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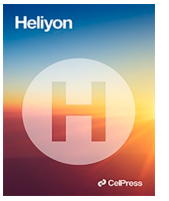

Research article

Long noncoding RNA LINC00858 aggravates the progression of esophageal squamous cell carcinoma via regulating the miR-425-5p/ABL2 axis

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ABSTRACT

Esophageal squamous cell carcinoma (ESCC) is one of the most fatal cancers with high morbidity and mortality, which severely affects people's lives. Long intergenic non-protein coding RNA 858 (LINC00858) was confirmed to promote the progression of colorectal cancer and lung cancer. However, the role of lncRNA LINC00858 is still unknown in ESCC. Herein, the main purpose of research was to explore LINC00858 function and its impact on ESCC cell biological behaviors. RT-qPCR was used to test the expression of LINC00858, miR-425-5p and ABL proto-oncogene 2 (ABL2) in ESCC cells. Functional experiments such as EdU assay, CCK-8 assay, transwell assay and Western blot assay were conducted to investigate the biological behaviors of ESCC cells. Luciferase reporter assay and RIP assay were implemented to determine the binding situation among RNAs. LINC00858 expression was abnormally high in ESCC cells and down-regulation of LINC00858 could restrain the proliferation, invasion, migration and EMT process of ESCC cells. Furthermore, miR-425-5p was proved to be sponged by LINC00858 and was down-regulated in ESCC cells. Besides, we discovered that miR-425-5p could target ABL2. Moreover, knockdown of ABL2 reversed the promoting function of miR-425-5p inhibitor on ESCC progression. LINC00858 aggravated ESCC progression via regulating the miR-425-5p/ABL2 axis.

1. Introduction

Esophageal cancer (EC) ranks as the sixth most fatal cancer worldwide with 450 thousand new cases and 400 thousand deaths every year [1]. Esophageal squamous cell carcinoma (ESCC) acts as one subtype of EC, accounting for about 90% of esophageal cancer cases [2]. Due to the late diagnosis and ESCC high invasiveness, the overall survival rate of ESCC is very low [3]. With the development of endoscopy, early screening and treatment have been improved to a certain extent. However, the survival rate and prognosis of most advanced patients are still unsatisfactory [4]. It is thus of necessity and importance to explore novel therapeutic targets for ESCC so that the overall survival rate of ESCC patients can be improved.

Long non-coding RNAs (lncRNAs) are the member of non-coding RNAs comprising more than 200 nucleotides, which cannot code proteins [5]. Previously, lncRNAs were considered as transcriptional noise without any biological function. However, a flow of researches have indicated that lncRNA not only has biological functions, but also participates in the development of various human

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diseases [6]. For instance, lncRNA SNHG14 has been reported to accelerate the development of cervical cancer and predict poor prognosis [7]. LncRNA LUCAT1 facilitates tumorigenesis of triple-negative breast cancer through regulating miR-5702 [8]. Also, lncRNA CASC9 can expedite tumorigenesis of ESCC by regulating EMT process and predict poor prognosis [9].

Long intergenic non-protein coding RNA 858 (LINC00858) is a novel lncRNA and has been confirmed to exert a carcinogenic effect on some human cancers. For example, LINC00858 can accelerate cell proliferation, migration and invasion via sponging miR-22-3p in colorectal cancer [10]. Also, LINC00858 is proved to promote cell growth of lung cancer through miR-3182/MMP2 axis [11]. LINC00858 promotes osteosarcoma through regulating miR-139-CDK14 axis [12]. In addition, LINC00858 has been reported to play a role in esophageal squamous cell carcinoma. LINC00858 promotes the development of ESCC through the ZNF184-FTO-m6A-MYC axis [13]. Nevertheless, there are still few studies on the function and mechanism of LINC00858 in ESCC at present.

MicroRNAs (miRNAs) are small non-coding RNAs with the ability of regulating gene expression at a post-transcriptional level through inhibiting messenger RNA (mRNA) translation or accelerating mRNA degradation [14]. Researches have indicated that miRNAs can participate in regulating the development and progression of different cancers, including ESCC. For example, miR-601 can inhibit cell proliferation in ESCC through targeting HDAC6 [15]. Also, miR-338-5p has been reported to repress cell proliferation, migration and cisplatin resistance in ESCC via targeting FERMT2 [16].

LncRNAs can act as competing endogenous RNAs (ceRNAs) that competitively bind to miRNAs to regulate post-transcriptional expression of genes, a mechanism that plays a role in various types of cancers [17,18]. LncRNA-CDC6 can act as a ceRNA to promote breast cancer progression by sponging miR-215 to regulate CDC6 expression [19]. LncRNA- MIAT can act as a ceRNA to promote thyroid cancer progression by sponging miR-150-5p to regulate EZH2 expression [20] LncRNA ITGB8-AS1 also acts as a ceRNA sponging miRNA to promote colorectal cancer [21]. In our research, the main purpose was to explore the detailed role and mechanism of LINC00858 in ESCC. Functional assays were utilized to determine its function in regulating the biological properties of ESCC cells, and mechanism assays were further employed to probe into the correlation between LINC00858 and other RNAs in ESCC, which may contribute to the identification of novel therapeutic targets of ESCC.

2. Materials and methods

2.1. Bioinformatics analysis

Differential expression of LINC00858 in non-tumor tissues and in ESCA tissues was predicted using GEPIA website (http://gepia.cancer-pku.cn/) and starBase database (http://starbase.sysu.edu.cn/index.php). And starBase and miRDB (https://mirdb.org/) databases predicted three possible miRNAs that might bind to LINC00858. The expression of LINC00858 in the normal esophagus tissues was predicted using UCSC website (http://genome.ucsc.edu/).

