***FAM83A-AS1* Promotes Tumor Progression Through MET Signaling in Lung Adenocarcinoma**

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Abstract

Background

Studies demonstrate that long non-coding RNAs (lncRNAs) play critical roles in the occurrence and development of cancer. However, many of the molecular mechanisms underlying lncRNAs role in this process remains unclear.

Methods

Here, we analyzed lncRNA expression in lung cancer tissues based on RNA-Seq analysis and found that lncRNA FAM83A-AS1 was one of the top up-regulated lncRNAs in lung adenocarcinoma and elevated expression of FAM83A-AS1 was significantly associated with poor patient survival. We validated these results using RT-PCR and an independent cohort of lung cancer.

Results

Functional studies indicated that knockdown of FAM83A-AS1 decreased cell proliferation, colony formation, migration and invasion in H1299 and H838 lung cancer cells. Knockdown of FAM83A-AS1 induced the autophagy and cell cycle arrest at G2. Mechanistically, we found that MET, p62 and phosphor S6K proteins were decreased upon FAM83A-AS1 knockdown.

Conclusion

In conclusion, FAM83A-AS1 may have potential as a diagnosis/prognosis marker and its oncogenic role could lead to potential targeting for lung cancer therapy.

Introduction

Lung cancer ranks second worldwide in morbidity and first in mortality [1]. The two major types are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which the latter including lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and lung large cell carcinoma (LLC). Lung cancer makes up approximately a quarter of all cancer-related deaths [1]. When most patients are diagnosed, 80% are at advanced stage where prognosis is relatively poor [2]. In recent years, breakthroughs in molecular diagnosis and targeted therapy of lung cancer have been made [3–5], but the five-year overall survival of lung cancer still remains at only about 19% [1].

Long non-coding RNAs (lncRNAs) are one type of RNA with nucleotides longer than 200 base pairs and are usually not translated into protein [6]. Recent studies have suggested that lncRNAs play critical roles in tumor development and progression via different mechanisms [7–10]. For example, lncRNA *PVT1*

promotes tumor progression via miR-143/HK2 axis in gallbladder cancer [7]. LncRNA *DNM3OS* contributes to tumor metastasis through regulation of the epithelial-to-mesenchymal transition in ovarian cancer [9]. Knock-down of lncRNA *MIR22HG* induces cell survival/death via YBX1-MET signaling in lung cancer [11]. With the advantage of applying next generation sequencing, we have uncovered several dysregulated lncRNAs in lung cancer [11–17]. However, the specific mechanisms of their actions are not completely understood.

There are several reports regarding lncRNA *FAM83A-AS1* in cancer [18–22]. It was reported *FAM83A-AS1* expression is higher in esophageal cancer and can promote cancer progression via binding of miR-495-3p [18]; whereas knockdown of *FAM83A-AS1* impaired cell growth and induced cell apoptosis through binding of NOP58 in liver cancer [19]. *FAM83A-AS1* was suggested to affect lung cancer cell migration and invasion via miR-150-5p/MMP14 signaling [20] and may promote lung cancer progression through increasing *FAM83A* expression [21]. However, the detailed mechanism of *FAM83A-AS1* in lung cancer remains unclear. In this study, by combining our data with publicly available RNA sequencing (RNA-Seq) data we uncovered and validated the up-regulation of *FAM83A-AS1* in lung cancer. Further, we found that elevated expression of *FAM83A-AS1* was significantly related to poor patient survival. Finally, the oncogenic role and underlying mechanism of *FAM83-AS1* action in lung cancer cells were investigated in this study.

Materials And Methods

Cell lines

We selected the three cell lines H1299, H838 and PC9 which express high levels of *FAM83A-AS1* to carry out all experiments. Cell culture medium contained RPMI 1640 (Invitrogen), 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cell culture was carried out using standard cell culture conditions at 37°C, 5% CO₂ and appropriate humidity. All the cell lines were purchased from the American Type Culture Collection and screened for mycoplasma contamination. Genotyping was performed at the University of Michigan Sequencing Core to confirm cell line identity.

