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LncRNA-CASC15 knockdown inhibits the progression of esophageal squamous cell carcinoma through targeting miR-33a-5p/PTGS2 axis

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Summary. LncRNA CASC15 has been determined as a novel tumor-related lncRNA in many cancers. However, the in-detail role of CASC15 remains elusive in esophageal squamous cell carcinoma (ESCC). CASC15 expression level was detected in 113 ESCC tissues and paired adjacent normal tissues and in human ESCC cell lines. The effects of CASC15 on ESCC proliferation, migration, and invasion were assessed using CCK-8 and transwell assays. In addition, the potential downstream molecules of CASC15 were searched and confirmed by software algorithms, RT-qPCR, western blot, dual-luciferase reporter, and rescue experiments. CASC15 was upregulated in ESCC tissues and cell lines. CASC15 overexpression was associated with poorer prognosis in ESCC patients. Functionally, CASC15 knockdown inhibited cell proliferation, migration, and invasion of ESCC cells. Mechanistically, it was confirmed that CASC15 acts as competing endogenous RNA for miR-33a-5p to regulate PTGS2 expression. In addition, rescue assay showed that miR-33a-5p knockdown or PTGS2 overexpression abolished the cell proliferation, migration, and invasion inhibition role of CASC15 knockdown. In conclusion, this study indicates that CASC15 was upregulated in ESCC and CASC15 knockdown hindered ESCC progression through targeting the miR-33a-5p/PTGS2 axis. CASC15 might serve as a novel biomarker and therapeutic target for ESCC.

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Introduction

Esophageal cancer ranks as the 6th primary cause for cancer-related mortality and the 8th most common cancer type worldwide (Uhlenhopp et al., 2020). It is estimated that almost 330,000 new cases and 270,000 death cases of esophageal cancer are reported all over the world each year (Bray et al., 2018). Remarkably, esophageal cancer is prevalent in China and esophageal squamous cell carcinoma (ESCC) is the predominant histological type (Abnet et al., 2018). Despite the considerable improvement in diagnosis and treatment of ESCC, the 5-year survival rate remains unsatisfactory at only 15–25% due to local invasion or distant metastasis (Jackie et al., 2016). Thus, it is of great importance to better elucidate the molecular mechanisms of ESCC tumorigenesis and develop novel therapeutical targets for patients with ESCC.

Long noncoding RNA (lncRNA) is a class of non-coding RNA at 200 bases, which have no ability to encode proteins (Prensner and Chinnaiyan, 2011). Recently, a great variety of lncRNAs have been shown to function as competing endogenous RNAs (ceRNAs) by acting as special miRNAs' sponges to regulate their targets and be involved in several physiological processes, including the development of many malignant tumors (Bhan et al., 2017; Peng et al., 2017; Sanchez et al., 2018). Notably, a great number of lncRNAs has also been identified to be associated ESCC, which shines a light to the research of ESCC carcinogenesis (Yao et al., 2016). LncRNA CASC15 is a novel tumor-related lncRNA, which has been reported to act as an oncogene in many cancers, including cervical cancer (Shan et al., 2019), gastric cancer (Wu et al., 2018), osteosarcoma (Zhang et al., 2020), colon cancer (Jing et al., 2018),

lung cancer (Bai et al., 2019), and tongue squamous cell carcinoma (Wu et al., 2019). Nevertheless, in ESCC, the biological function and related mechanisms of CASC15 remain unclear.

In the present study, we firstly reported that CASC15 was upregulated in human ESCC and that CASC15 overexpression was correlated with poorer prognosis. We also demonstrated that CASC15 knockdown inhibited the proliferation, migration, and invasion of ESCC cells. Moreover, CASC15 knockdown hindered ESCC development through regulating prostaglandin-endoperoxide synthase expression 2 (PTGS2) expression by interacting with miR-33a-5p. Overall, this study demonstrated that CASC15 can act as an oncogene via targeting the miR-33a-5p/PTGS2 axis.

Materials and methods

Human clinical samples and cell lines

From January 2012 to December 2015, a cohort of 113 patients with ESCC were recruited from The First Affiliated Hospital of the Navy Medical University. All the ESCC patients underwent primary surgery, and tumor tissues and pair adjacent normal tissues were collected after surgery. The inclusion criteria were: (1) all patients were histologically proven by at least two pathologists; (2) no other associated malignancies; (3) with complete clinical information. Patients with any neoadjuvant treatment (chemotherapy or radiotherapy) before surgery were excluded. All enrolled patients signed informed consents for their tissue samples and clinical information to be used in this study. This study was approved by the ethics committee of The First Affiliated Hospital of the Navy Medical University and was conducted in accordance with the Declaration of Helsinki. human ESCC cell lines (EC9706, EC109, KYSE150, and KYSE450) and one human normal esophagus epithelial cell line HEEC were provided by the American Type Culture Collection (ATCC). All cells were cultured in RPMI-1640 medium (Hyclone, USA) containing 10% fetal bovine serum (FBS, GIBCO) and 1% Penicillin-Streptomycin (GIBCO), at 37°C in a 5% CO_2 humidified incubator.

Cell transfection

The CASC15 siRNA (si-CASC15), over-expression plasmids (pcDNA3.1-PTGS2), miR-33a-5p mimics, miR-33a-5p inhibitors, and the corresponding negative control oligonucleotides or plasmids were purchased from GenePharma Company (Shanghai, China). Cell transfections were performed using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the standard user's manual. 48h later, cells were extracted for subsequent experiments.

Reverse transcription quantitative polymerase chain reaction (RT qPCR)

Total RNA was extracted using the TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Then, 5 μL RNA sample was collected, and diluted 20 times using RNase‑free ultrapure water. Next, OD values at excitation wavelengths of 260 and 280 nm were determined using an ultraviolet spectrophotometer, and the purity and concentration of RNA were detected. An OD260/OD280 ratio value between 1.7 and 2.1 indicated that purity was high enough for further experimentation. Subsequently, complete DNA (cDNA) was synthesized using the M-MLV Reverse Transcriptase (Promega,

Table 1. The sequences of PCR primers.

