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LAMC2 regulated by microRNA-125a-5p accelerates the progression of ovarian cancer via activating p38 MAPK signalling

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ABSTRACT

Aims: Laminin γ2 (LAMC2) is over-expressed in ovarian cancer, and its high expression facilitates cell invasion. Nevertheless, the effects of LAMC2 on other ovarian cancer cell functions and its underlying mechanism remain largely unclear. Bioinformatics analysis shows that LAMC2 is a predicted target of miR-125a-5p and miR-193a-3p. Therefore, the present study aimed to investigate the effects of LAMC2 in ovarian cancer progression and determine whether LAMC2 expression is under the regulation of miR-125a-5p or miR-193a-3p in ovarian cancer.
Materials and methods: Immunohistochemistry staining, western blot and qPCR were used to detect LAMC2 expression profiles. CCK-8, flow cytometry and tumour formation assays were used to assess cell proliferation, apoptosis and tumorigenesis. The interaction between miR-125a-5p/miR-193a-3p and LAMC2 were determined by the luciferase gene reporter assay.
Key findings: The results showed that LAMC2 was over-expressed in ovarian cancer tissues and cell lines. Over-expression of LAMC2 significantly promoted cell proliferation and repressed cell apoptosis, as well as increased the expression levels of p38, p-p38, c-myc and CREB, and translocated p38 protein to the nucleus. In addition, the promotion of cell proliferation and repression of cell apoptosis mediated by LAMC2 over-expression were all weakened when p38 was downregulated. Moreover, LAMC2 expression was negatively regulated by miR-125a-5p, which inhibited the nuclear accumulation of p38 protein. Upregulation of LAMC2 significantly abolished the effects of miR-125a-5p on cell proliferation inhibition and cell apoptosis promotion, as well as tumourigenesis repression.
Significance: The present study clarified that LAMC2 functioned as an oncogene in ovarian cancer through up-regulating p38 under the regulation of miR-125a-5p.

1. Introduction

Ovarian cancer is one of the three predominant malignant tumours affecting the female reproductive system, accounting for ~25% of tumours, and ~90% of ovarian cancer cases are epithelial ovarian cancer (EOC) [1,2]. It has been reported that approximately 70% of patients with EOC reached a median or advanced stage when they were first diagnosed [3]. Although much progress has been made in developing methods of treatment for EOC, the majority of EOC patients inevitably undergo recurrence at the postoperative stage, with the 5-year survival rate being only 30–40% [4]. Therefore, it is necessary to deeply explore the mechanisms underlying ovarian cancer, aiming to find potent targets for ovarian cancer treatment.

Laminins are extracellular glycoproteins that are important components of all basement membrane zones and are strongly implicated in some biological processes, including wound healing, tissue development, and tumourigenesis [5]. Laminin γ2 (LAMC2), encoding laminin γ2, together with LAMB3 (encoding β3) and LAMA3 (encoding α3), constitutes the heterotrimeric glycoprotein laminin-332 (formerly known as laminin-5), which helps to regulate the migration of epithelial cells and is upregulated in several kinds of cancers to promote cancer invasion [6]. Additionally, increasing evidence has revealed the important role of LAMC2 in the tumourigenesis of many types of cancers, including lung cancer [6], oral squamous cell carcinoma [7], pancreatic cancer [8], anaplastic thyroid carcinoma [9], colorectal carcinoma [10], and ovarian cancer [11,12]. For instance, Kato et al. [11,12] revealed that the LAMC2 chain accumulates in the stroma of ovarian clear cell carcinoma, enhancing cell migration in vitro. Upregulation of LAMC2 protein is also involved in epididymis protein 4-induced malignant properties of ovarian cancer cells [13]. However, the other effects of LAMC2 on ovarian cancer cell function and its underlying mechanisms remain largely unclear.