Tissue samples

Clinical samples included lung cancer tissues and paired non-tumoral lung tissues from patients undergoing curative cancer surgery during the period from 1992 to 2015 at the University of Michigan Health System. The study was reviewed and approved by the University of Michigan Institutional Review Board and Ethics Committee. All of the patients were without preoperative chemoradiotherapy treatment and had provided informed consent. The median follow-up time was 8.5 years. Surgical samples were quickly frozen in liquid nitrogen and maintained at –80 °C prior to both cryostat sectioning and pathological analysis and RNA extraction. All patient samples used in qRT-PCR validation (101 LUADs) were associated with clinical information and reported in our previous studies [11, 12].

RNA extraction and qRT-PCR

The RNeasy Mini kit (Qiagen) was used to isolate total RNA according to the manufacturers' instructions. For quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR), the isolated total RNA was subjected to reverse transcription to cDNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). The qRT-PCR reactions were carried out with the Power SYBR Green master mix which purchased from Life Technology Inc. The cDNA was amplified by real-time quantitative PCR using a ABI StepOne Real-Time PCR System (Applied Biosystems) following the manufacturers instruction. Each reaction used contained 10ng cDNA in a final reaction volume of 15μL. The oligonucleotide primers for *FAM83A-AS1* included: forward, 5'-GGAAGCAGGGCTCTTCAGTT, and reverse, 5'-AGGGCCGTCTGTGTTTACTG. GAPDH served as housekeeping normalization control to ensure equal loading.

Published RNA-Seq data collections

RNA sequencing data sets were downloaded from previously published papers [12, 23-26], which included UM, Seo, TCGA and CCLE cell lines [26] data portals. Lung samples consists of 6 normal lung tissues, 67 LUAD, 36 SCC and 10 LLC in the UM dataset [25]; 77 normal lung tissues and 85 LUADs in Seo dataset [24]; 73 normal lung tissues and 309 LUADs in TCGA dataset [23]. FPKM was calculated to identify transcripts and to measure their relative expression levels [25, 27].

siRNA-mediated knockdown

PC-9, H838 and H1299 cells were plated at the appropriate cell concentration and were separated into non-target control group and *siFAM83A-AS1* group. *FAM83A-AS1* siRNA sequences were all located in the first exon of this lncRNA. A mixture of 10nM experimental siRNA oligonucleotides or non-targeting controls, Lipofectamine® RNAiMax Reagent (Invitrogen, USA) and Opti-MEM were included in each respective 96-well at the appropriate proportion. Knockdown was performed 24 h after plating and qRT-PCR was used to detect the knockdown efficiency.

Cell proliferation assay

Cell proliferation experiments were analyzed in 96-well plates. H838 and H1299 were plated at a desired concentration. Once cells were attached to the substratum, medium was changed to antibiotic-free culture medium, transfected with 10nM negative control siRNA oligonucleotides or *siFAM83A-AS1* oligonucleotides. Transfection was implemented as described above. The proliferation rate was measured 72 h after transfection. Cell viability was measured using WST-1 reagent (Roche). Percentage of cellular viability was calculated as a percentage relative to the control group.

Colony formation assay

Forty-eight hours after treatment with *FAM83A-AS1* siRNAs and control siRNAs, H1299 and H838 cells were suspended with trypsin-treatment and then plated in 6-well plates (500 cells per well). After 10 to 14 days of incubation, 20% methanol was used to fix the clones for 20 minutes and 0.1% crystal violet was

used to stain the clones for 30 minutes. After washing away the excess crystal violet, the colonies were photographed and counted. More than 50 cells were regarded a colony.

Migration and invasion

For invasion assays, Matrigel (BD company) was maintained at 4°C overnight for dissolution prior to dilution with cold serum-free RPMI 1640 (Matrigel: RPMI 1640=1:8). Basement membrane matrix Boyden chambers (8-mm pore size) were purchased from BD Company and 100 ml Matrigel were added to each upper chamber. Resuspended cells in serum-free RPMI-1640 were adjusted to a cell density of $1-5 \times 10^5$ /ml, with total cells being $2-10 \times 10^4$ per well (H1299: 5×10^4 per well; H838: 10×10^4 per well). 200µl of RPMI-1640 with cells was added into the upper chamber and 600µl of 20% FBS-containing RPMI-1640 was added into the lower chamber. Cells were incubated for 48 h at 37 °C, then fixed, stained, and counted. For invasion assays, the only difference was that no Matrigel was included in the basement membrane matrix Boyden chambers.