Name		Sequence (5'-3')
CASC15	Forward primer	CACACGCATGGAAAACC
	Reverse primer	GAGGACCTGAGCTGTAAGCC
miR-33a-5p	Forward primer	GATCCTCAGTGCATTGTAGTTGC
	Reverse primer	CTCTGTCTCTCGTCTTGTTGGTAT
PTGS2	Forward primer	GGGGATCAGGGATGAACTTT
	Reverse primer	TGGCTACAAAAGCTGGGAAG
GAPDH	Forward primer	CCTTCATTGACCTCAACTACA
	Reverse primer	GCTCCTGGAAGATGGTGAT
U6	Forward primer	CTCGCTTCGGCAGCACATATACT
	Reverse primer	ACGCTTCACGAATTTGCGTGTC

Table 2. Relationship between CASC15 expression and clinicopathological features in patients with ESCC.

Variables	Cases (n=113)	CASC15, n (%)		
		High expression (n=56)	Low expression (n=57)	
Age, year				
<60	68 (60.2)	35 (62.5)	33 (57.9)	0.617
≥60	45 (39.8)	21 (37.5)	24 (42.1)	
Gender				
Male	79 (69.9)	41 (73.2)	38 (66.7)	0.448
Female	34 (30.1)	15 (26.8)	19 (33.3)	
Smoking				
Yes	72 (63.7)	39 (69.6)	33 (57.9)	0.194
No	41 (36.3)	17 (30.4)	24 (42.1)	
Drinking				
Yes	68 (60.2)	31 (55.4)	37 (64.9)	0.300
No	45 (39.8)	25 (44.6)	20 (35.1)	
Differentiation grade				
Well/moderate	87 (77.0)	40 (71.4)	47 (82.5)	1.164
Poor	26 (23.0)	16 (28.6)	10 (17.5)	
Primary tumor invasion depth				
T1/T2	52 (46.0)	20 (35.7)	32 (56.1)	**0.029**
T3/T4	61 (54.0)	36 (64.3)	25 (43.9)	
Lymph node invasion				
Negative	47 (41.6)	17 (30.4)	30 (52.6)	**0.016**
Positive	66 (58.4)	39 (69.6)	27 (47.4)	
TNM Stages				
I-II	79 (69.9)	31 (55.4)	48 (84.2)	**0.001**
III-IV	34 (30.1)	25 (44.6)	9 (15.8)	

$P<0.05$ is shown in bold.

Madison, WI) based on the manufacturer's protocol. The reaction conditions for reverse transcription were 37°C for 15 min and 85°C for 5 sec. The generated cDNA was preserved at -20°C, and then collected for RT-qPCR, which was performed using CellDirectTM One-Step qRT-PCR Kit (Invitrogen; Thermo Fisher Scientific, Inc.) on an FTC-3000TM System (Funglyn Biotech Inc., Toronto, Canada). The thermocycling conditions were as follows: 95°C for 2 min, followed by 45 cycles at 95°C for 30 sec (denaturation), 56°C for 60 sec (annealing), and 72°C for 1 min (extension), followed by 72°C for 6 min. Relative expression of miR-33a-5p was normalized to that of U6, and CASC15 and PTGS2 were normalized to that of GAPDH using $2^{-\Delta\Delta Ct}$ method (Livak et al., 2001). All used PCR primers are shown in Table 1.

Western Blotting

Total proteins were extracted from cells by incubating with RAPI buffer (Bio Rad Laboratories, Inc.) on ice for 10 min and sonication 5 sec each time 4 times (200 W with 2 sec interval). Then, the protein extracts form each sample were measured using the bicinchoninic acid method and separated on 10% SDS-PAGE. A total of 30 µg proteins were run on gels and transferred to PVDF membranes (Millipore, Bedford, MA, USA). The membranes were blocked in 5% bovine serum albumin (Gibco; Thermo Fisher Scientific, Inc.) at room temperature for 1 hour and then incubated with anti-PTGS2 antibody (1:1000; ab179800; Abcam Ltd., Cambridge, UK) and anti-GAPDH antibody (1:1000, AF0006; Beyotime) overnight at 4°C. After the membranes were washed with TBST three times, they were then incubated with corresponding horseradish peroxidase (HRP) linked second antibodies (Beyotime, Shanghai, China) for 1h at room temperature. GAPDH was used as a loading control. The blots were fixed and visualized using Pierce™ ECL solution (Thermo Fisher Scientific, Inc.).

CCK-8 assay

Cell proliferation was measured using CCK-8 assay (Beyotime, Shanghai, China). In brief, ESCC cells were diluted to a final concentration of 2×10^4 cells/ml, and then 100 µl of diluted cells were seeded into each well of a 96-well plate. At 0, 1, 2, and 3 days after transfection, 10 µl of CCK-8 solution was added to each well and cells were incubated for 2h at 37°C. Optical density at 450 nm was measured using a spectrophotometer (Bio-Rad, Hercules, CA, USA).

Transwell assay

Cell migration and invasion abilities were detected using a Transwell chamber with 8 µm aperture (Costar, Corning, NY, USA). For migration assay, a total of 3×10^4 ESCC cells suspended in serum-free medium were seeded to the upper chamber, and 600 µl

RPMI1640 medium supplemented with 10% FBS was added in the lower chamber. For invasion assay, the upper surface of the membrane was precoated with Matrigel (BD Biosciences). A total of 5×10^4 cells were seeded on the upper chamber. After incubation in a 37°C incubator for 24h, cells on the upper surface of the membrane were gently removed with a cotton swab. Cells that adhered to the lower surface of the membrane were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and then randomly counted from at least five fields (magnification, x200) under a microscope (DM6M, Leica, Wetzlar, Germany).

