

Our previous studies showed *FAM134B* DNA copy number aberration and altered mRNA expression in ESCC [9,16]. For example, 69% of ESCC cell lines and 30% of cancer tissues display overexpression of FAM134B at the mRNA level [9]. One-third of ESCC tissues (n = 38/102) exhibit *FAM134B* DNA copy number amplification in comparison with noncancer control tissue samples [16]. In the present study, 31% (n = 29/93) and 37% (n = 41/112) of tissues revealed FAM134B overexpression at the mRNA and protein level, respectively. In addition, 3 of 5 tested ESCC cell lines exhibited FAM134B overexpression at both the mRNA and protein level. Thus, these results concur with the previous findings. On the other hand, approximately 39% (n = 37/93) and 63% (n = 71/112) of the cancer tissues showed lower FAM134B expression at the mRNA and protein level, respectively. This underexpression of FAM134B in ESCC might be

related to other genetic (eg, chromosomal deletion) or epigenetic (ie, promoter hypermethylation) alterations [32,33]. It is worth noting that *FAM134B* promoter hypermethylation induces suppression of FAM134B mRNA and protein expression in colorectal adenocarcinoma [34].

Downregulation of *FAM134B* is associated with adverse clinicopathologic factors and biological aggressiveness of cancer in patients with colorectal adenocarcinoma and breast carcinoma [11,12,27,30,34]. On the contrary, in this study, we noted that overexpression of FAM134B protein correlated with high histologic grade of ESCC. In addition, a majority of the patients with ESCC in advanced stages showed low FAM134B protein expression compared with patients with ESCC in early stages. The association of FAM134B overexpression with tumor stage and histologic type is consistent with our previous findings [9]. These correlations may indicate that there are 2 distinct groups of ESCC in term of biological behavior. The findings imply that even at an identical pathologic stage, lower–histologic grade (well or moderately differentiated) squamous cell carcinomas are biologically more aggressive than higher–histologic grade squamous cell carcinomas [6].

In the present study, in vitro downregulation of *FAM134B* in ESCC cells (KYSE-450$^{FAM134B-}$ and KYSE-150$^{FAM134B-}$) induced significant alterations in cell growth and proliferative properties, including reduced proliferation and colony formation, slower wound healing, and lower migration and invasion ability than control cells (Figs. 5 and 6). Overall, overexpression in cancer cells and tissues, as well as simultaneously reduced tumorigenic potential of cancer cells after FAM134B suppression, indicated the oncogenic feature of *FAM134B* in ESCC. These changes in cellular and tissue concentrations could be involved in the molecular pathogenesis of ESCC. However, the protein functions as a tumor inhibitor in colorectal and breast carcinoma [11,12]. In colorectal adenocarcinoma, FAM134B interacts with end-binding protein 1 and modulates the expression of APC/β-catenin, thereby regulating the growth and proliferation of cells [35], whereas in ESCC, the exact mechanism and the signaling networks with which FAM134B interacts have not been identified. Further research is imperative to identify the interacting partners of FAM134B in ESCC to clarify its roles in the pathogenesis of ESCC.

In conclusion, FAM134B overexpression is a common event in ESCC and is associated with the biological aggressiveness of this cancer. Exogenous downregulation of *FAM134B* caused increased proliferation, migration, and invasion as well as colony formation, thereby promoting cancer development. Thus, overexpression of this gene could provide tumor growth advantages in ESCC by itself or by switching on other regulatory molecules. FAM134B protein expression may have predictive value, as it is related to cancer differentiation and pathologic stage. The results from the present study raise the possibility that anti-FAM134B agents have therapeutic implications for patients with ESCC.

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