LncRNA FAM83A-AS1 Dives ESCC Progression by Regulating miR-214/CDC25B Axis

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Abstract

Background Recently, extensive researches have established that long non-coding RNA (lncRNA) was an important factor that is strongly related to carcinogenesis. However, the function of lncRNAs in esophageal cell squamous carcinoma (ESCC) remains to be explored. In the current study, we assessed the expression pattern and the biological function of FAM83A-AS1 in ESCC.

Methods qRT-PCR was used to detect the expression of FAM83A-AS1,miR-214, and CDC25B expression in ESCCtissues and cell lines. Cell counting kit 8 (CCK-8, Transwell, apoptosis, and cell cycle assays were performed to define the function of FAM83A-AS1 in ESCC cell. Furthermore, the regulation of miR-214 by FAM83A-AS1 was defined by qRT- PCR and rescue assays.In addition, the association between CDC25B,miR-214,CDC25B were performed with qRT-PCR.

Results: Here we discovered that FAM83A-AS1 was strongly expressed in ESCC tissues. FAM83A-AS1 abundance was associated with TNM stages and the differentiation grade of ESCC patients. The receiver operating characteristic curve (ROC) analysis indicated the high accuracy of FAM83A-AS1 in ESCC diagnosis. Functionally, inhibiting FAM83A-AS1 repressed cell proliferation, migration, and invasion in ESCC. In addition, we found that FAM83A-AS1 accelerated cell cycle and inhibited cell apoptosis. Mechanistically, we found that FAM83A-AS1 regulated miR-214 expression and there was a negative correlation between miR-214 and FAM83A-AS1 in ESCC. Rescue assay indicated that miR-214 could impair the suppressing effect of cell migration induced by FAM83A-AS1 depletion. Furthermore, CDC25B was a direct target of miR-214 and FAM83A-AS1 enhanced CDC25B expression while miR-214 positively CDC25B expression in ESCC.

Conclusions Collectively, we concluded that FAM83A-AS1 facilitated ESCC progression by regulating the miR-214/CDC25B axis. Our study showed FAM83A-AS1 may act as a target for ESCC diagnosis and therapy.

1. Introduction

Esophageal cancer is a progressive cancerous swelling worldwide, which is always accompanied by high morbidity and mortality [1]. Approximately 80% of EC is clarified as ESCC [2]. Despite greatly advanced progress that has been achieved in the diagnostic and treatment of ESCC over the past decades, the high occurrence and poor survival of ESCC patients remains a public health concern [3, 4]. And the molecular mechanism of ESCC development remains to be largely elucidated. Therefore, it is urgent to find out the precise molecular mechanism underlying ESCC pathogenesis, which can provide novel sights into the effective therapeutic strategy for ESCC.

Recently, great attention has been paid to lncRNA, because of their diverse effect in hallmarks of cancer [5]. lncRNA is a kind of non-coding transcripts, whose length is longer than 200 nucleotides (nt) and lncRNA could participate in numerous biological processes that regulate the tumorigenesis and metastasis procedure of ESCC [6]. It is established that lncRNA functions through the epigenetic,

transcriptional, and post-transcriptional mechanisms [7, 8]. Emerging studies have highlighted that lncRNA could serve as a miRNA sponge to regulate downstream gene expression and activity[9, 10]. The ceRNA (competing endogenous RNA) mechanism has been widely recognized as an important and common working mechanism of lncRNA. For instance, MNX1-AS1 promoted cell growth and metastasis activity by binding to miR-34a in ESCC[11]. LEF1-AS1 served as the sponge of miR-489-3p, increased the HIGD1A expression level, ultimately accelerating the malignancy phenotype of glioma[12]. Though many lncRNAs have been defined as the crucial modulators in cancer, the association between lncRNA and ESCC pathogenesis remains largely to be explored.

FAM83A-AS1 was reported to be an important oncogene of lung cancer [13]. FAM83A-AS1 was significantly increased in lung cancer tissues and FAM83A-AS1 promoted cell proliferation and migration potential by targeting the miR-150-3p/MMP14 axis [14]. In addition, FAM83A-AS1 facilitated tumorigenesis through interacting with NOP58 to increase the FAM83A stability in hepatocellular carcinoma [15]. Herein, we found FAM83A-AS1 was dramatically overexpressed in ESCC tissues. The FAM83A-AS1 expression level was associated with differentiation grade and advanced stages of ESCC patients. Besides, there was a high accuracy of FAM83A-AS1 in ESCC diagnosis. We investigated the effect of FAM83A-AS1 in ESCC progression. FAM83A-AS1 promoted cell proliferation, migration, invasion, and apoptosis. We also observed that FAM83A-AS1 increased the count of ESCC cells in the S phase. Notably, we identified that FAM83A-AS1 could function as the molecular sponge to regulate ESCC progression through the miR-214/CDC25B axis. These findings showed that FAM83A-AS1 was a promising target gene of diagnosis and treatment in ESCC, which deepened our understanding of the epigenetic regulations during the ESCC carcinogenesis.

2. Materials And Methods

2.1 Tissues specimens

ESCC tissues and matched adjacent tumor tissues were obtained from 51 patients who were newly diagnosed and histologically confirmed ESCC by more than three pathologists at the department of pathology, the First Affiliated Hospital of Zhengzhou university during 2019. All the enrolled patients in this research signed written informed consent and the study project got the approval of the ethics committee of the first affiliated hospital of Zhengzhou University. Fresh cancer tissues and matched adjacent non-tumoral tissues were immediately collected after surgery and stored at -80°Cwith RNA later treated until RNA derivation. Then lncRNA, miRNA, and mRNA expression levels were detected with qRT-PCR assays. Meanwhile, we collected the clinical features of these 51 participants for further analysis.

2.2 Cell lines

KYSE30 and EC109 cells were obtained from the Shanghai institutes of life science cell bank center (Shanghai, China). ESCC cell lines were maintained in DMEM (Hycolne, UT, USA) adding 10% fetal bovine

serum along with 1% penicillin-streptomycin. Besides, cells were routinely cultured in an atmosphere of 5% CO_2 at 37°C.

2.3 RNA extraction and qRT-PCR

Total RNAs isolated from ESCC tissues and cell lines were obtained with Trizol reagent (Takara, Dalian, China)according to the manufacturer's protocol. The quality of RNA was assessed by the NanoDrop 2000 spectrometer. The reverse transcription reaction was implemented by the PrimeScript™RT reagent Kit with gDNA Eraser (TakaRa, Dalian, China) to acquire the cDNAs of lncRNA and mRNA. The reverse transcription reaction of miRNA was performed with the miRNA 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). RT-PCR was used to detect the relative expression of lncRNA, miRNA, and mRNA in the QuautStudio-5 system. β-Actin or U6 were defined as the endogenous controls for normalization. We adopted $2^{-\triangle\triangle ct}$ method to analyze the differences.

2.4 Cell transfection

Three small interfering RNA sequences targeting FAM83A-AS1 and corresponding negative control sequence, along with miR-214 inhibitor and control were gained from GenePharma (Shanghai, China). KYSE30 and EC109 cells in the logarithmic growth phase were cultured in a six-well plate at a suitable density before transfection. Then cells were treated siRNA or miRNA inhibitor with Lipofectamine 3000 reagents (Invitrogen) according to the manufacturer's instruction. The cells were cultured for 48 h at 37°C after transfection for further assays.