2.2. Cell culture

Human ESCC cell lines (ECa-109, EC9706, KYSE30 and KYSE450) and normal cell line (Het-1A) were utilized for this study and all of them were deposited with 5% CO_2 at 37 °C. Het-1A cells were purchased from ATCC (Manassas, VA, USA). ECa-109 cells were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). EC9706 cells were purchased from Fuxiang Biotechnology Co., Ltd (Shanghai, China). KYSE30 cells were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). KYSE450 cells were purchased from Cobioer Biosciences Co., Ltd (Nanjing, China). Het-1A cells were cultured in BEGM medium (Gibco, Grant Island, NY, USA). ESCC cell lines were all cultivated in RPMI-1640 medium with 10% FBS and 1% p/s (Gibco). All cell lines have been certified using STR profiling.

2.3. RT-qPCR

Total RNA extracted from ECa-109 and EC9706 cells utilizing TRIzol reagent (Invitrogen, Carlsbad, CA) was then subjected to reverse transcription to cDNA through the Reverse Transcription Kit (Takara, Tokyo, Japan). After that, SYBR-Green Real-Time PCR Kit (Takara) was applied for RT-qPCR reaction. Gene expression was calculated via the $2^{-\Delta\Delta Ct}$ method. GAPDH or U6 acted as the control.

2.4. Cell transfection

For silencing LINC00858 or ABL2 expression in cells, we obtained the structured specific shRNAs targeting LINC00858 or ABL2 and its negative control (NC) from GeneChem company (Shanghai, China). Meanwhile, miR-425-5p mimics/inhibitor and its NC were designed by GenePharma (Shanghai, China). As to cell transfection, Lipofectamine 3000 (Invitrogen) was utilized to transfect these plasmids into cells. After 48 h, we collected cells for later experiments. Assays were conducted for at least three times.

2.5. EdU assay

The utilization of EdU kit (RiboBio, Guangzhou, China) was to detect cell proliferation in line with the protocols of supplier. First of all, we placed ESCC cells in 96-well plates and added EdU staining for cultivation. After staining, nuclei were dyed with DAPI. In the end, we used the fluorescence microscope (Olympus, Tokyo, Japan) for observation. Assays were conducted for at least three times.

2.6. CCK-8 assay

After transfection, cells were seeded in a 96-well plate at a density of 2×10^4 cells per well. Then 10 μL of CCK-8 reagent was added to each well. Following cultivation for 2 h, microplate reader was utilized to examine the absorbance value of each well at 450 nm. Assays were conducted for at least three times.

2.7. Transwell assay

Cell invasion was measured with Transwell chambers (Corning, NY). The upper chamber pre-coated with matrigel was added with cells in serum-free medium, while complete medium was supplemented into the lower chamber. 24 h later, the invaded cells were subjected to fixation and staining. Finally, invaded cells were observed through the microscope (Olympus Corporation). Assays were conducted for at least three times.

2.8. Western blot

The total proteins were extracted from cells by RIPA lysis buffer. Subsequently, they were isolated on 10% SDS-PAGE and transferred onto the PVDF membrane. Next, membranes were sealed with 5% nonfat milk, and then incubated with the diluted primary antibodies (Abcam, Cambridge, MA) overnight at 4 °C. After that, they were rinsed and then incubated with secondary antibodies (Abcam) conjugated with HRP. In the end, the band density was analyzed by ECL detection kit (Invitrogen). Assays were conducted for at least three times.

2.9. Subcellular fractionation assay

On the basis of the user guide, we applied Cytoplasmic and Nuclear RNA Purification Kit (Norgen, Ontario, Canada) to isolate cytoplasm and nucleus. The level of LINC00858 in different parts was determined via RT-qPCR. U6 was the nuclear control while GAPDH was the cytoplasmic control. Assays were conducted for at least three times.

2.10. RNA pull down assay

The miR-425-5p fragments covering wild-type and mutated ABL2 binding sites were biotinylated into Bio-miR-425-5p-WT/MUT. Also, the LINC00858 fragments covering wild-type and mutated miR-425-5p binding sites were biotinylated into Bio-LINC00858-WT/MUT. After culturing with cell protein extracts, the streptavidin agarose beads were added for collecting the pull-downs. Enrichment of RNAs was analyzed via RT-qPCR. Assays were conducted for at least three times.

2.11. RIP

Magna RNA-binding protein immunoprecipitation kit (Millipore, Bedford, MA) was employed for this assay on the basis of supplier protocols. ECa-109 and EC9706 cells in RIP buffer were immunoprecipitated with Anti-Ago2 or Anti-IgG (Millipore). Then the immunoprecipitates were subjected to analysis with RT-qPCR. Assays were conducted for at least three times.

2.12. Luciferase reporter assay

The wild type or mutant type target sequences of miR-129-5p in LINC00858 or ABL2 fragments were inserted to pmirGLO luciferase reporter vector (Promega, Madison, WI). Then they were co-transfected into cells with miR-425-5p mimics or NC mimics for 48 h. In the end, the luciferase activity was detected via dual luciferase system (Promega). Assays were conducted for at least three times.

2.13. Statistical analyses

Each experiment in our study was performed for at least three times. Statistical analysis was processed with GraphPad Prism 5 software (GraphPad Software, San Diego, CA). Data were displayed as means ± SD. Group differences were analyzed by Student's *t*-test or one way ANOVA. $P < 0.05$ was considered as statistical significance.

3. Results

3.1. LINC00858 expression is significantly up-regulated in ESCC tissues

First of all, we detected LINC00858 expression in ESCC cells by utilizing several bioinformatics tools. Through GEPIA (http://gepia.cancer-pku.cn/) database, we found that LINC00858 expression was significantly up-regulated in ESCA tissues compared with non-tumor tissues (Fig. 1A). Besides, the predicted outcome from starBase (http://starbase.sysu.edu.cn/index.php) also showed a notable overexpression of LINC00858 in ESCA tissues compared with normal tissues (Fig. 1B). Furthermore, in the normal esophagus tissues, we found that LINC00858 expression was low, as searched from UCSC (http://genome.ucsc.edu/) (Fig. 1C). In a word,

LINC00858 expression was significantly up-regulated in ESCC tissues.