Luciferase reporter assay

MiR-299-5p mimic or NC mimic (10 nmol/L), miR-299-5p inhibitor or NC inhibitor (10 nmol/L), luciferase reporter vectors wild-type CASC15 and mutant CASC15 (CASC15-WT and CASC15-MUT, 30 ng, Promega Corporation), PTGS2-WT and PTGS2-MUT (20 ng, Promega Corporation), as well as pRL-TK (Renilla Luciferase protein as the internal reference, 30 ng) were co-transfected into ESCC cells with Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were cultured for 48h prior to measurement of luciferase intensity. At 48h post-transfection, the cells were lysed using radioimmunoprecipitation assay buffer (Beyotime, Shanghai, China) based on the manufacturer's protocol. The luciferase activity was measured using the Dual Luciferase Reporter Assay Kit (Promega) and normalized to that of Renilla luciferase.

Statistical analysis

Results are presented as mean ± standard deviation of at least three independent experiments. Statistical evaluations were performed using SPSS 20.0 (IBM SPSS Inc., Chicago, IL, USA). Differences between different groups were analyzed using Student's t-test or ANOVA test. Categorical data were compared using chi-square test. Overall survival (OS) was evaluated using Kaplan-Meier curves and log-rank test. Prognostic factors associated with OS were assessed by both univariate and multivariate Cox regression analyses using a forward step-wise approach. Prognostic factors with statistical significance ($P<0.05$) after univariate analysis were included in the Cox proportional hazards regression model for multivariate analysis. The correlation analysis was performed using Pearson's method. Differences were considered statistically significant when $P< 0.05$.

Results

CASC15 is upregulated in ESCC and indicates poor prognosis for ESCC patients

By analyzing the clinical tumor tissues and matched normal tissues from 113 ESCC patients with RT-qPCR

analysis, we revealed that CASC15 was significantly upregulated in ESCC tissues (*P*<0.001, Fig. 1A). In addition, an obviously higher CASC15 expression was observed in ESCC tissues with advanced tumor invasion depth (*P*<0.001, Fig. 1B), lymph node invasion (*P*<0.05, Fig. 1C), and TNM stage (*P*<0.001, Fig. 1D). Next, we

profiled CASC15 expression in some human ESCC cell lines (EC9706, EC109, KYSE150, and KYSE450) and one human normal esophagus epithelial cell line HEEC, and the results showed that CASC15 was much more enriched in ESCC cells (*P*<0.001, Fig. 1E).

To explore the clinicopathological role of CASC15

Table 3. Univariate and multivariate analysis for overall survival in in patients with ESCC.

Variables	Univariate		Univariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, ≥60 vs. <60	1.012 (0.862-1.251)	0.651		
Gender, male vs. female	1.007 (0.996-1.174)	0.367		
Smoking, yes vs. no	1.314 (0.852-1.684)	0.574		
Drinking, yes vs. no	1.121 (0.941-1.284)	0.274		
Differentiation grade, poor vs. well/moderate	1.334 (0.874-1.875)	0.117		
Primary tumor invasion depth, T3/T4 vs. T1/T2	1.625 (1.514-1.869)	**0.005**	1.485 (1.261-1.715)	**0.012**
Lymph node invasion, positive vs. negative	1.985 (1.684-2.125)	**0.001**	1.842 (1.574-1.984)	**0.005**
TNM stage, III-IV vs. I-II	2.541 (2.124-2.945)	**<0.001**	1.876 (1.745-2.175)	**0.001**
CASC15 expression, high vs. low	1.857 (1.674-1.987)	**0.001**	1.542 (1.418-1.793)	**0.009**