2.5 Cell count Kit assay

We investigated cell proliferation rate by CCK8 kit as previously described. ESCC cells treated with siRNAs were collected and seeded into a 96-well plate at a density of 2000 cells/well. Next, we added 10 μl CCK8 reagent into each well and incubated for 24, 48, 72, 96 h. We detected the absorbance at 450 nm after incubation for 2h at 37°C. Data were collected by a microplate reader for statistical analysis.

2.6 Transwell assays

For the transwell migration and invasion assays, the top chambers were coated with or without the matrigel. Subsequently, 2×10^4 cells were seeded in the upper chamber containing 200 μl serum-free DMEM. In contrast, the 650 ul DMEM supplementing 15% FBS was added into the lower chamber carefully. After incubation for 24-48h, those cells which did not migrate into the undersurface in the upper chamber were cleared away. Cells were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, imaged with the microscope.

2.7 Flow cytometry

The cell cycle and apoptosis analysis kit was utilized to detect the cell cycle distribution and apoptosis, respectively. For cell cycle assay, 5×10 cells seeded in the six-well plate were digested with trypsin and

washed by PBS. Then we fixed cells with cooling 70% ethanol overnight at 4℃. On the next day, we washed cells with PBS and acquired single-cell suspensions. Propidium iodide (PI) was used to stain the detached cells. Finally, we tested the cell cycle with the BD Accuri™ C6 Plus system and analyzed it with modfit 4.0. For apoptosis assay, single-cell suspensions were labeled with FITC/PI double staining for 15 min at room temperature. Subsequently, we used flow cytometry to detect early and late apoptosis of ESCC cells. FlowJo was used to analyze the percentages of apoptosis events.

2.8 Statistical analysis

All data in the current study were displayed as mean ± standard deviation (S.D.). Student's t-test and Chi-square analysis were employed to examine the differences between the two groups according to the data types. One-way analyses of variance was adopted to analyze the differences between serval groups. Kaplan-Meier and ROC analysis was utilized to analyze the survival status and specificity and sensitivity, respectively. Pearson Correlation Coefficient was used to calculate the association. All statistical analyses were performed in GraphPad prism 8. P□0.05 was defined as statistical significance.

3. Results

3.1 FAM83A-AS1 is up-regulated in ESCC tissues

The bioinformatics data analysis retrieved from GEPIA (http://gepia2.cancer-pku.cn/#index) and lnCAR[16] revealed that FAM83A-AS1 was remarkably increased in ESCC (Fig. 1A-B). Our results also corroborated in the high expression of FAM83A-AS1 in 51 paired ESCC tissues compared with adjacent non-tumor tissues (Fig. 1C). Next, we collected the clinical data of these independent ESCC cases and assessed the association between FAM83A-AS1 level and clinical parameters of ESCC patients. Here, we identified FAM83A-AS1 expression was significantly correlated with differentiation grade (P = 0.0209 and advanced stages (P=0.0104, Table 1)). However, there was no obvious association in other clinical parameters, including gender, age, and so on. Additionally, we assessed the diagnostic and prognostic effects of FAM83A-AS1 in ESCC. Kaplan-Meier statistics suggested that patients with high expression of FAM83A-AS1 exhibited a similar overall survival time with those with low FAM83A-AS1 levels (Fig. 1E). Whereas ROC analysis showed that FAM83A-AS1 gained high accuracy in distinguishing ESCC (Fig. 1D). The AUC(Area under the curve) was 0.954 (95% CI 0.895–0.985) and sensitivity and specificity were 92.59% and 90.57%, respectively. Taken together, these findings implied that FAM83A-AS1 plays a tumorigenic role in ESCC development and progression.

3.2 FAM83A-AS1 accelerated ESCC cell growth.

To seek for the precise function of FAM83A-AS1 in ESCC cells, we designed three siRNA targeting FAM83A-AS1 and a negative control sequence and named them as si-FAM83A-AS1#1, si-FAM83A-AS1#2, si-FAM83A-AS1#3, and si-NC. Next, we assessed the gene silencing efficiency by qRT-PCR assays in KYSE30 and EC109 cells. Clearly, si-FAM83A-AS1#2 and si-FAM83A-AS#3 exhibited higher gene silencing efficiency (Fig. 2A). Consequently, we focused on these two siRNAs for the following experiments. CCK-8

results demonstrated that cell proliferation ability was dramatically blunted after depleting FAM83A-AS1 in KYSE30 and EC109 cells (Fig. 2B-C). These data suggested that FAM83A-AS1regulated cell proliferation of ESCC cells in vitro.

3.3 FAM83A-AS1 enhances ESCC cells metastasis capabilities

Given the importance of FAM83A-AS1 in ESCC, we assessed the effect of FAM83A-AS1 on ESCC cell migration and invasion. Transwell migration and invasion assays were performed according to the description before [17]. Interestingly, we observed that KYSE30 cell migration capacity was significantly attenuated following knocking down FAM83A-AS1. Similarly, the migration assays in EC109 cells produced the same results (3A-C). Furthermore, the Transwell invasion assay indicated that the invasive capabilities of both KYSE30 and EC109 cells exhibited a decreasing trend in si-FAM83A-AS1 compared to the si-NC group, which was consistent with the migration assays. Overall, these investigations revealed that FAM83A-AS1 promoted ESCC metastasis process.

3.4 FAM83A-AS1 amplifies ESCC cells apoptosis events

Annexin V/PI double staining assay was adopted to evaluate the influence FAM83A-AS1 on ESCC cells apoptosis progress. Correspondingly, we examined apoptosis events in ESCC cells using flow cell cytometry. Notably, as shown in Fig. 4A-D, we discovered that the percentage of cells in KYSE30 cells had an increase in KYSE30 cells after FAM83A-AS1 depletion. Meanwhile, inhibiting FAM83A-AS1 also led to the growth of the proportion of apoptosis cells in EC109 cells. (Fig. 4E-H). Beyond these findings, we argued that FAM83A-AS1 regulated ESCC cell apoptosis.

3.5 FAM83A-AS1 expediates ESCC cells cycle distribution

To explore the potential mechanism of FAM83A-AS1 in promoting ESCC cell proliferation, we performed cell cycle assays based on PI staining with flow cell cytometry. The analysis results illustrated that FAM83A-AS1 remarkably regulated cell cycle distribution. Specifically, the population of G1 phase cells was strikingly augmented in the FAM83A-AS1-deficient group compared with the control group. In contrast, the cells in the S phase were sharply reduced after the silencing of FAM83A-AS1 (Fig. 5A-D). Taken together, these observations suggested that FAM83A-AS1 promoted cell proliferation through accelerating cell cycle process.