Flow cytometry for cell cycle analysis

Forty-eight hours after treatment with *FAM83A-AS1* siRNAs and control siRNAs, H1299 and H838 cells were suspended with trypsin treatment, washed twice with ice-cold PBS and the supernatant removed after centrifugation at 1000×r for 10 min. The cells were fixed with 70% ice-cold ethanol, and then stored overnight at 4°C. After washing with PBS, 1ml of propidium iodide (PI) staining solution (containing 0.1% (v/v) Triton X-100, 10 µg/mL PI, and 100 µg/mL DNase-free RNase A in PBS) was added into the tubes and incubated for 30 min at room temperature in the dark. Flow cytometer was used to measure the cell cycle. The experimental operation was as gentle as possible to reduce cell damage.

Autophagy measurement

Autophagic flux was detected using Premo Autophagy Tandem Sensor RFP-GFP-LC3 (Life Technologies, P36239). Cells were plated at the required concentration, transfected at 24h, and adenovirus added at 48h. The culture medium containing adenovirus was replaced with new medium after 6-8 h. Autophagy fluorescence was observed using an Olympus IX71 microscope 24 and 48 h after incubation. Autolysosomes (red dots in fusion images) and autophagosomes (yellow dots in fusion images) were counted in at least 100 cells in a 200X field.

Western blot analysis

Protein collected for western analysis was with RIPA lysis buffer at 72h after siRNAs transfection. Cells were lysed on ice and the supernatant was collected following centrifugation for 20 min at 14000g. The extracted protein was quantified, and 10 mg protein per sample were separated according to their molecular weight by 10% polyacrylamide gel electrophoresis. Proteins were transferred onto polyvinylidene difluoride membrane and blocking for 1 h with 5% non-fat milk prior to adding primary antibodies and incubating overnight at 4 °C. Antibodies and concentration were as follows: total MET

(Cell Signaling #8198, at a 1:1000 dilution), phospho-MET (MET Proto-Oncogene, Receptor Tyrosine Kinase, Cell Signaling #3077, at a 1:1000 dilution), GAPDH (at a 1:10000 dilution), p62 (Sequestosome 1, SQSTM1, Cell Signaling #5114, at a 1:1000 dilution), phospho-s6k (p70 S6 Kinase, Cell Signaling #2708, at a 1:1000 dilution). The second antibody (1:2000 dilution) was then added the following day and incubated at room temperature for 1h. The membranes were developed using Luminata Crescendo Western HRP Substrate (Millipore Sigma, WBLUR0500) and exposed with the ChemiDoc MP Imaging System (BIO-RAD).

Statistical analysis

Data were collected and statistically analyzed using GraphPad Prism 6 and R software. Receiver Operating Characteristic (ROC) curves of the results were created and the area under the curve (AUC) was calculated to describe diagnostic efficacy. The Kaplan-Meier method was utilized to plot survival curves of patients and a log-rank test was used to compare survival times between different groups. The expression level of *FAM83A-AS1*, cell proliferation, colony formation, migration and invasion was evaluated with unpaired Student's t-test. The cut-off for considering a significant difference was established with a p -value < 0.05.

Results

FAM83A-AS1 is increased in lung cancer and associated with poor patient survival

RNA-Seq data was acquired from previously published papers including the UM, Seo and TCGA data sets [12, 23–25]. FPKM values for lncRNA expression were calculated to identify transcripts and measure their relative expression levels. The results showed that *FAM83A-AS1* was up-regulated in lung adenocarcinoma (LUAD) (Fig. 1A-C). Receiver Operating Characteristic (ROC) curves of the results were created based on the expression of *FAM83A-AS1* in LUAD vs. normal lung and the area under the curve (AUC) in these 3 data sets were 0.95, 0.93 and 0.95, respectively (Fig. 1D-F). To verify these results, *FAM83A-AS1* expression from an independent cohort of lung adenocarcinoma tissues including 101 LUADs and 27 normal lung samples was analyzed by qRT-PCR. *FAM83A-AS1* expression was higher in LUAD relative to normal lung tissues and the area under the curve (AUC) was 0.97 (Fig. 1G and H). Further, the high expression of *FAM83A-AS1* was significantly related to poor prognosis in patients with lung cancer (Fig. 1I). Collectively, these data suggest that *FAM83A-AS1* is highly expressed in lung adenocarcinoma and is associated with poor patient survival. *FAM83A-AS1* may have potential as a biomarker for lung cancer.