P<0.05 is shown in bold.
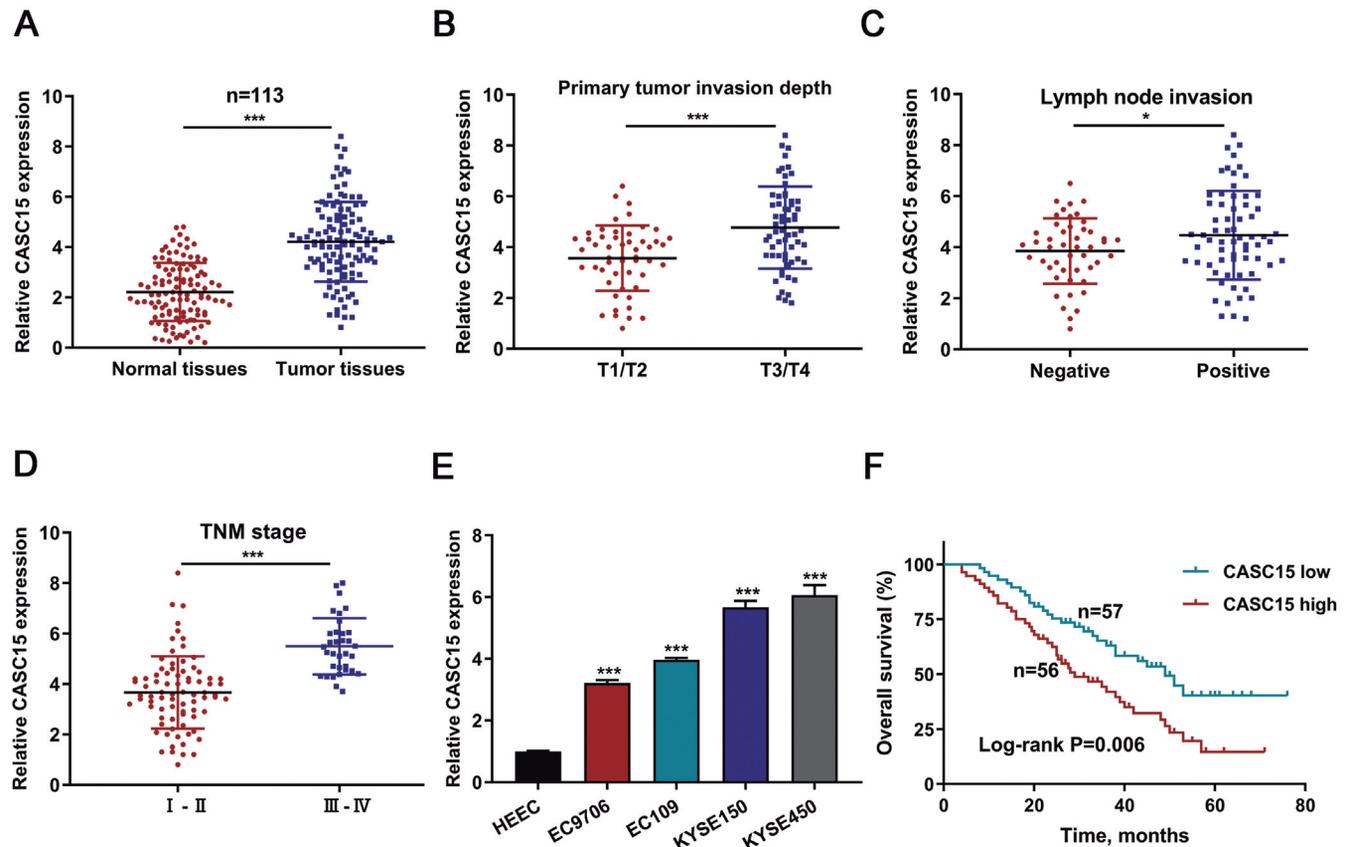
Fig. 1. CASC15 expression is upregulated in ESCC and correlates with poorer prognosis. **A-D.** CASC15 expression in 113 pairs of ESCC tissues and adjacent normal tissues **(A)**, ESCC tissues with primary tumor invasion T1/T2 or T3/T4 **(B)**, ESCC tissues with or without lymph node invasion **(C)**, ESCC tissues with TNM stage I-II or III-IV **(D)**. **E.** Relative CASC15 expression in human ESCC cells and one human normal esophagus epithelial cell line. **F.** Kaplan-Meier curves for overall survival in ESCC patients stratified by CASC15 expression. **P<0.05, ***P<0.001.
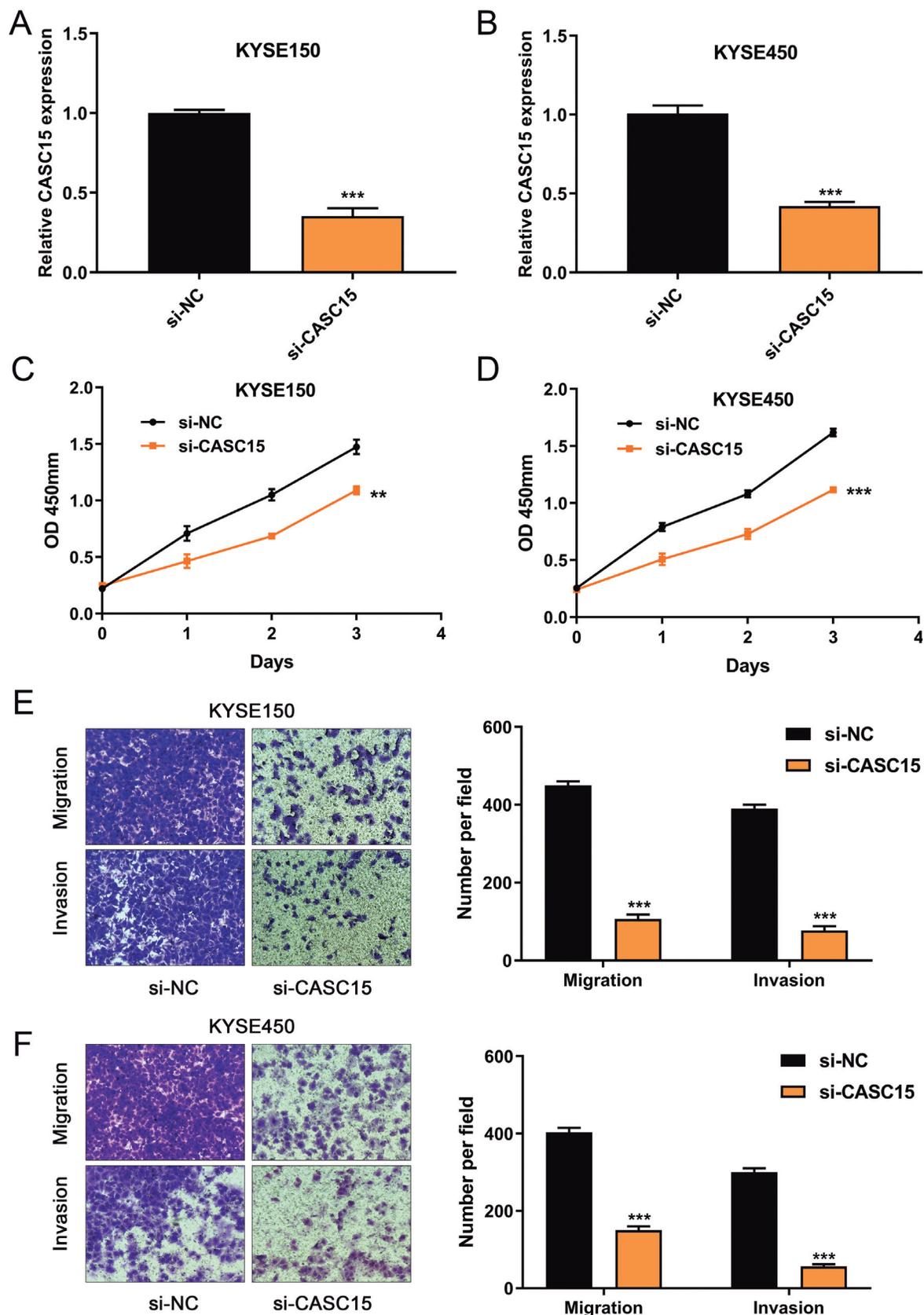
Fig. 2. CASC15 knockdown inhibits ESCC proliferation, migration and invasion. **A-B.** Relative CASC15 expression levels in KYSE150 and KYSE450 cells after transfection with si-CASC15 and its negative control (si-NC). **C-D.** KYSE150 and KYSE450 cell proliferation ability following transfection with si-CASC15 evaluated by CCK8 assays. **E-F.** KYSE150 and KYSE450 cell migration and invasion ability following transfection with si-CASC15 evaluated by transwell assays. **P<0.01, ***P<0.001.