3.6 FAM83A-AS1 regulates miR-214 expression pattern in ESCC cells

It was conclusively established that lncRNA could act as competing endogenous RNA by binding miRNAs, leading to the liberation of target mRNAs [18,19]. First, we analyzed the location of FAM83A-AS1 in cells. Results indicated that FAM83A-AS1 was mainly accumulated in the cytoplasm (Fig. 6A). Moreover, we predicted potential miRNA candidates of FAM83A-AS1 by the Annolnc website. Here we found that miR-214 was a target miRNA of FAM83A-AS1. Additionally, it was reported that miR-214 suppressed ESCC progression by regulating cell proliferation, migration, and invasion [20]. Therefore, we speculated that FAM83A-AS1 could regulate miR-214 expression to promote ESCC progression. As

illustrated in 6B, there was an impressive increase of miR-214 expression level after FAM83A-AS1 depletion in KYSE30 and EC109 cells. Subsequently, miR-214 expression was negativity correlated with FAM83A-AS1 in ESCC tissues (6C, R^2=0.6836, P<0.0001). To confirm the role of miR-214 in ESCC cells, we designed the miR-214 inhibitor and the corresponding control sequence. As shown in 6D, miR-214 expression was decreased after transfected with miR-214 inhibitor compared with the control group. Interestingly, we noted that cell migration potential was increased after FAM83A-AS1 knockdown combined with miR-214 inhibition compared with FAM83A-AS1 depletion (Fig. 6E-F). Collectively, these results illuminated that miR-214 could partially rescue the inhibition of cell migration caused by FAM83A-AS1 silencing. Overall FAM83A-AS1 drives ESCC progression by regulating miR-214.

3.7 The role of CDC25B in ESCC and the association between CDC25B, miR-214, and FAM83A-AS1

CDC25B was reported to be the direct target of miR-214 [21]. CDC25B was dramatically elevated in multiple cancer, which was validated by the pan-cancer analysis from GEDS [22] and Uclcan [23] (Fig. 7A,7C). Notably, the CDC25B expression level was significantly higher in ESCC tissues. In addition, the data retrieved from GEPIA, Uclcan, and oncomine supported that CDC25B was predominantly accumulated in ESCC tissues compared to normal esophagus tissues (Fig. 7B, 7D, 7E). These investigations provided evidence that CDC25B was an important driving force in ESCC development. Moreover, we found that CDC25B obtained a high diagnostic accuracy for ESCC (Fig. 7F, AUC=0.9263, P<0.0001). Interestingly, the CDC25B expression pattern was increased after inhibiting miR-214 in ESCC cells (Fig. 7G). In contrast, the CDC25B level exhibited a decreasing trend when ESCC cells were treated was si-FAM83A-AS1. In summary, FAM83A-AS1 up-regulated CDC25B expression by regulating miR-214 as molecular sponge.

4. Discussion

Recently, accumulating literature have pointed out lncRNA is responsible for multiple cancer progression [24, 25]. It was widely observed that the ectopic expression pattern of lncRNAs occurred in tumor tissues and cells. More importantly, the dysregulation of lncRNAs was often associated with some clinical features, such as tumor size, lymphatic metastasis,and tumor stage [25]. For example, SNHG3 was significantly up-regulated in thyroid carcinoma [26], lung cancer [27], gastric cancer [28]. PVT1 expression level is associated with TNM stages, differentiation grade, and distant metastasis in gallbladder carcinoma [29]. Moreover, lncRNAs exhibited great potential to be the candidate of biomarkers for diagnostic and prognosis evaluation of cancer. For instance, DLEU1 expression was correlated with the survival time of colorectal cancer patients [30]. These evidence indicated lncRNA may play an oncogenic or tumor-suppressing role in different tumors. Specifically, they can serve as the key regulators of cancer-related signal pathways to control the biological function and cellular process. Hu et al. manifested that HOXC-AS3 promoted gastric cancer cell proliferation and metastasis by interacting with YBX1 at the transcriptional level [31]. However, the expression pattern and molecular mechanism of many lncRNAs in ESCC remains elusive.

Here, we first defined the expression pattern of FAM83A-AS1 in ESCC. FAM83A-AS1 was strikingly augmented in ESCC tissues and the FAM83A-AS1 level was tightly associated with differentiation grade and advanced stages. In addition, FAM83A-AS1 had great potential to be the candidates of biomarkers in ESCC diagnosis. However, there was no evident relationship between FAM83A-AS1 and survival time. More following-up investigations and survival data need to be required to definite it. Our research demonstrated that FAM83A-AS1 promoted proliferation, migration, and invasion of ESCC cells. Moreover, flow cell cytometry analysis showed that inhibiting FAM83A-AS1 led to the remarkable increase of cells in the S phase accompanied by the decrease of cells in the G1 phase, which indicated that FAM83A-AS1 promoted cell proliferation by expediating cell cycle distribution. Notably, we noticed that the apoptosis events had progressively increased after knocking down FAM83A-AS1 in ESCC cells. Collectively, our work demonstrated that FAM83A-AS1 is a pivotal driver during ESCC development.

Accumulating evidence documented that lncRNA executed their function through the diverse mechanisms including ceRNA, leading to the abnormal expression of the gene [32]. Binding with miR-let-7 in the cell cytoplasm, lncRNA GSTM3TV2 served as ceRNA to up-regulate LAT2 and OLR1 expression, promoting gemcitabine resistance in pancreatic cancer. In the current study, we found that miR-214 was a direct target miRNA of FAM83A-AS1 in ESCC [33]. It was reported that miR-214 inhibited cell proliferation, migration, and invasion process in ESCC [20]. Here we discovered that there was a negative relationship between FAM83A-AS1 and miR-214 expression in ESCC tissues. And miR-214 expression was significantly elevated after knocking down FAM83A-AS1 in ESCC cells, suggesting that FAM83A-AS1 regulated miR-214 expression in ESCC. In terms of function, miR-214 inhibition partly comprised the ablation of cell migration activity induced by FAM83A-AS1 silencing. Combined with the current research investigations, we concluded that FAM83A-AS1 promoted ESCC progression by regulating miR-214. However, the luciferase reporter assay will be needed to confirm the exact binding site between FAM83A-AS1 and miR-214.

Given the regulation between miR-214 and FAM83A-AS1, we were determined to identify the downstream target genes of miR-214 to get a deeper understanding of the molecular mechanism of FAM83A-AS1 in ESCC. CDC25B was reported to be a direct target mRNA of miR-214 and it was strongly expressed in various cancers including ESCC [21]. It was evident that CDC25B was an important oncogene to improve cancer progression [34]. CDC25B was significantly elevated in sera and cell lines of ESCC, and there was a strong relationship between CDC25B expression and prognosis of patients with advanced ESCC [35]. In ovarian cancer, CDC25B inhibitor, WG-391D blunted cell proliferation, migration, resulting in cell cycle arrest at the G2/M and apoptosis [36]. Herein, we discovered that CDC25B was significantly higher in ESCC tissues and CDC25B could serve as an independent biomarker in ESCC diagnosis. These analyses disclosed that the tumor-promoting effects of CDC25B in ESCC development. Notably, we found that FAM83A-AS1 knockdown impeded CDC25B expression. In contrast, CDC25B was strikingly increased in the ESCC cells treated with miR-214 inhibitor. Considering the regulation of FAM83A-AS1 on miR-214, we concluded that FAM83A-AS1 promoted ESCC development by regulating the miR-214/CDC25B axis for the first time.