FAM83A-AS1 expression is higher in different type of lung cancers and is localized in the cytoplasm

RNA-Seq results of lung cancer samples from UM cohort, which including 6 normal lung tissues, 67 lung adenocarcinoma tissues, 10 large cell lung cancer tissues and 36 lung squamous cell carcinoma tissues [12], showed that *FAM83A-AS1* expression is not only higher in lung adenocarcinoma, but also higher in large cell lung cancer (LLC) and squamous cell lung cancer (LUSC) as compared to normal lung tissue. Of these, the highest expression was found in lung adenocarcinoma (LUAD) (Fig. 2A). We then analyzed the expression of *FAM83A-AS1* in different lung cancer cell lines [26], which included 42 lung adenocarcinoma cell lines, 11 large cell lung cancer cell lines, 22 lung squamous cell lines and 48 small cell lung cancer cell lines. *FAM83A-AS1* remained highly expressed in LUAD, LUSC and LLC, but was lower in SCLC (Fig. 2B). In order to determine the cellular location of *FAM83A-AS1*, and thus its potential site of action, qRT-PCR was performed in H1299 and H838 cells. GAPDH is used as cytoplasmic control and PVT1 lncRNA as nuclear control. The results showed that *FAM83A-AS1* was primarily expressed in the cell cytoplasm (61% – 77%) (Fig. 2C and D). This suggests some its action may influence signaling occurring in this location.

Fam83a-as1 Knockdown Reduces Cell Proliferation And Colony Formation

FAM83A-AS1 knockdown efficiency by siRNAs in PC-9, H1299 and H838 cell lines was measured by qRT-PCR. We found that *FAM83A-AS1* was decreased by 80% at 48 h after siRNAs treatment (Fig. 3A). After *FAM83A-AS1* siRNA treatment, cell proliferation was decreased in PC-9, H1299 and H838 cell lines ($p \leq 0.05$) (Fig. 3B). The colony formation ability and cell cycle were measured at 48 h after siRNAs transfection. The results showed that colony formation ability was decreased in H1299 and H838 cell lines ($p \leq 0.01$) (Fig. 3C and D), and cell cycle was arrested at the G2 phase in H1299 cells upon knockdown of *FAM83A-AS1* (Fig. 3E and F). This G2 arrest suggests *FAM83A-AS1* involves cell cycle regulation.

Knockdown of FAM83A-AS1 impairs cell migration and invasion, as well as induces autophagy

In order to investigate the potential effect of *FAM83A-AS1* on cancer cell behavior, cell migration and invasion assays were performed in H1299 and H838 cell lines. After silencing of *FAM83A-AS1* by siRNAs, cellular migration and invasion ability were significantly decreased in these two cell lines (Fig. 4A-D), suggesting that *FAM83A-AS1* could promote lung cancer invasiveness or metastasis.

Autophagy is a significant cellular degradation process, and closely related to cancer progression. In this study, autophagic flux was detected using Premo Autophagy Tandem Sensor RFP-GFP-LC3. In Fig. 4E, the green signal represents GFP fluorescence, red puncta represent autolysosomes, and yellow puncta represents autophagosomes. Following silencing of *FAM83A-AS1* by siRNA, both autophagosomes and

autolysosomes were increased in H838 cells (Fig. 4E and F), indicating that *FAM83A-AS1* inhibits autophagy as one of the mechanisms in regulating cell death signaling in lung cancer.

MET, p62 and p-S6K proteins are decreased upon FAM83A-AS1 silencing

MET is reported to play a critical role in cancer progression including cell growth and death (REFS). To explore whether MET is involved in the underlying mechanism of *FAM83A-AS1* in regulating cancer progression, Western blot analysis was performed following siRNA-mediated knock-down of *FAM83A-AS1*. We found that both total and phosphor MET were decreased upon *FAM83A-AS1* knockdown in PC-9 and H1299 cells (Fig. 5A) suggesting that MET signaling is regulated by *FAM83A-AS1*. Since we found that autophagy was induced by *FAM83A-AS1* silencing, we examined whether the autophagy marker p62 and the autophagy inhibitor phosphor-S6K protein were affected. After knocking down *FAM83A-AS1*, we found that the autophagy-related protein p62 and phosphor p-S6K were decreased (Fig. 5A), which further confirmed that *FAM83A-AS1* is involved in regulation of autophagy, while more its more detailed mechanism will need to be investigated further.