3.2. LINC00858 accelerates cell proliferation, migration, invasion and EMT process in ESCC

Next, we detected LINC00858 expression in ESCC cell lines (ECa-109, EC9706, KYSE30 and KYSE450) and normal cell line (Het-

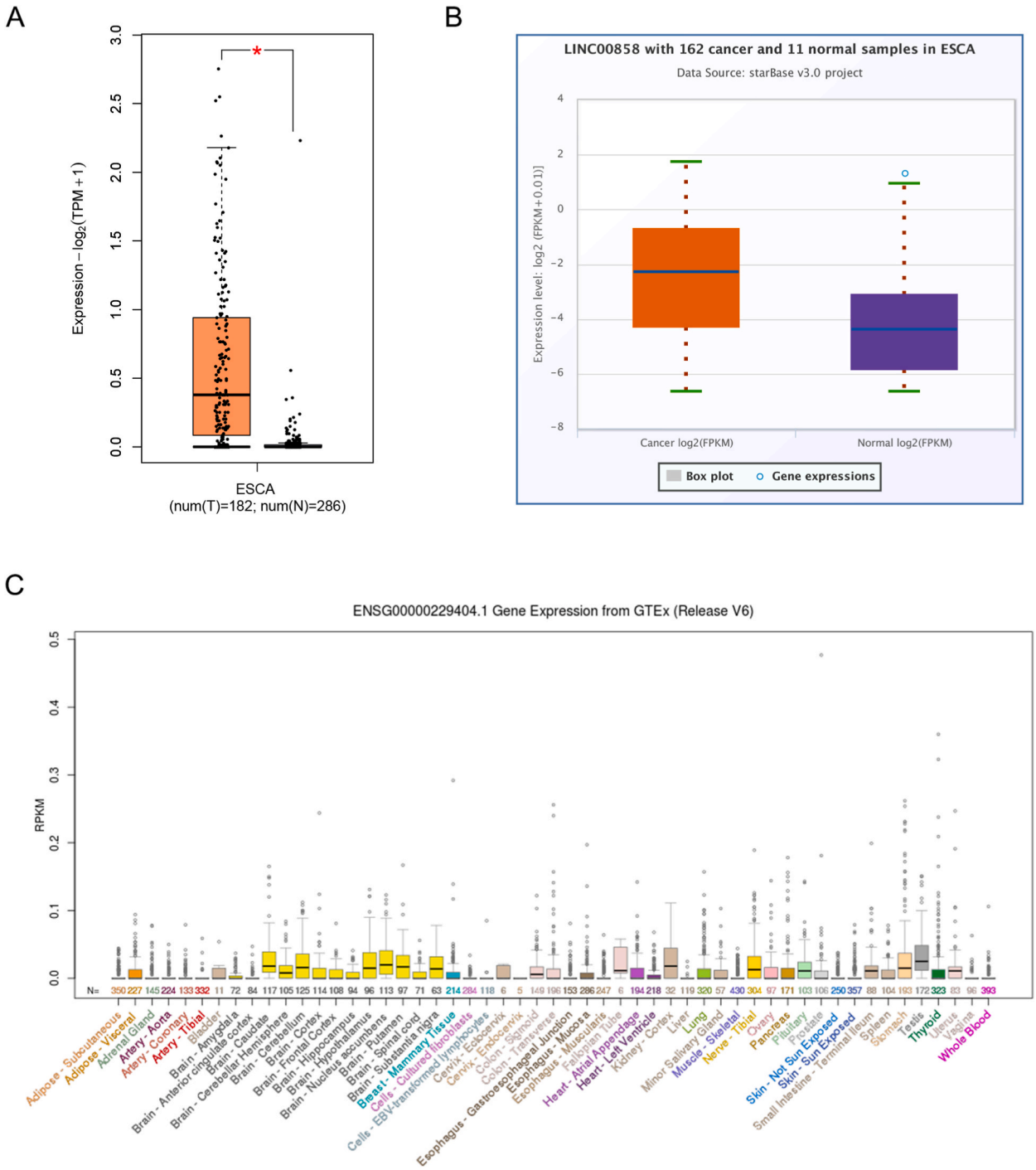

Fig. 1. LINC00858 expression is significantly up-regulated in ESCC tissues A. TCGA database of LINC00858 expression in ESCA tissues predicted by GEPIA website. Left: tumor tissue (T), right: normal tissue (N). B. StarBase database of LINC00858 expression in ESCA tissues. C. LINC00858 expression in the normal esophagus tissues was predicted by UCSC. *P < 0.05.

1A) through RT-qPCR. Results displayed that LINC00858 was highly expressed in ESCC cell lines, especially in ECa-109 and EC9706 cells (Fig. 2A). We silenced LINC00858 in ECa-109 and EC9706 cells by transfecting specific shRNAs targeting LINC00858 and performed related loss-of-function assays. RT-qPCR results showed a favorable interference efficiency of LINC00858 after the sh-RNA transfection (Fig. 2B). Through EdU assay, we observed that the EdU positive cells were obviously decreased in sh-LINC00858 transfection groups compared with the negative control, suggesting cell proliferation was inhibited by LINC00858 depletion (Fig. 2C). The optical density (OD) value at 450 nm was declined obviously after LINC00858 inhibition in ESCC cells, as shown from CCK-8 assay (Fig. 2D). It was then indicated from transwell assay that cell invasion capability was hampered by LINC00858 depletion (Fig. 2E). Finally, Western blot assay detected the level change of E-cadherin, N-cadherin and Vimentin proteins and also MMP2 and MMP7 associated with cell invasion [22]. The outcomes illustrated that only E-cadherin level was elevated by LINC00858 inhibition, while other proteins were reduced, proving that cell migration and EMT process was repressed by LINC00858 silencing (Fig. 2F). Taken together, LINC00858 accelerated cell proliferation, migration, invasion and EMT process in ESCC.