5. Conclusion

Taken together, our study revealed that FAM83A-AS1 exerted the oncogenic effect in ESCC through enhancing cell growth and metastasis while inhibiting cell apoptosis. We identified the regulatory mechanism of the FAM83A-AS1/miR-214/CDC25B axis in ESCC development and progression. These investigations suggested that FAM83A-AS1 might be a promising biomarker for ESCC diagnosis and therapy.

Declarations

Ethics approval and consent to participate

This study got the approval of the ethics committee of the First Affiliated Hospital of Zhengzhou University and the informed consent was gained from all enrolled patients

Consent for publication

Not applicable.

Availability of data and materials

Data generated or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest.

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Authors' contribution

HL, JJ, and JC conducted the experiments and data analysis. JX and CW collected the clinical samples. HQ , FC and JH helped to conduct the experiment. HL wrote the manuscript. All authors read and approved the final manuscript.

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Table

Table1

The association of FAM83A-AS1 expression and clinical parameters in 51 ESCC patients.

Variable	N		FAM83A-AS1 expression		P-value
			High (26)	Low (25)	
Gender	Male	21	13	8	0.1917
	Female	30	13	17	
Age	≤ 65 years	24	10	14	0.2097
	> 65 years	27	16	11	
Tumor size	≤ 3 cm	15	7	8	0.6908
	> 3 cm	36	19	17	
Differentiation grade	moderate	40	17	23	0.0209*
	Poor	11	9	2	
TNM stage	□+□	39	16	23	0.0104*
	□+□	12	10	2	
Lymphatic metastasis	negative	27	13	14	0.6678
	positive	24	13	11	

*P < 0.05

Figures

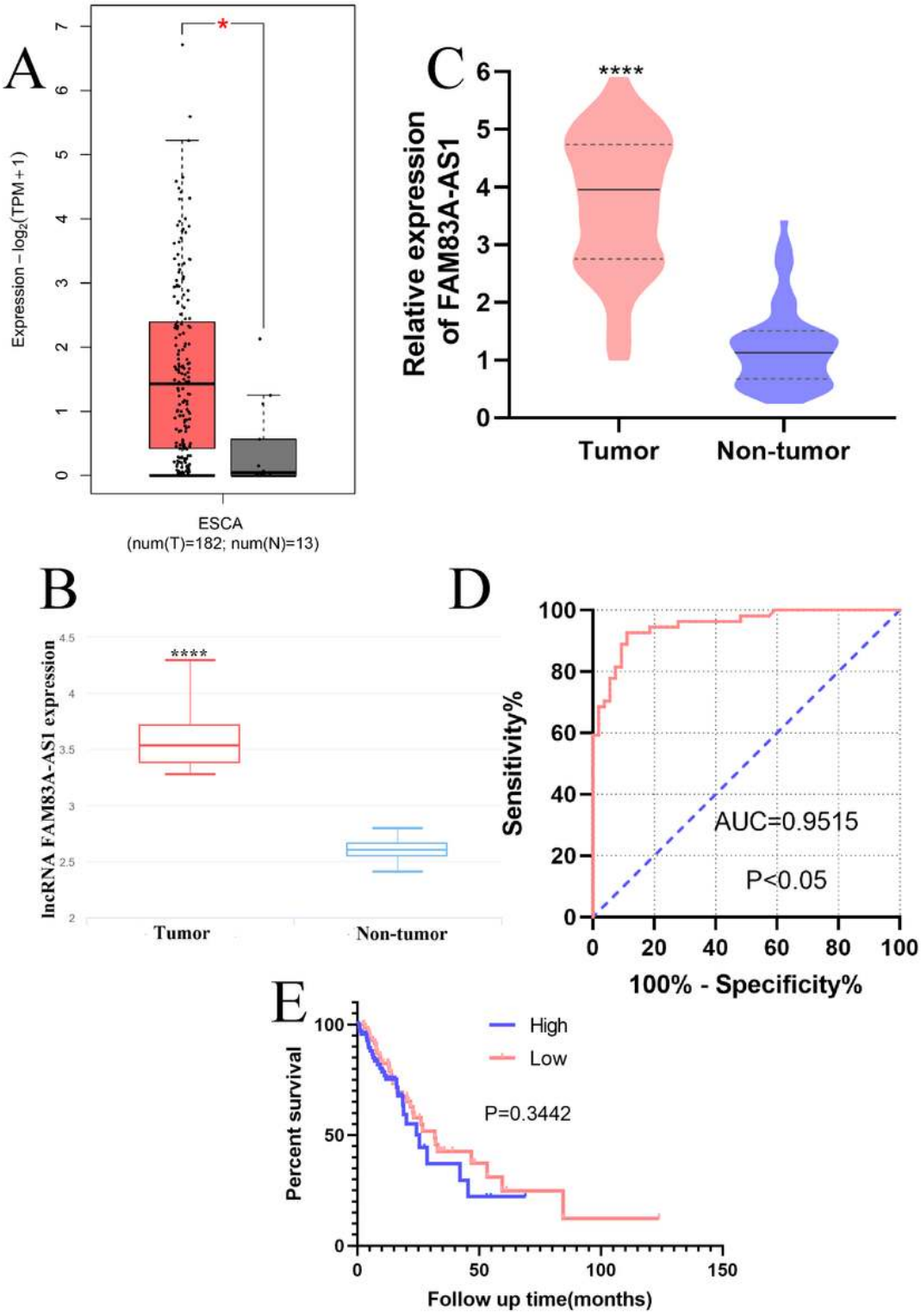

Figure 1

FAM83A-AS1 was strongly overexpressed in ESCC tissues. The bioinformatic analysis retrieved from GEPIA and lnCAR illuminated that FAM83A-AS1 was strikingly accumulated in ESCC tissues (A, C). Indeed, FAM83A-AS1 was significantly increased in 51 ESCC tissues compared with the adjacent tumor tissues (B). ROC analysis showed that high accuracy of FAM83A-AS1 in ESCC diagnosis (D). Kaplan-Meier analysis revealed the association between FAM83A-AS1 expression and survival time of ESCC

patients (E). FAM83A-AS1 level was correlated with tumor differentiation grade and advanced stages in ESCC patients (Table 1). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