Discussion

LncRNAs have been reported to play critical roles in cancer immunity, cancer metabolism, cancer metastasis and drug resistance [28–31]. *FAM83A-AS1* has been found to play an oncogenic role in several cancers including esophageal, liver and lung cancer [18–22], however, the underlying mechanisms of this lncRNA is not clear. In this study, we found that lncRNA *FAM83A-AS1* expression was up-regulated in lung adenocarcinoma, and increased expression of this lncRNA was significantly related to poor patient survival. Functional investigation indicated that knockdown of *FAM83A-AS1* can decrease cell proliferation, migration and invasion, as well as induce the autophagy in lung cancer cells. Mechanistically, *FAM83A-AS1* promotes tumor progression may through oncogene MET signaling in lung adenocarcinoma (Fig. 5B).

So far, there are only four reports examining expression and roles of lncRNA *FAM83A-AS1* in cancers. Huang *et al.* reported that *FAM83A-AS1* expression was higher in 62 squamous esophageal cancers and higher expression was related to poorer patient survival [18]. He *et al.* reported that *FAM83A-AS1* expression was higher in 60 hepatocellular carcinomas [19]. Through the analysis of TCGA RNA-Seq data, Xiao and Shi groups [20, 21] found that the level of *FAM83A-AS1* was up regulated in both LUAD and LUSC and higher expression was related to unfavorable survival in LUAD. In this study, we analyzed the expression of *FAM83A-AS1* not only in the TCGA RNA-Seq data used in Xiao and Shi's studies [20, 21], but also in another two RNA-Seq data sets including UM (67 LUAD) [12, 25] and Seo (85 LUAD) [24] datasets, as well as an independent validation data set (101 LUAD) with qRT-PCR assay. We found that the expression of *FAM83A-AS1* was higher in all four data sets with AUC 0.93–0.97 (Fig. 1A-H), and higher expression was related to poor patient survival in our qRT-PCR validation set (Fig. 1I). In addition,

we found *FAM83A-AS1* level was higher in LUSC and LLC (Fig. 2A). Further, through the analysis of CCLE lung cancer cell line's RNA-Seq data [26], we confirmed that *FAM83A-AS1* level was higher in LUAD, LUSC and LLC, but not higher in SCLC (Fig. 2B). This suggests a cell type specific role although the basis for this remains unknown. We found *FAM83A-AS1* expression was mainly located in cytoplasm which may represent some of where it influences cell function and a finding consistent with Xiao's report [20].

FAM83A-AS1 plays oncogenic roles in esophageal, liver and lung cancers [18–21]. We found that knockdown of *FAM83A-AS1* decreased the cell proliferation, colony formation, migration and invasion in H1299 and H838 lung cancer cells (Figs. 3 and 4), which were consistent with others [18–21]. In addition, we found that the cell autophagy was induced upon *FAM83A-AS1* knockdown in H838 cells (Fig. 4E), which may be related to p-S6K signaling.

The molecular mechanisms of *FAM83A-AS1* in promoting of lung cancer progression is not clear. MET, also known as c-MET or the Receptor of Hepatocyte Growth Factor (HFG), is a tyrosine kinase and proto-oncogene located on chromosome 7q31.2 [32–34]. Its dysregulation in lung cancer was first discovered in the 1990s [35, 36]. Since then, further research has examined MET signaling pathways [37–39]. Changes in MET have become a classic signal pathway for the lung cancer progression including cell proliferation, migration, invasion and tumor growth in our previous studies and others [11, 17, 40]. In this study, we found that both total and phosphor MET proteins were decreased after knocking down of *FAM83A-AS1*. This suggests a possible connection between the oncogenic role of *FAM83A-AS1* and MET signaling. Further detailed mechanistic studies will be needed in order to use *FAM83A-AS1* as a potential diagnosis/prognosis marker or therapeutic target.

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by the University of Michigan Institutional Review Board and Ethics Committee. All of the patients were without preoperative chemoradiotherapy treatment and had provided informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SBB, HJZ, XFZ and BYL conducted the experiments and data analysis, YHW, SMH, SLZ and YL performed statistical analysis, ZQZ and CZD conceived and designed the study, DGB drafted the manuscript, GAC revised the manuscript. All authors read and approved the final manuscript.