3.3. *LINC00858 acts as a sponge of miR-425-5p in ESCC cells*

Competing endogenous RNA (ceRNA) network represents a regulatory mechanism between RNAs, which refers to that lncRNA can sponge miRNA by acting as a ceRNA to regulate mRNA expression [23]. Thus, we guested that LINC00858 could act as ceRNA in ESCC cells. Firstly, we detected LINC00858 distribution in ESCC cells. Through subcellular fractionation experiment, LINC00858 was found

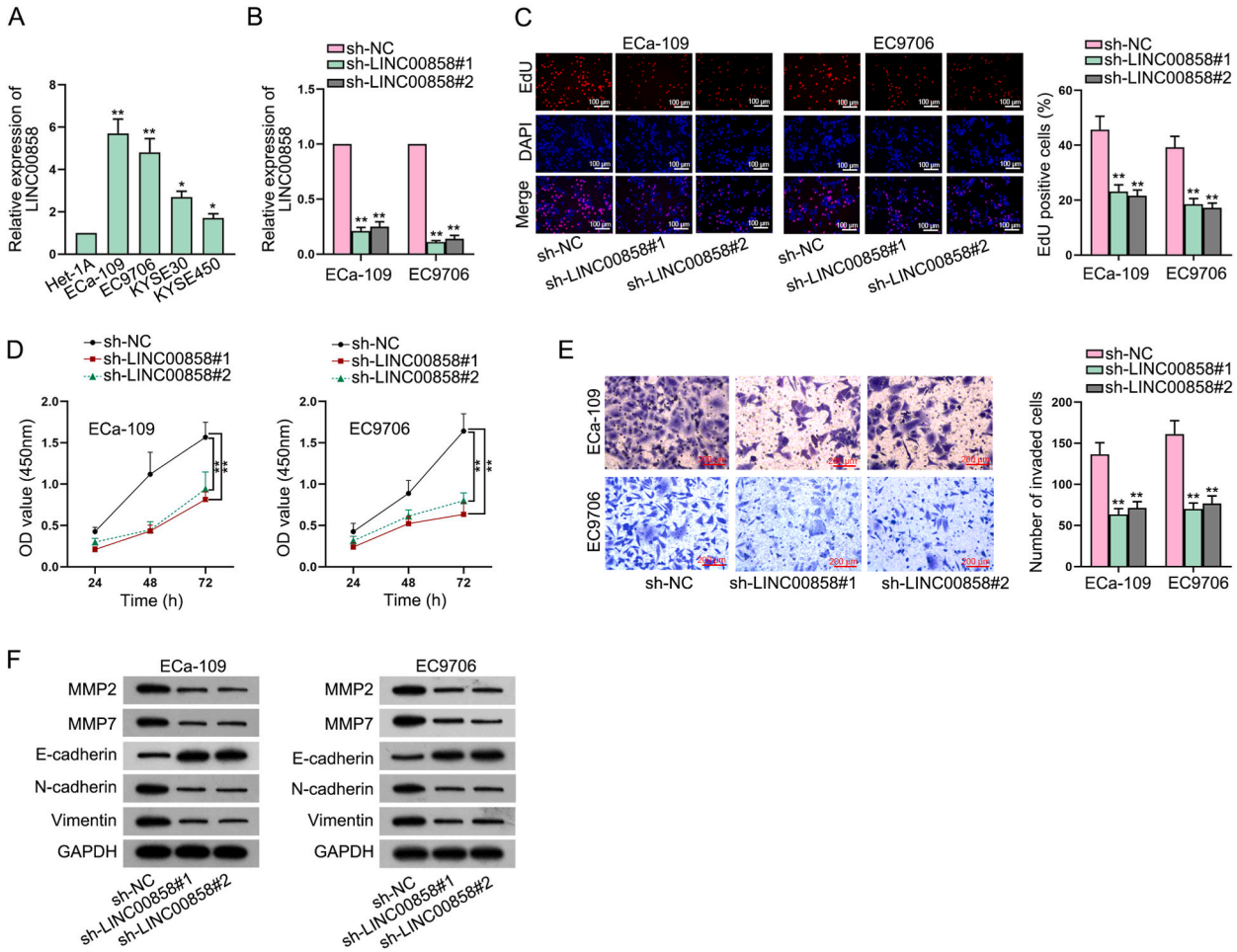

Fig. 2. LINC00858 accelerates cell proliferation, migration, invasion and EMT process in ESCC A. LINC00858 expression in ESCC cell lines (ECa-109, EC9706, KYSE30 and KYSE450) and normal cell line (Het-1A) was tested via RT-qPCR. One-way ANOVA was used for statistical comparison. B. LINC00858 expression was reduced in ECa-109 and EC9706 cells via the transfection of sh-RNAs targeting LINC00858. One-way ANOVA was used for statistical comparison. C-D. EdU (scale bar: 100 μm) and CCK-8 assays detected cell proliferation after sh-LINC00858 transfection in ESCC cells. One-way ANOVA was used for statistical comparison in EdU assay while two-way ANOVA was used in CCK-8 assay. E. Transwell assays (scale bar: 200 μm) detected the number of invaded cells after LINC00858 was silenced in ESCC cells, with one-way ANOVA used for statistical comparison. F. The protein level of MMP2, MMP7, E-cadherin, N-cadherin and Vimentin was measured by Western blot when LINC00858 was depleted. *P < 0.05, **P < 0.01.