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MicroRNAs (miRNAs) are small, non-coding RNAs that negatively regulate gene expression at the posttranscriptional level by binding to the 3′-untranslated region (3′-UTR) of their target mRNAs, subsequently regulating numerous physiological processes, such as cell differentiation and development [14]. Accumulated evidence has illustrated that miRNAs are deregulated in a majority of cancers, including ovarian cancer, and contribute to cancer progression by regulating their target genes [15,16]. Bioinformatics analysis with miRanda, TargetScan and Targets and Expression online software shows that LAMC2 is a predicted target for miR-125a-5p and miR-193a-3p. However, whether miR-125a-5p/miR-193a-3p is involved in the progression of ovarian cancer via targeting LAMC2 remains unknown.

Overall, the current study was performed to explore the effects of LAMC2 in ovarian cancer progression and determine whether LAMC2 expression is under miR-125a-5p/miR-193a-3p regulation in ovarian cancer progression.

2. Materials and methods

2.1. Patients

A total of 20 paired ovarian cancer tissues and their adjacent normal tissues were obtained from patients with EOC. Following ovariectomy, the tissue samples were immediately stored at −80 °C until further study. Tissues isolated from ≥3 cm of cancer tissues margin were considered as normal tissues. All patients with ovarian cancer had undergone ovariectomy before chemo- and/or radiotherapy and signed informed consent. The expression of LAMC2 in the ovarian tissue was determined by immunohistochemistry staining score. LAMC2 was thought to be highly expressed in tissues if the score was ≥3. Experiments involving human samples were performed in accordance with the Helsinki Declaration and were approved by the ethical committee of the First Affiliated Hospital of Zhengzhou University.

2.2. Immunohistochemistry staining

Samples of formalin-fixed, paraffin-embedded ovarian cancer tissue and adjacent normal tissue were cut into 5-μm sections. After deparaffinization, hydration, and blocking with 5% goat serum (AmyJet Scientific Inc., Wuhan, China), the sections were probed with the primary antibody against LAMC2 (No. ab210959, Abcam, MA, USA) and the corresponding secondary antibody (Cell Signalling Technology, CA, USA), followed by incubation with Chromogen 3, 30-diaminobenzidine tetrachloride (DAB) (R&D Systems, MN, USA). Cell nuclei were stained with Harris' haematoxylin solution.

To analyse the expression level of LAMC2 in different ovarian cancer tissues or adjacent normal tissues, we evaluated LAMC2 staining by multiplying the staining extent and intensity according to a previous study [17]. The staining extent was scored according to the percentage of the positively stained area with the following scale: 0 (< 5%), 1 (5–25%), 2 (25–50%), 3 (50–75%), and 4 (> 75%). The staining intensity was scored as 0 (no staining), 1 (weak), 2 (distinct) and 3 (strong). The scoring was determined by two evaluators who did not know the pathological and clinical characteristics of the patients.

2.3. Cell lines and culture conditions

Human ovarian cancer cell lines CAOV3, A2780 and HO-8910 and the human transformed ovarian epithelial cell line IOSE80 were all obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). A2780, CAOV3 and IOSE80 cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, CA, USA); HO-8910 cells were cultured in RPMI 1640 medium with 10% foetal bovine serum (FBS, Gibco, CA, USA), 50 U/ml penicillin and 50 μg/ml streptomycin in a humidified atmosphere at 37 °C with 5% CO_2.

2.4. Cell transfection

Small interference RNAs (siRNAs) used to downregulate LAMC2 (si-LAMC2; No. SR302651) and p38 (si-p38; No. SR301010) and the lentivirus vector used to over-express LAMC2 (OE-LAMC2, No. RC222076L4) were purchased from OriGene (Beijing, China); lentivirus vectors used to upregulate human miR-125-5p or miR-193-3p, referred to as mimic-miR-125-5p and mimic-miR-193-3p, respectively, together with the lentivirus vectors used to silence miR-125-5p or miR-193-3p, referred to as inhibitor-miR-125-5p and inhibitor-miR-193-3p, respectively, together with their negative controls (NC) were designed and synthesized by GenePharma (Shanghai, China).

Cell transfection was performed when cell confluence reached 60–70% using lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Cell transfection efficiency was measured by western blot or real-time quantitative PCR assay (qPCR). For lentivirus infections, cells were treated with the lentivirus vectors with the help of polybrene (7 μg/ml) and selected by G418 (100 μg/ml) or puromycin (5 μg/ml) for 14 days after 24 h of cell infection to build stable cell lines if necessary.