in ESCC, we divided the 113 ESCC patients into two groups based on the median CASC15 expression in tumor tissues. As shown in Table 2, CASC15 expression was closely associated with primary tumor invasion depth (P=0.029), lymph node invasion (P=0.016), and TNM stage (P=0.001). The Kaplan-Meier analysis indicated that high CASC15 expression correlated with poorer OS for ESCC patients (P<0.006, Fig. 1F). Furthermore, as shown in Table 3, the multivariate analysis demonstrated that primary tumor invasion depth (P=0.012), lymph node invasion (P=0.005), TNM stage

P=0.001), and CASC15 expression (P=0.009) were independent impact factors for ESCC patients. Therefore, all the above findings indicated that CASC15 was a poor prognostic factor in ESCC patients, which might be involved in the progression of ESCC.

Knocking down CASC15 inhibited the proliferation, migration and invasion of ESCC cells

We firstly downregulated CASC15 expression in KYSE150 and KYSE450 cells with si-CASC15, and the
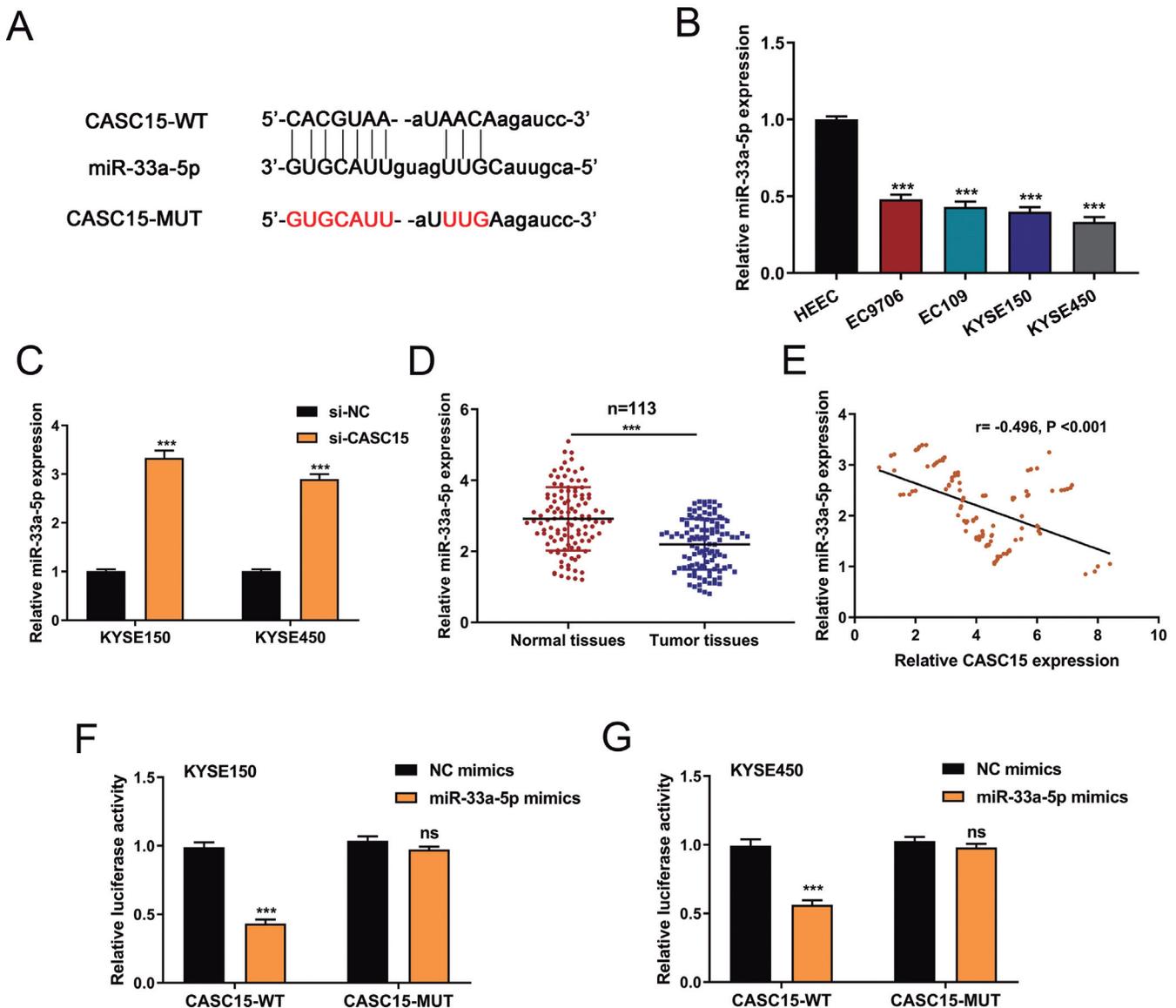
Fig. 3. CASC15 directly interacts with miR-33a-5p. **A.** Potential binding sites between CASC15 and miR-33a-5p. **B.** Relative miR-33a-5p expression in human ESCC cells and one human normal esophagus epithelial cell line. **C.** Relative miR-33a-5p expression in KYSE150 and KYSE450 cells after transfection with si-CASC15. **D.** Relative miR-33a-5p expression in ESCC tissues and adjacent normal tissues. **E.** Pearson correlation analysis between CASC15 and miR-33a-5p expression levels in ESCC tissues. **F-G.** Inhibition of luciferase activity by miR-33a-5p mimics in KYSE150 and KYSE450 cells. ***P<0.001, ns, not significant.