to be mostly located in the cytoplasm of cells, implying its possible regulation at the post-transcriptional level (Fig. 3A). Then RIP assay was implemented to test the correlation of LINC00858 and Ago2 protein. The results indicated that LINC00858 was abundantly enriched in Anti-Ago2 groups, suggesting LINC00858 existed in RISC complex (Fig. 3B). After that, we searched potential miRNAs that could bind to LINC00858. Through starBase and miRDB databases, three miRNAs were selected (Fig. 3C). For further screening, miR-153-3p, miR-4761-5p and miR-425-5p levels in ESCC cells were respectively detected. Results showed that only miR-425-5p expression was down-regulated in ESCC cells, while the other miRNAs exhibited no obvious changes (Fig. 3D). Accordingly, miR-425-5p was selected for later experiments. Through RNA pull down assay, we discovered that miR-425-5p was enriched in the pull-down of biotinylated LINC00858-wt group, which proved that LINC00858 could bind to miR-425-5p (Fig. 3E). Furthermore, after we overexpressed miR-425-5p in cell by transfection of miR-425-5p mimics (Fig. 3F), we found that the luciferase activity of LINC00858-wt was declined by miR-425-5p mimics, while that of LINC00858-mut was not affected (Fig. 3G). Overall, LINC00858 acted as a sponge of miR-425-5p in ESCC cells.

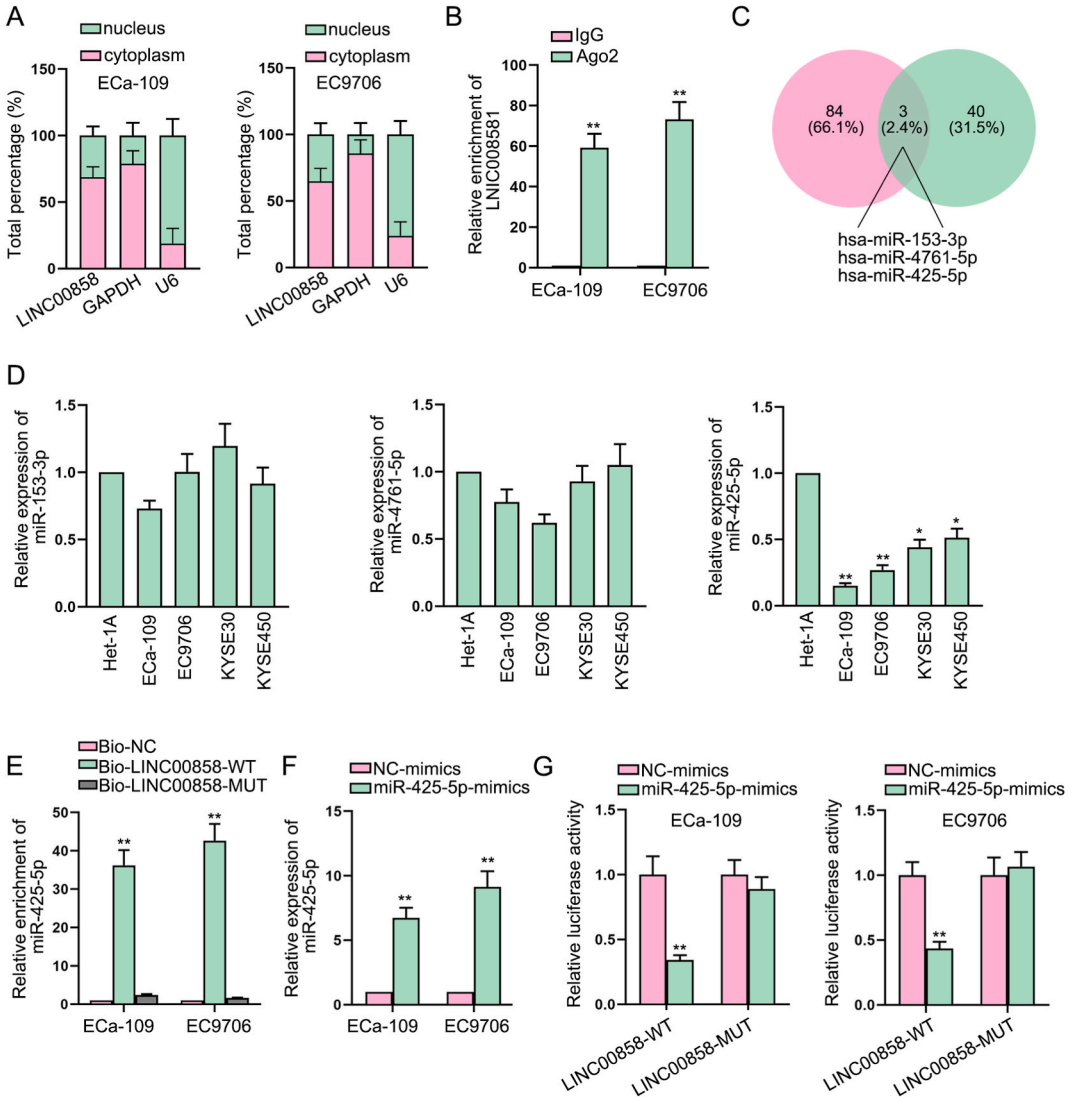

Fig. 3. LINC00858 acts as a sponge of miR-425-5p in ESCC cells A. Subcellular fractionation assays determined the distribution of LINC00858 in ESCC cells. B. The enrichment of LINC00858 in anti-Ago2 and control IgG group was measured via RIP assays. Student's *t*-test was applied for statistical comparison. C. StarBase and miRDB databases predicted three possible miRNAs that might bind to LINC00858. D. MiR-153-3p, miR-4761-5p and miR-425-5p levels in ESCC cells were detected by RT-qPCR, with one-way ANOVA used for statistical analysis. E. RNA pull down assay was utilized to test the enrichment of miR-425-5p in biotinylated LINC00858 groups. One-way ANOVA was used for statistical analysis. F. MiR-425-5p expression was enhanced by the transfection of miR-425-5p mimics in ESCC cells, with student's *t*-test used for statistical analysis. G. Luciferase reporter assays were utilized to measure the luciferase activity of ESCC cells transfected with miR-425-5p mimics in the wild and mutated LINC00858 groups. Two-way ANOVA was applied for statistical comparison. *P < 0.05, **P < 0.01.