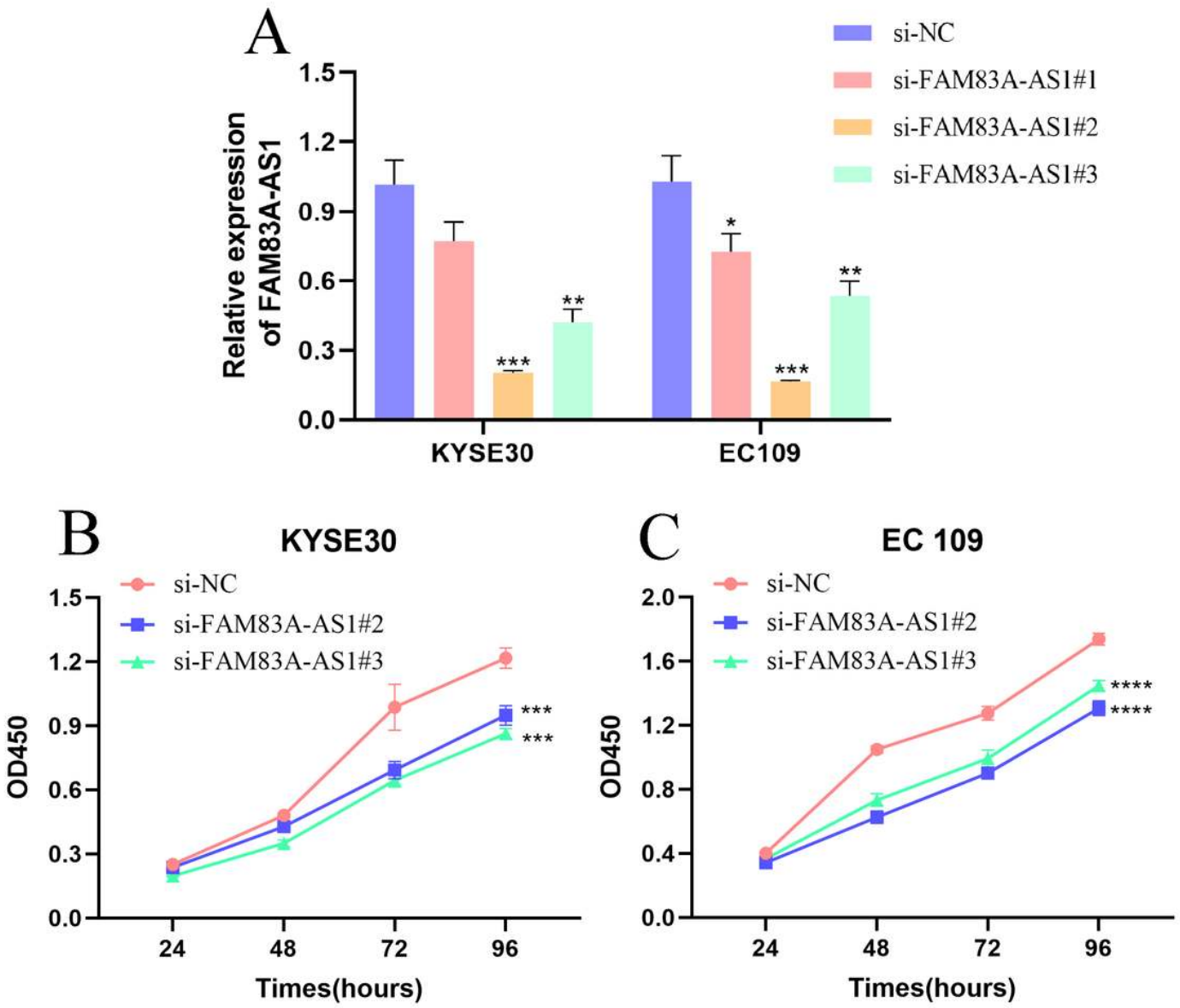

Figure 2

inhibiting FAM83A-AS1 impeded ESCC cell proliferation. The treatment of si-FAM83A-AS1 in KYSE30 and EC109 cells hampered FAM83A-AS1 expression (A). CCK8 assay results demonstrated that FAM83A-AS1 promoted ESCC cell generation (B-C). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

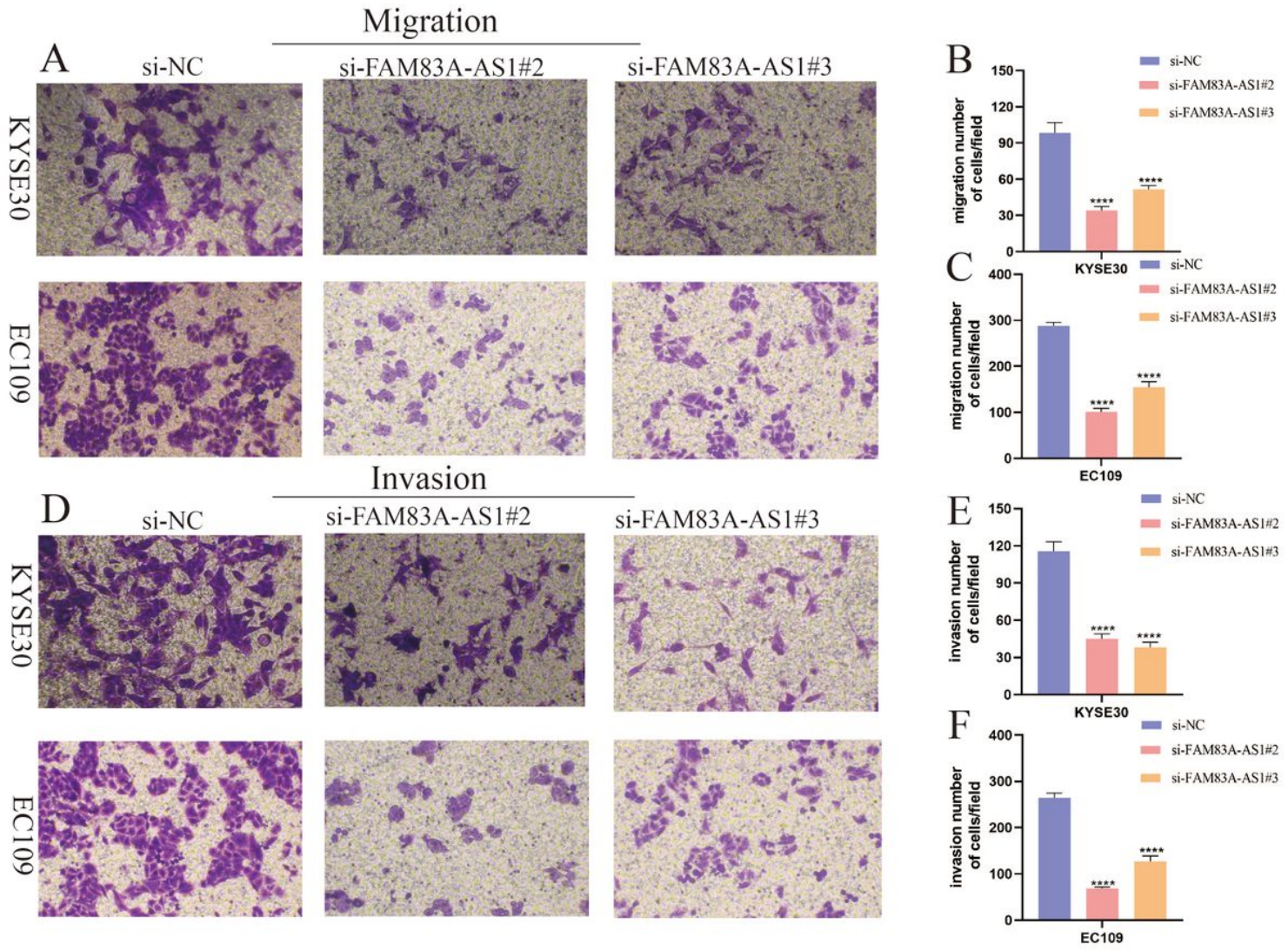

Figure 3

FAM83A-AS1 enhanced ESCC cell metastasis capacity. It was clear that FAM83A-AS1 depletion suppressed cell migration activity in KYSE30 and EC109 cells (A-C). Besides, it was notable that the invasive ability was significantly increased after knocking down FAM83A-AS1 in KYSE30 and EC109 cells (D-F). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