transfection efficiency was confirmed by RT-qPCR (all *P*<0.001, Fig. 2A,B). Subsequently, CCK-8 assays showed that CASC15 knockdown led to significantly decreased cell viability of KYSE150 and KYSE450 cells (all *P*<0.001, Fig. 2C,D). Then, transwell assays showed that CASC15 knockdown markedly inhibited the migration and invasion abilities of KYSE150 and KYSE450 cells (all *P*<0.001, Fig. 2E,F).

CASC15 acted as a sponge for miR-33a-5p in ESCC

Based on bioinformatics tools DIANA-LncBase v2, we identified that miR-33a-5p was a potential target of CASC15, which possessed a complementary binding

with CASC15 (Fig. 3A). Next, the expression of miR33a-5p was determined in human ESCC cell lines, and the results indicated that miR33a-5p was markedly downregulated in ESCC cells (*P*<0.001, Fig. 3B). Moreover, CASC15 knockdown significantly upregulated the expression of miR33a-5p in KYSE150 and KYSE450 cells (*P*<0.001, Fig. 3C). The analysis of clinical tissues showed that miR-33a-5p was obviously downregulated in ESCC tissues (*P*<0.001, Fig. 3D). Pearson's correlation analysis further revealed a negative relationship between CASC15 and miR-33a-5p expression in ESCC tissues (*P*<0.001, Fig. 3E). To assess the potential physical interactions between CASC15 and miR-33a-5p, the wild-type CASC15
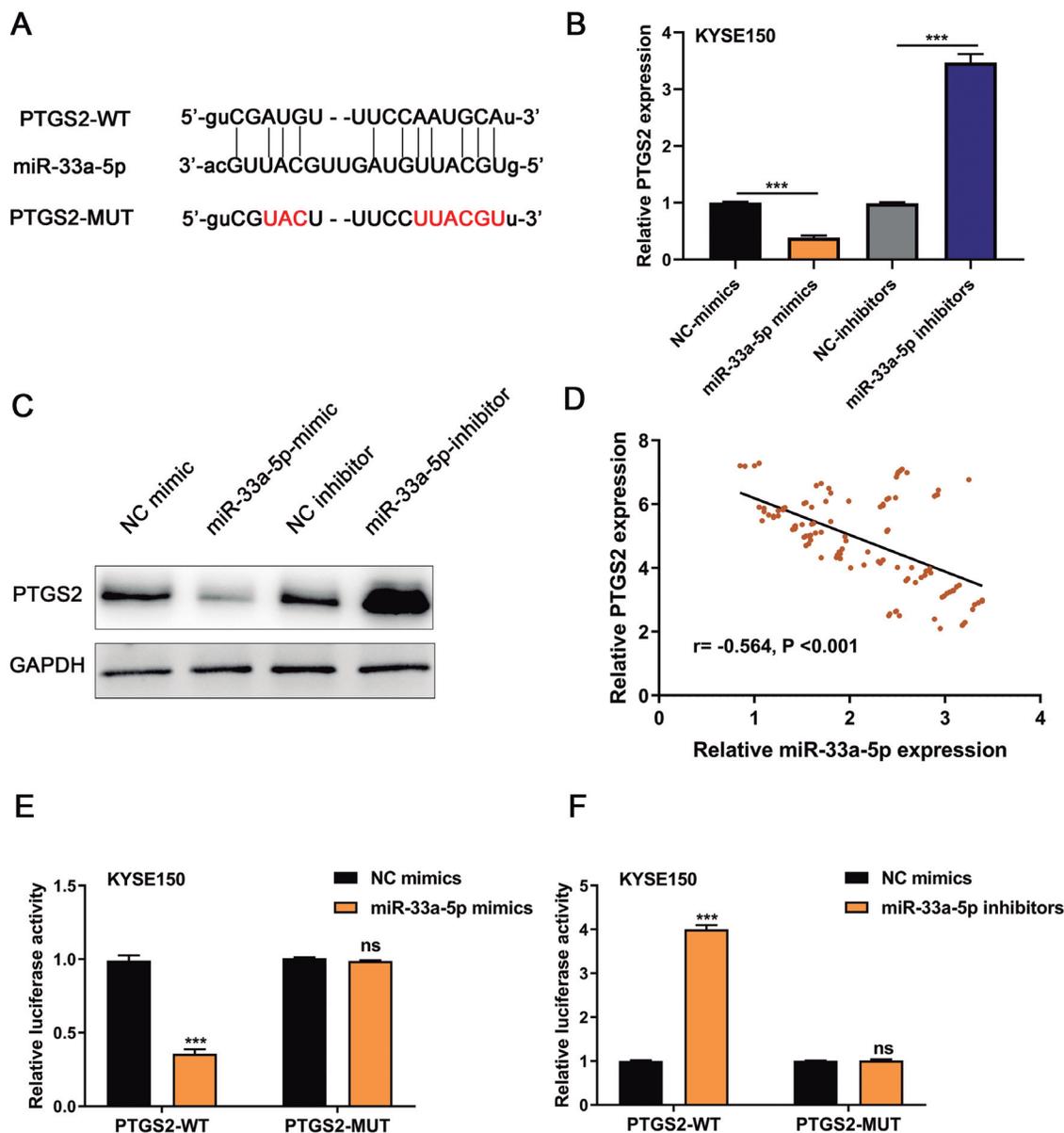