3.4. ABL2 is the target gene of miR-425-5p in ESCC cells

Next, we tried to search the target gene of miR-425-5p. By utilizing starBase, two mRNAs were discovered to combine with miR-425-5p under the prediction of PITA, miRmap, microT and RNA22 databases (Fig. 4A). Then we detected their expression in ESCC cells transfected with miR-425-5p mimics. RT-qPCR data indicated that only ABL proto-oncogene 2 (ABL2) expression was reduced by miR-425-5p overexpression (Fig. 4B). Western blot data further proved that ABL2 level was repressed in miR-425-5p mimics-transfected cells (Fig. 4C). We also discovered that both of mRNA level and protein level of ABL2 was inhibited by the silenced LINC00858 expression (Fig. 4D and E). These findings implied that ABL2 was negatively regulated by miR-425-5p, but positively regulated by LINC00858 in ESCC cells. Next, observed a notable up-regulation of ABL2 in ESCC cells (Fig. 4F). We then discovered through RNA pull down assay that ABL2 was enriched in biotinylated miR-425-5p-WT group (Fig. 4G). Moreover, the luciferase activity of ABL2-WT was reduced by miR-425-5p mimics (Fig. 4H). These results proved that miR-425-5p could bind to ABL2 in ESCC cells. RT-qPCR results further illustrated that the inhibited ABL2 expression caused by the silenced LINC00858 could be recovered by miR-425-5p inhibitor (Fig. 4I). In short, ABL2 was targeted by miR-425-5p in ESCC cells.

3.5. The progression of ESCC is regulated by LINC00858/miR-425-5p/ABL2 axis

In the end, we conducted rescue assays to verify the regulatory mechanism in cells transfected with sh-LINC00858. In EdU assay