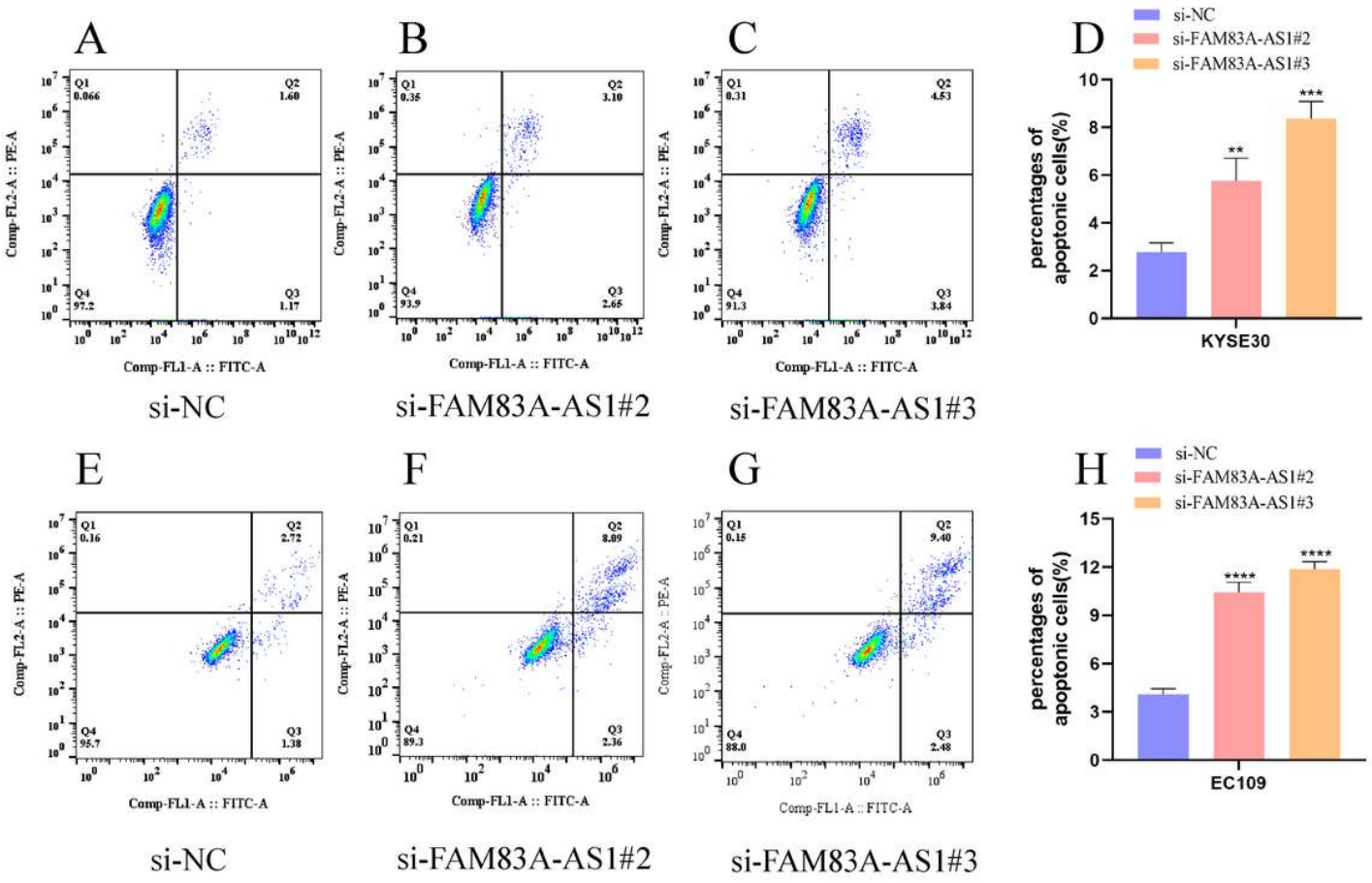

Figure 4

FAM83A-AS1 positively regulated cell apoptosis in ESCC. Flow cell cytometry was used to examine the apoptosis events in ESCC cells with different treatments. The total number of apoptosis cells was growing after inhibiting FAM83A-AS1 in KYSE30 cell (A-D). Similarly, the flow cell cytometry analysis based on EC109 treated with si-FAM83A-AS1 produced the resembling results(E-H). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