Fig. 4. PTGS2 is a direct target of miR-33a-5p. **A.** Potential binding sites of PTGS2 in the 3′-UTR region with miR-33a-5p. **B-C.** miR-33a-5p mimics-induced inhibition and miR-33a-5p inhibitors-induced promotion of PTGS2 expression at mRNA **(B)** and protein levels **(C)**. **D.** Pearson correlation analysis between miR-33a-5p and PTGS2 expression in ESCC tissues. **E.** Inhibition of luciferase activity by miR-33a-5p mimics in KYSE150 cells. **F.** Promotion of luciferase activity by miR-33a-5p inhibitors in KYSE150 cells. ***P*<0.001, ns, not significant.
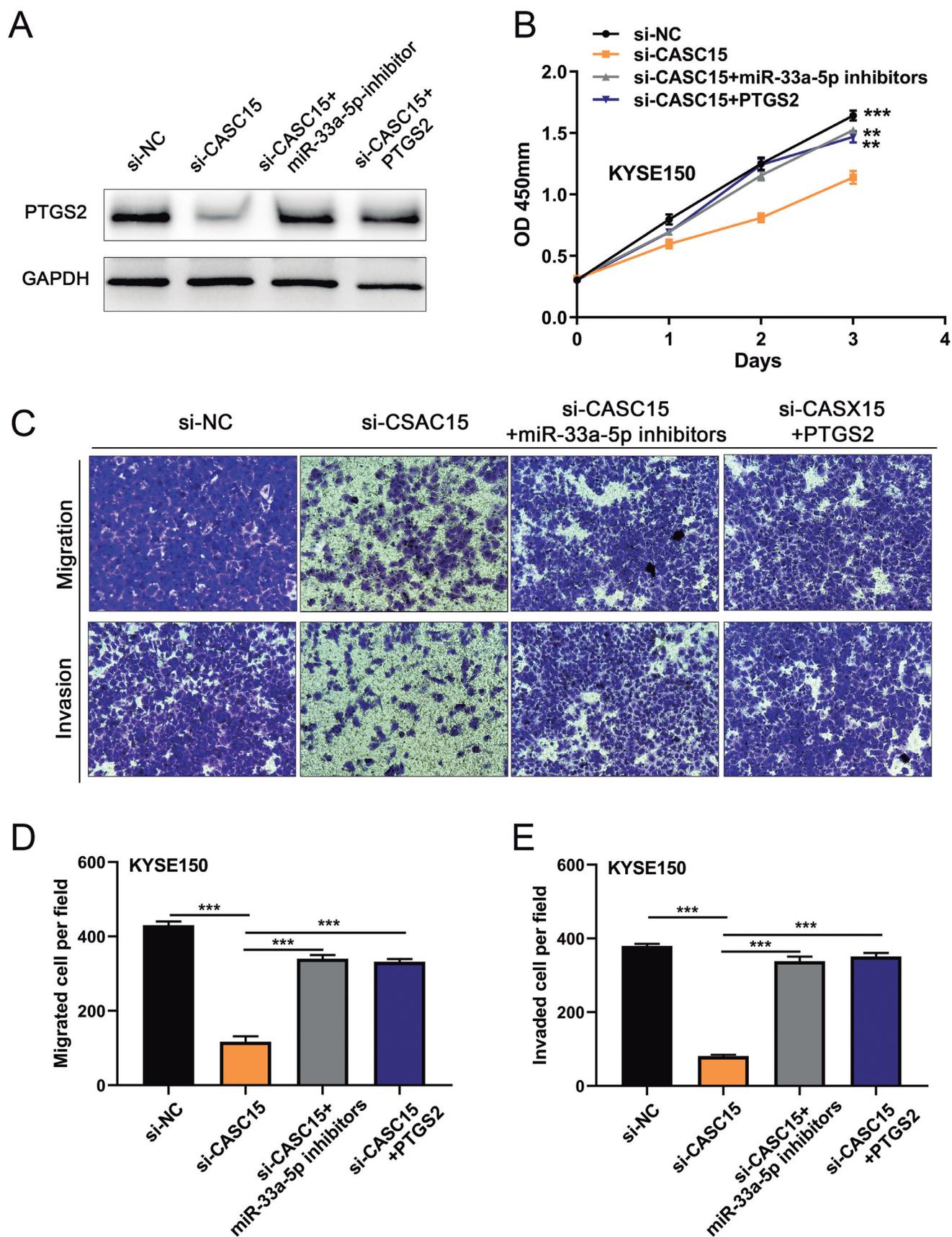
Fig. 5. MiR-33a-5p knockdown or PTGS2 overexpression reconciles the tumor-suppressive effects of CASC15 knockdown in ESCC cells. **A.** Western blot analysis of PTGS2 expression in KYSE150 cells after transfection with si-NC, si-CASC15, si-CASC15+miR-33a-5p inhibitor, or si-CASC15+PTGS2. **B-D.** CCK-8 and transwell assays were performed to analyze the cell proliferation **(B)**, migration **(C)**, and invasion **(D)** capacity in KYSE150 cells after transfection with si-NC, si-CASC15, si-CASC15+miR-33a-5p inhibitor, or si-CASC15+PTGS2. **$P<0.01, ***P<0.001.

(CASC15-WT) and mutant CASC15 (CASC15-MUT) were established. The luciferase reporter showed that miR-33a-5p mimics led to a significantly lower luciferase activity of the CASC15-WT, but exerted no effect on the CASC15-MUT in KYSE150 and KYSE450 cells (all *P*<0.001, Fig. 3F,G). All the above data indicated that miR-33a-5p was a direct target of CASC15 in ESCC.