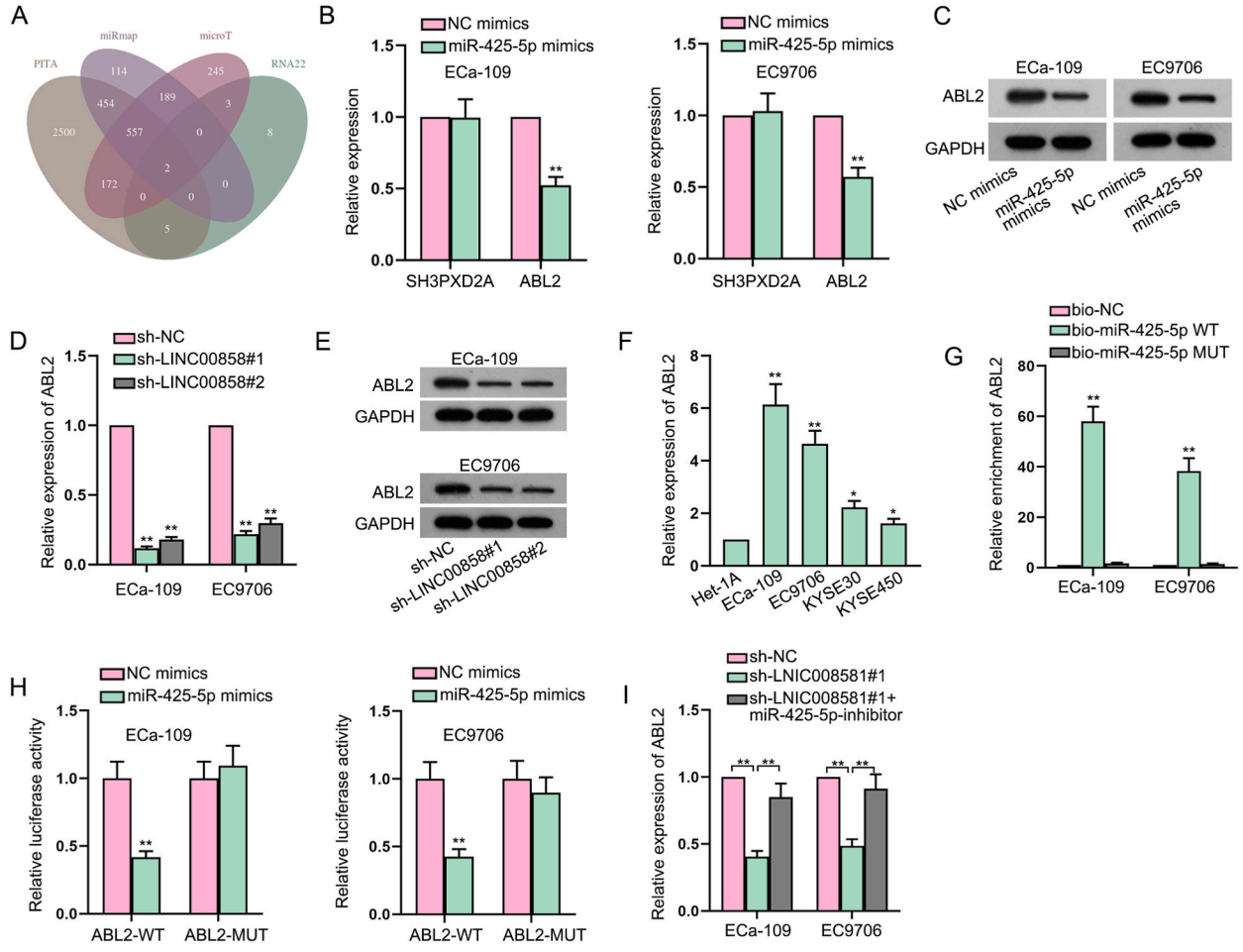

Fig. 4. ABL2 is targeted by miR-425-5p A. MiRmap, microT, PITA and RNA22 databases predicted 2 possible mRNAs in starBase. B. RT-qPCR tested the expression of SH3PXD2A and ABL2 in cells transfected with miR-425-5p mimics, with one-way ANOVA used for statistical comparison. C. Western blot assay measured ABL2 level in miR-425-5p mimics-transfected ESCC cells. D-E. ABL2 level was tested by Western blot and RT-qPCR when LINC00858 was inhibited in cells, with one-way ANOVA used for statistical comparison. F. RT-qPCR result of ABL2 expression in ESCC cells. One-way ANOVA was used for statistical comparison. G. RNA pull down assay tested the enrichment ABL2 in bio-miR-425-5p WT/MUT groups. One-way ANOVA was used for statistical analysis. H. Luciferase reporter assay further confirmed the interplay between ABL2 and miR-425-5p in ESCC cells. Two-way ANOVA was used for statistical analysis. I. ABL2 expression was measured by RT-qPCR when LINC00858 and miR-425-5p were inhibited in cells. Two-way ANOVA was used for statistical analysis. *$P < 0.05$, **$P < 0.01$.

and CCK-8 assay, we discovered that cell proliferation inhibited by sh-LINC00858 could be accelerated by miR-425-5p inhibitor, while co-transfection of sh-ABL2 repressed cell proliferation again (Fig. 5A and B). Then transwell and Western blot assay also proved that cell migration, invasion and EMT process which were suppressed by sh-LINC00858 could be promoted by miR-425-5p inhibitor, while co-transfection of sh-ABL2 reversed these effects (Fig. 5C and D). In addition, according to EdU assay results, we found that transfection of sh-LINC00858 inhibited ESCC cell proliferation, but co-transfection of ABL2 overexpression vector reversed this inhibitory effect (Fig. S1A). Transwell assay also showed that transfection of sh-LINC00858 inhibited the migration and invasion of ESCC cells, but co-transfection of ABL2 overexpression vector reversed this inhibitory effect (Fig. S1B). All in all, we verified that LINC00858 could regulate ESCC progression via targeting miR-425-5p and ABL2.

4. Discussion

Esophageal squamous cell carcinoma (ESCC) is one of the most fatal cancers with high morbidity and mortality, which severely affects people's lives [1]. With the biological function of lncRNA gradually revealed, accumulating researches have confirmed the role of lncRNAs in various types of cancer, including ESCC [24]. For example, it has been reported that SNHG16 predicts the poor prognosis in ESCC and accelerates cell proliferation via regulating Wnt/β-catenin pathway [25]. Besides, CCAT1 is confirmed as a biomarker for the proliferation and drug resistance of ESCC via miR-143/PLK1/BUBR1 axis [26]. Moreover, PART1 is proved to induce gefitinib resistance in ESCC through functioning as a competing endogenous RNA [27]. The first common feature of these oncogenes is that they exert abnormally high expression in cancer cells. LncRNA LINC00858 has been verified to be highly expressed in cells of colorectal cancer [10], lung cancer [11] and osteosarcoma [12], and it exerts a carcinogenic effect on the biological phenotypes of cancer cells. In our study, we first verified a significantly high expression of LINC00858 in ESCC cells through RT-qPCR, which were consistent with our predictions on bioinformatics tools. Accordingly, we conducted a series of functional assays and discovered that LINC00858 could accelerate cell proliferation, migration, invasion and EMT process in ESCC. Thus, LINC00858 as an oncogene in ESCC, along with its tumor-promoting impact on the biological properties of ESCC cells was proved.

MiRNAs are endogenous RNA molecules with about 22 nucleotides, which regulate the expression of downstream target mRNAs through binding to its 3′UTR [28]. In recent years, competing endogenous RNA (ceRNA) network has been proved to be involved in human cancers by increasing researches, which refers to that lncRNA can act as a ceRNA to sponge miRNA to regulate mRNA