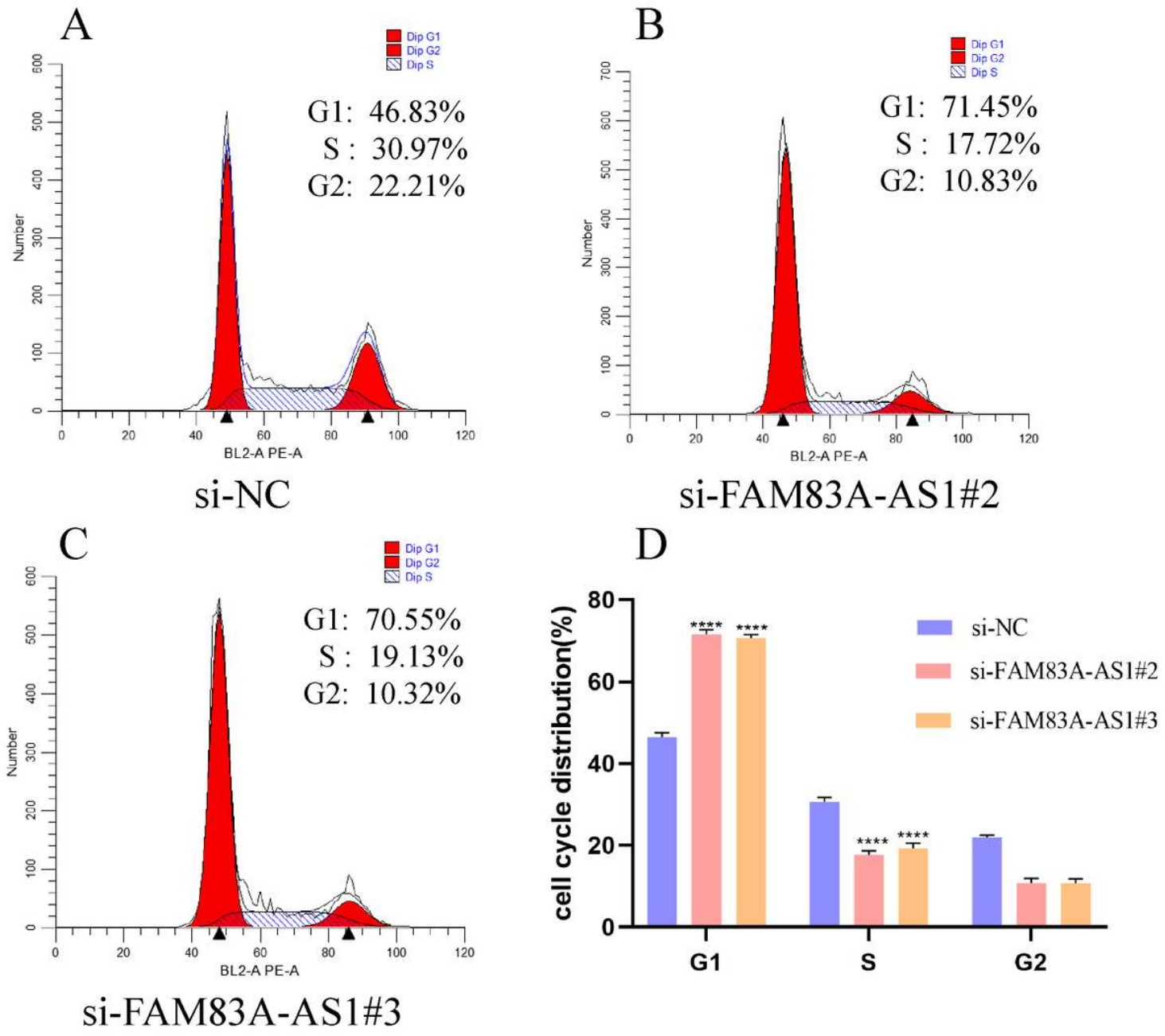

Figure 5

FAM83A-AS1 regulated cell cycle distribution in ESCC. We assessed the cell cycle distribution with flow cell cytometry according to the instruction of the cell cycle detection kit. As shown, there was a remarkable rise of cells in the G1 phase while the count of cells in the S phase was reduced(A-D). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

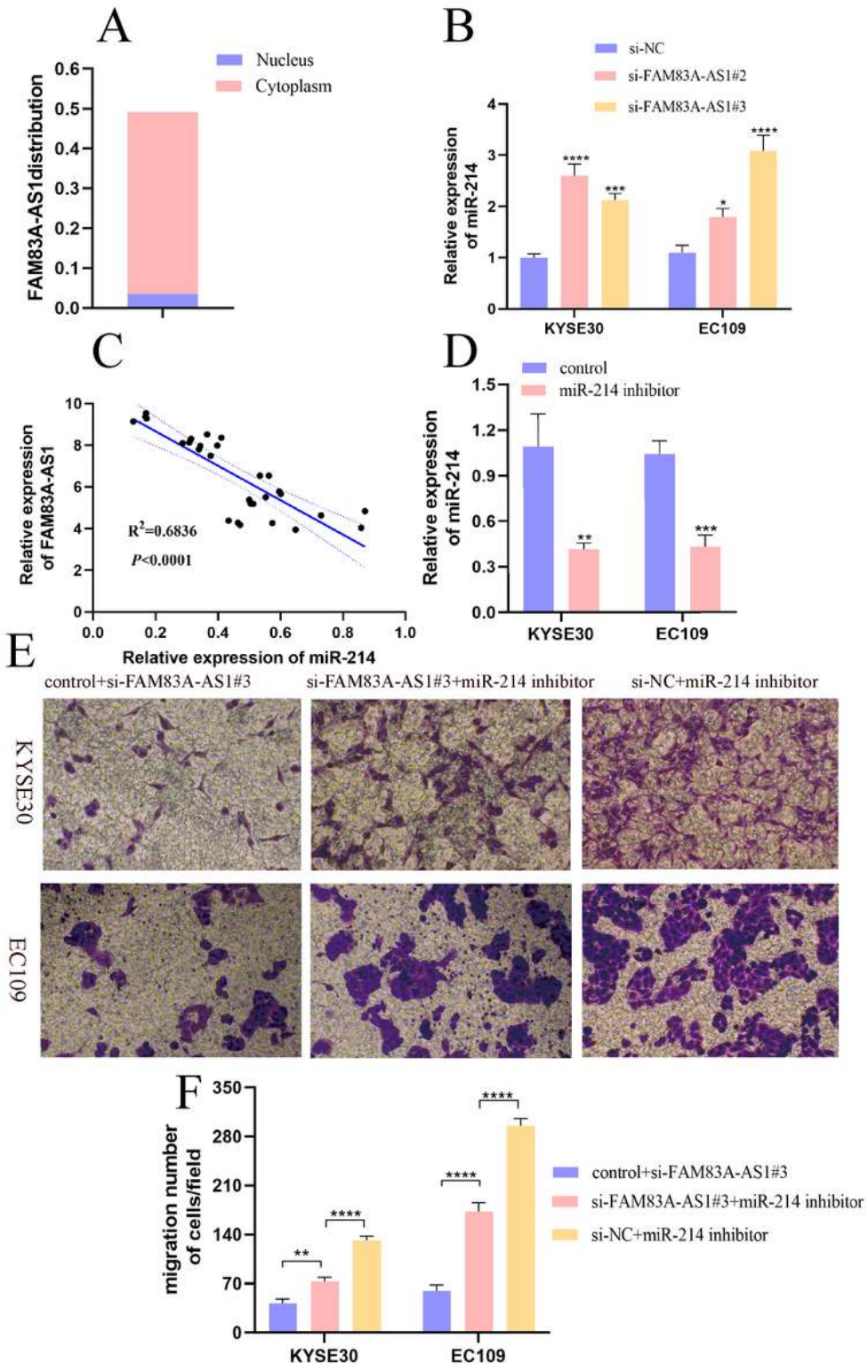

Figure 6

FAM83A-AS1 promoted ESCC development by regulating miR-214. The subcellular localization of FAM83A-AS1 in the cell (A). FAM83A-AS1 silence produced an increase of miR-214 in ESCC cell (B). The miR-214 expression pattern was negatively related to FAM83A-AS1 in ESCC tissues (C). miR-214 inhibitor repressed miR-214 expression in ESCC cell (D). The weakening of cell migration in ESCC cells induced by

FAM83A-AS1 depletion could be reversed by miR-214 inhibitor partly (E-F). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