MiR 33a 5p directly targets PTGS2 in ESCC

To explore potential targets of miR-33a-5p in ESCC, bioinformatics online tool (Starbase v2.0) determined the putative binding sites between miR-33a-5p and PTGS2 (Fig. 4A). As revealed by RT-qPCR and western blot, miR-33a-5p overexpression significantly inhibited the PTGS2 mRNA and protein expression in KYSE150 cells, while miR-33a-5p knockdown strikingly promoted their expression levels (all *P*<0.001, Fig. 4B,C). In addition, the Pearson's analysis between miR-33a-5p and PTGS2 mRNA expression in ESCC tumor tissues showed a consistent trend (*P*<0.001, Fig. 4D). Besides, luciferase reporter determined the direct interaction between miR-33a-5p and PTGS2 (*P*<0.001, Fig. 4E,F). Thus, it was confirmed that PTGS2 was a direct target of miR-33a-5p in ESCC.

CASC15 knockdown hindered ESCC progression via the miR-33a-5p/PTGS2 axis

Then, to further confirm whether the suppression of cell proliferation, migration, and invasion effects of CASC15 knockdown was mediated by miR-33a-5p/PTGS2 axis, a "rescue" strategy was adopted. The miR-33a-5p inhibitor or pc-DNA 3.1 PTGS2 was co-transfected with si-CASC15 into KYSE150 cells, and the western blotting results indicated that miR-33a-5p knockdown or PTGS2 overexpression remarkably abrogated the inhibition of PTGS2 expression induced by CASC15 knockdown (Fig. 5A). CCK8 assays showed that miR-33a-5p knockdown or PTGS2 overexpression rescued the CASC15 knockdown-induced inhibition on cell proliferation (Fig. 5B). Meanwhile, transwell assays demonstrated that miR-33a-5p knockdown or PTGS2 overexpression reversed CASC15 knockdown-mediated inhibition on cell migration and invasion (Fig. 5C-E). Overall, these observations indicated that CASC15 knockdown exerts its tumor-inhibiting effects on ESCC progression through the miR-33a-5p/PTGS2 axis.

Discussion

Several studies have demonstrated that CASC15 plays a significant oncogenic role in various cancers. Specifically, Zhang et al. demonstrated that CASC15 was upregulated in osteosarcoma and its knockdown can inhibit the proliferation, migration, and invasion of osteosarcoma cells (Zhang et al., 2020). Wu et al. reported that CASC15 was enriched in gastric cancer

and promoted tumor progression by targeting CDKN1A and ZEB1 (Wu et al., 2018). In colon cancer, Jing et al. demonstrated that CASC15 was upregulated in colon cancer and knockdown of CASC15 impeded the progression of colon cancer cells in vitro and in vivo (Jing et al., 2018). However, the potential biological function of CASC15 in ESCC progression is unknown. In this study, we found that CASC15 was upregulated in ESCC, and high expression of CASC15 was closely associated with poor clinicopathological features and survival outcome in ESCC patients. Functional experiments showed that CASC15 knockdown inhibited cell proliferation, migration, and invasion of ESCC cells. Our data revealed the oncogenic role of CASC15 in ESCC, which was consistent with previously reported findings.

Mounting evidence has revealed that lncRNAs can act as ceRNAs for miRNAs to regulate tumorigenesis (Huarte, 2015). In this study, bioinformatics analysis presented that miR-33a-5p might be a target of CASC15. Notably, a negative relationship between CASC15 and miR-33a-5p was determined in ESCC. Subsequently, luciferase reporter assays further confirmed the direct interaction between CASC15 and miR-33a-5p. Hence, CASC15 exerted an oncogenic effect on ESCC by negatively regulating miR-33a-5p expression. It has also been reported that miR-33a-5p was downregulated in ESCC and enhanced expression of miR-33a-5p can inhibit cell proliferation and metastasis of ESCC cells, which echoes with the findings in this study (Zhang et al., 2019). Moreover, the anti-cancer function of miR-33a-5p has been previously determined in melanoma (Zhang et al., 2020), colorectal cancer (Yan et al., 2019), lung cancer (Pan et al., 2018), osteosarcoma (Zhang et al., 2015), and pancreatic ductal adenocarcinoma (Lian et al., 2021).

To further examine the potential downstream targets of miR-33a-5p, bioinformatics tools and related functional assays elucidated that PTGS2 was a downstream target of miR-33a-5p. PTGS2, also known as cyclooxygenase-2 (COX-2), is an enzyme that mediates the synthesis of prostaglandins and thromboxanes, which is a critical regulator of cell proliferation, differentiation, transformation, and apoptosis (Goradel et al., 2019). PTGS2 overexpression has been reported to be associated with tumorigenesis and progression of many cancers, including colorectal cancer (Kunzmann et al., 2013), gastric cancer (Thiel et al., 2011), and breast cancer (Hoellen et al., 2011). Notably, several studies reported that COX-2 is overexpressed in ESCC and positively associated with progression of ESCC (Zhang et al., 2011; Hu et al., 2016; Tasneem et al., 2018). Moreover, the rescue assays showed that miR-33a-5p knockdown or PTGS2 overexpression reversed the cell proliferation, migration, and invasion inhibition role of CASC15 knockdown, which illuminated that CASC15 knockdown was able to restrain ESCC progression via CASC15/miR-33a-5p/PTGS2 axis.

In conclusion, this study has firstly demonstrated

that CASC15 was upregulated in ESCC, and CASC15 knockdown inhibited progression of ESCC cells via targeting the miR-33a-5p/PTGS2 axis. The present study highlights a tumor-promoting function and potential therapeutic approach of CASC15 to ESCC.

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