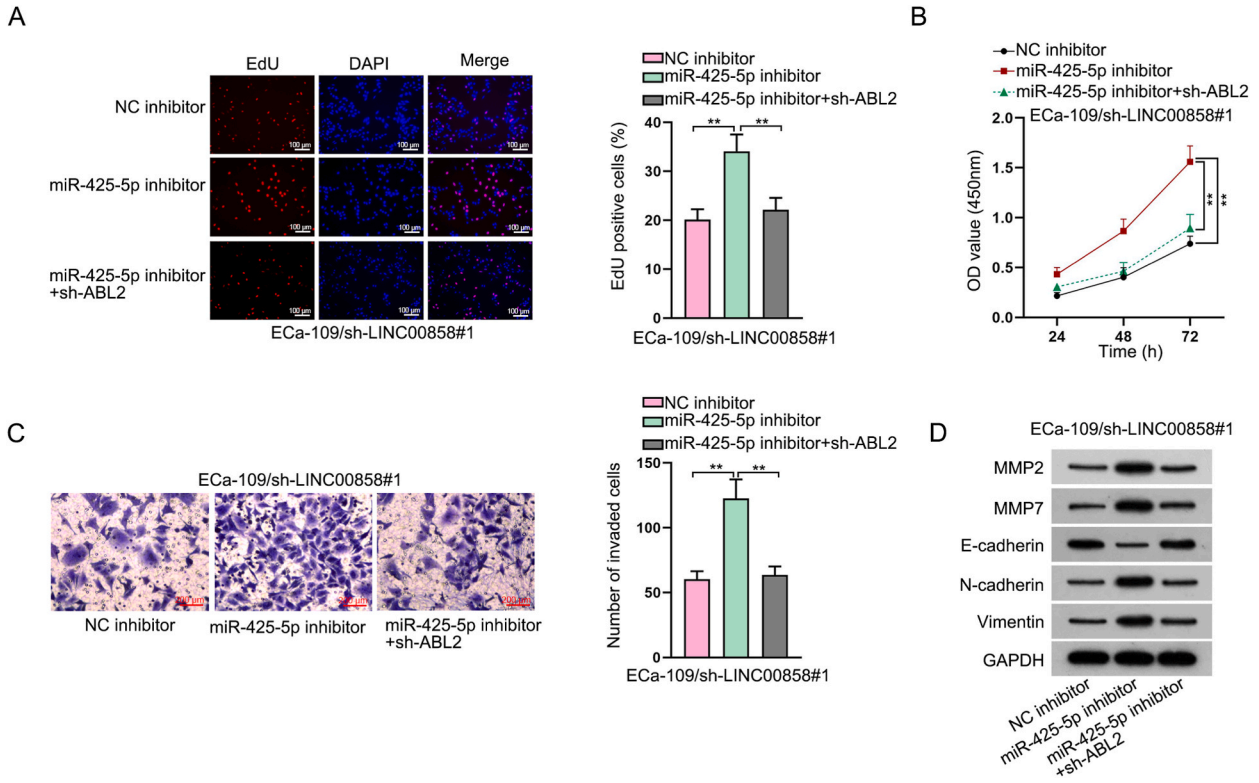

Fig. 5. The progression of ESCC is regulated by LINC00858/miR-425-5p/ABL2 axis A-B. EdU (scale bar: 100 μm) and CCK-8 assay detected cell proliferation after LINC00858 was silenced in different groups (NC inhibitor, miR-425-5p inhibitor, miR-425-5p inhibitor + sh-ABL2). Two-way ANOVA was applied for statistical analysis. C. Transwell (scale bar: 200 μm) assays detected the number of invaded cells in different transfection groups in contrast to negative control groups. Two-way ANOVA was applied for statistical analysis. D. Western blot was employed for measuring the protein level of MMP2, MMP7, E-cadherin, N-cadherin and Vimentin in different transfection groups. $^{**}P < 0.01$.

expression [29]. For example, FLVCR1-AS1 promotes cell proliferation in lung cancer via sponging miR-573 to up-regulate the expression of E2F3 [30]. IGFL2-AS1 can function as a ceRNA in regulating ARPP19 through competitive binding to miR-802 in gastric cancer [31]. As a mechanism of post transcriptional regulation, ceRNA requires lncRNA to be distributed in the cytoplasm. Thus, we performed subcellular fractionation assay and RIP-Ago2 assay to detect the ceRNA possibility of LINC00858. As we expected, LINC00858 was mainly located in the cytoplasm and existed in RISC complex. Thus, we utilized the bioinformatics tools to predict the possible miRNAs of LINC00858, and miR-425-5p was proved to be the target miRNA in our research. The ceRNA model of LINC00858 has been illustrated in the regulation of many cancers. For example, LINC00858 is a ceRNA for miR-422a to facilitate the cell growth in non-small cell lung cancer [32], it sponges miR-139 to elevate the expression of CDK14 in osteosarcoma [12], and it promotes the malignant development of colorectal cancer cells via acting as a ceRNA of miR-22-3p to further modulate the downstream gene YWHAZ expression [10]. For another molecule miR-425-5p, it has been identified as a molecular marker for cancer development, including ovarian cancer [33], hepatocellular carcinoma [34] and breast cancer [35]. MiR-425-5p is verified to be with high expression in some cancers while is down-regulated in other cancers. Previous reports indicate that miR-425-5p suppresses tumorigenesis of prostate cancer via targeting GSK3β [36], while it can also promote prostate cancer progression via targeting FOXJ3 [37]. What's more, miR-425-5p has been verified to participate in the ceRNA model to regulate cancer development such as glioma [38] and gastric cancer [39].

The non-receptor tyrosine kinase Abelson (Abl) is known as a key regulator of cell behaviors, and along with its mammalian paralogue, Abl-related gene (Arg), is part of a kinase superfamily which can phosphorylate protein targets that regulate cell behavior, thus altering their function [40–43]. Abl's structure facilitates the link between cell signaling and cytoskeletal regulation, and all Abl family members share a highly conserved set of N-terminal domains with Src [44]. As a member of the Abelson family, ABL2 has been confirmed to exert a carcinogenic effect in different kinds of cancers. For example, high expression of ABL2 is identified in gastric cancer cells and suppresses cell apoptosis in gastric cancer [45]. Besides, up-regulation of ABL2 is reported to predict poor prognosis of hepatocellular carcinomas and accelerate cell migration and invasion [40]. In our research, we confirmed ABL2 as the downstream target of miR-425-5p, and revealed a positive correlation between ABL2 and LINC00858 expression in ESCC cells. Moreover, in rescue assays, we discovered that miR-425-5p inhibitor could reverse the inhibitory function of silencing LINC00858 on ESCC cells, while knockdown of ABL2 could normalize such affect again. We also found that overexpression of ABL2 reversed the inhibitory effect of LINC00858 silencing on ESCC cells.

5. Conclusion

In conclusion, it was suggested by our research that LINC00858 promoted the malignant cell behaviors in ESCC by regulating the miR-425-5p/ABL2 axis. High LINC00858 expression has been verified to be closely related to the clinical staging and metastasis in many types of cancer [46], but how it may exert certain regulatory function in ESCC clinical samples is still unknown. As we did not collect relevant ESCC patient samples for clinical analysis, we put this point as a limitation of this study, and we will focus more on this aspect to further verify the clinical value of LINC00858 in ESCC. LncRNAs can affect cancer progression through a variety of approaches, but in this study we focus more on the ceRNA pattern of LINC00858. Therefore, we will try to explore more about the regulatory mechanism of LINC00858 by identifying other effective downstream targets and potential regulatory pathways. All in all, we hope that these discoveries may supply the novel targets and directions for ESCC therapies.

Additional information

No additional information is available for this paper.

Data availability statement

The data used to support the findings of this study are included within the article.

CRediT authorship contribution statement

Pengfei Li: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Hui Ding:** Writing – review & editing, Writing – original draft, Software, Resources, Formal analysis. **Shuangyin Han:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Songze Ding:** Writing – original draft, Supervision, Formal analysis. **Yuxiu Yang:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that there are no competing interests in this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27337.

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