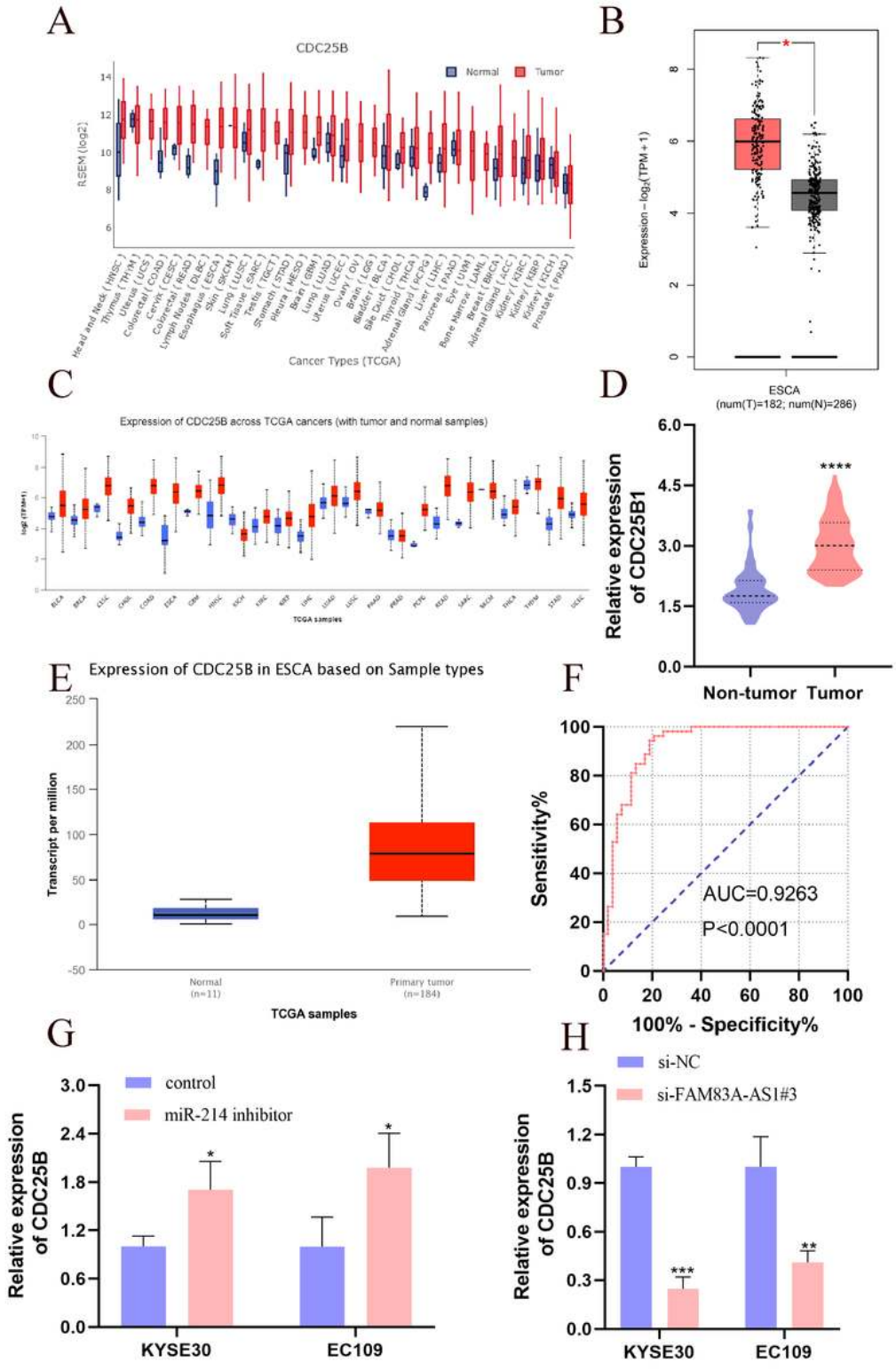

Figure 7

The high expression of CDC25B in ESCC and the regulations between CDC25B, miR-214, and FAM83A-AS1. The pan-cancer analysis of CDC25B (A, C). CDC25B was significantly over-expressed in ESCC tissues, which was validated by three databases: GEPIA, oncomine, and Uclcan (B, D, E,). The diagnostic

value of CDC25B based on the ROC analysis in ESCC (F). miR-214 inhibitor engendered the augment of CDC25B in ESCC cell (G). In contrast, FAM83A-AS1 knockdown caused the reduction of CDC25B expression in ESCC cell (H). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

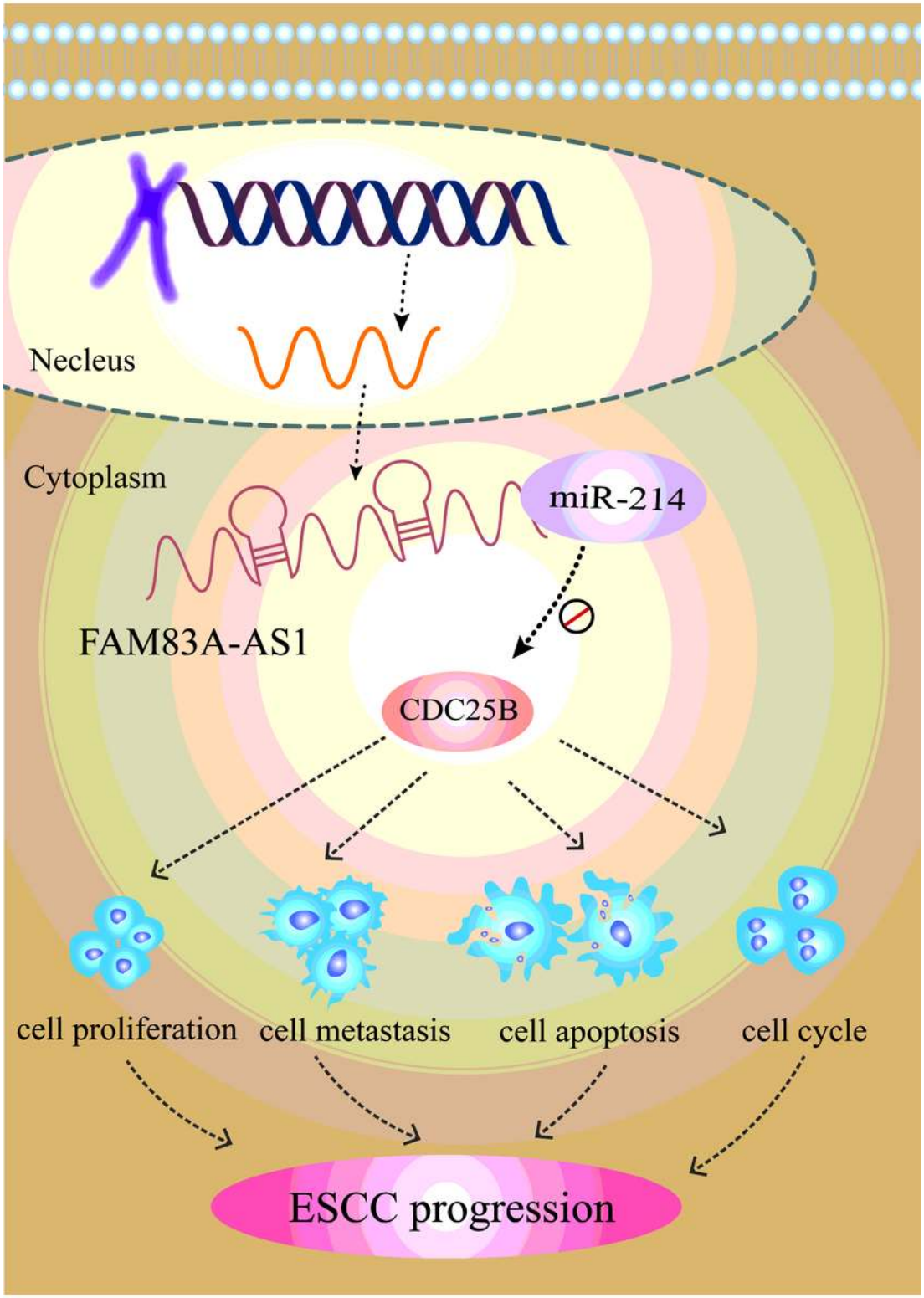

Figure 8

lncRNA FAM83A-AS1 dives ESCC progression by regulating miR-214/CDC25B1 axis