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Figures

Fig. 1

Figure 1

FAM83A-AS1 is highly expressed in lung adenocarcinomas and is associated with poorer patient survival. (A-C), FAM83A-AS1 expression levels are shown as Scatter plots. RNA-Seq (6 normal samples and 67 LUADs) results of UM, Seo, and TCGA show that FAM83A-AS1 is up-regulated in lung adenocarcinoma (log2 of FPKM value as y-axis, groups and number of samples in x-axis; ** represents $p < 0.01$, LUAD vs. normal); (D-F), ROC curves and corresponding AUC values of FAM83A-AS1 in UM (6 N vs 67 LUAD), Seo (77 N vs 85 LUAD), and TCGA (73 N vs 309 LUAD) RNA-Seq data sets; (G), Box plot indicating FAM83A-AS1 expression is higher in tumor in an independent validation set (101 LUAD and 27 normal lung tissues) verified by RT-PCR (fold-change of FPKM to mean of all tissues as y-axis, loading control of PCR is ACTB; ** represents $p < 0.01$ by t test, LUAD vs. normal); (H), ROC analysis revealed that FAM83A-AS1 was a good predictor (AUC is 0.97) for classifying the normal lung tissues and LUAD; (I), Kaplan-Meier survival curve indicated higher FAM83A-AS1 expression is unfavorable for patient survival in the validation set (log-rank test, $p = 0.002$).

Fig. 2

Figure 2

FAM83A-AS1 expression in different type of lung cancers and cell lines as well as cellular location. (A), FAM83A-AS1 expression is higher in lung adenocarcinoma (LUAD), large cell lung cancer (LLC) and squamous cell lung cancer (LUSC) as compared to normal lung tissues (**, $p < 0.01$ by t test); (B), FAM83A-AS1 expression in different type of lung cancer cell lines (CCLE RNA-Seq data set). FAM83A-AS1 is higher in AD as compared to LLC, LUSC and small cell lung cancer (SCLC) (*, $p < 0.05$ by t test); (C, D), The location of FAM83A-AS1 was shown by qRT-PCR in H1299 and H838 cells. PVT1 snRNA as nuclear control and GAPDH was used as cytoplasmic control. FAM83A-AS1 is mainly in cytoplasm (61% - 77%).

Fig. 3

Figure 3

FAM83A-AS1 knockdown impairs cell proliferation, colony formation and cell cycle regulation. (A), Knockdown efficiency of FAM83A-AS1 siRNA at 48 h in PC-9, H1299 and H838 cell lines verified by qRT-PCR. GAPDH expression selected as the loading control; (B), Cell proliferation is decreased after FAM83A-AS1 siRNAs treatment in PC-9, H1299 and H838 cell lines. * $p < 0.05$ by t test; (C, D) Silencing of FAM83A-AS1 by siRNA decreased cell colony formation ability in H1299 and H838 cell lines, D is the relative quantified value from C image; (E, F) Silencing of FAM83A-AS1 by siRNA caused cell cycle arrested at G2 phase in H1299 cells.

Fig. 4

Figure 4

FAM83A-AS1 knockdown decreases cell migration and invasion, as well as induces autophagy. (A, B), Silencing of FAM83A-AS1 by siRNA decreased cellular migration ability in H1299 and H838 cell lines (10X), B is the relative quantified value from A; (C, D) FAM83A-AS1 knockdown by siRNA decreased cellular invasion ability in H1299 and H838 cell lines (10X), D is the relative quantified value from C; (E, F), H838 was treated with FAM83A-AS1 siRNAs for 48h and infected with Premo Autophagy Tandem Sensor RFP-GFP-LC3B for 24h. Cells were visualized alive with fluorescence microscope. Autophagosomes and

autolysosomes in each 200X field were counted, at least 100 cells were counted for siRNA or NT treatment cells. Autophagic flow was increased upon FAM83A-AS1 silencing. F is the relative quantified value from image E.

Fig. 5

Figure 5

Proteins changed upon FAM83A-AS1 knockdown. (A), Silencing of FAM83A-AS1 by siRNA, total MET and phosphor MET proteins were decreased in PC9 and H1299. The autophagy related proteins p62 and phosphor S6K were also decreased, especially in PC-9 cells. (B), the schematic of FAM83A-AS1-MET signaling in promoting tumor progression in lung adenocarcinoma.