2.5. Western blot analysis

Cells were collected and lysed with PIPA lysis buffer (BestBio, Nanjing City, Jiangsu province, China), supplemented with protease inhibitors. After being centrifuged at 12,000 rpm for 30 min at 4 °C and quantified with a BCA Kit (Thermo Fisher Scientific, MA, USA), 20 μg of protein from each sample was loaded into the 10% SDS-PAGE gel, followed by electrophoresis and transfer onto PVDF membranes (Millipore, Billerica, MA, USA) in transfer buffer. Next, the membranes were blocked with 5% non-fat milk diluted in TBS solution containing 0.5% Twen-20 (TBST) at 4 °C overnight and then incubated with anti-LAMC2 (1:1000 dilution; No. ab210959, Abcam, MA, USA), anti-p38 (1:1000 dilution; No. ab31828, Abcam, MA, USA), anti-p-p38 (1:2000 dilution; No. #9216, Cell Signalling Technology, CA, USA), anti-ERK1/2 (1:1000 dilution; No. MA5-15134, Thermo Fisher Scientific, MA, USA), anti-p-ERK1/2 (1:2000 dilution; No. #4370, Cell Signalling Technology, CA, USA), anti-JNK (1:500 dilution; No. 44-690G, Thermo Fisher Scientific, MA, USA), anti-p-JNK (1:2000 dilution; No. #9255, Cell Signalling Technology, CA, USA), anti-ERK5 (1:1000 dilution; No. 44-688G, Thermo Fisher Scientific, MA, USA), anti-p-ERK5 (1:1000 dilution; No. #3371, Cell Signalling Technology, CA, USA), anti-c-myc (1:1000 dilution; No. ab32072, Abcam, MA, USA), anti-CREB (1:1000 dilution; No. ab32515, Abcam, MA, USA) and anti-GADPH (1:10000 dilution; No. ab8245, Abcam, MA, USA) antibodies at 4 °C overnight. After washing with TBST three times, the membranes were probed with horseradish peroxidase-labelled secondary antibodies (Proteintech, USA) for 1 h at room temperature and measured with ECL detection reagents (Millipore, Billerica, MA, USA). GAPDH was used as a loading control.

2.6. RNA isolation and qPCR

All kits used in this experiment were purchased from TaKaRa (Dalian, China). In detail, total RNA was isolated from cells or tissues with RNAiso plus and reverse transcribed (RT) into cDNA using the PrimeScript RT reagent Kit. Finally, the cDNA was quantified by qPCR using SYBR Premix Ex Taq. The mRNA expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method after normalization to the expression of U6 or GAPDH. Primer sequences are listed in Table 1.

2.7. CCK-8 assay

Cell proliferation was measured by a Cell Counting Kit-8 (Dojindo, Tokyo, Japan) according to the manufacturer's instructions. Briefly, 100 μl of cell suspension containing 2000 cells was placed into a 96-

Table 1
Sequences for qPCR.

Gene	Sequence (5′-3′)
LAMC2	Forward: TACCAGAGCCAAGAACGCTG
	Reverse: CGCAGTTGGCTGTTGATCTG
p38	Forward: GGGGCTGAGCTTTTGAAGAA
	Reverse: GTGGTGGCACAAAGCTGATG
GAPDH	Forward: CCACTAGGCGCTCACTGTTCTC
	Reverse: CATGGTGGTGAAGACGCCAG
miR-125a-5p	RT:GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCACAG
	Forward: TCCCTGAGACCCTTTAAC
	Reverse: GTGCAGGGTCCGAGGT
miR-193a-3p	RT:GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC ACTGGG
	Forward: GCAACTGG CCTACAAAGT
	Reverse: GTGCAGGGTCCGAGGT
U6	RT: AACGCTTCACGAATTTGCGT
	Forward: CTCGCTTCGGCAGCACA
	Reverse: AACGCTTCACGAATTTGCGT

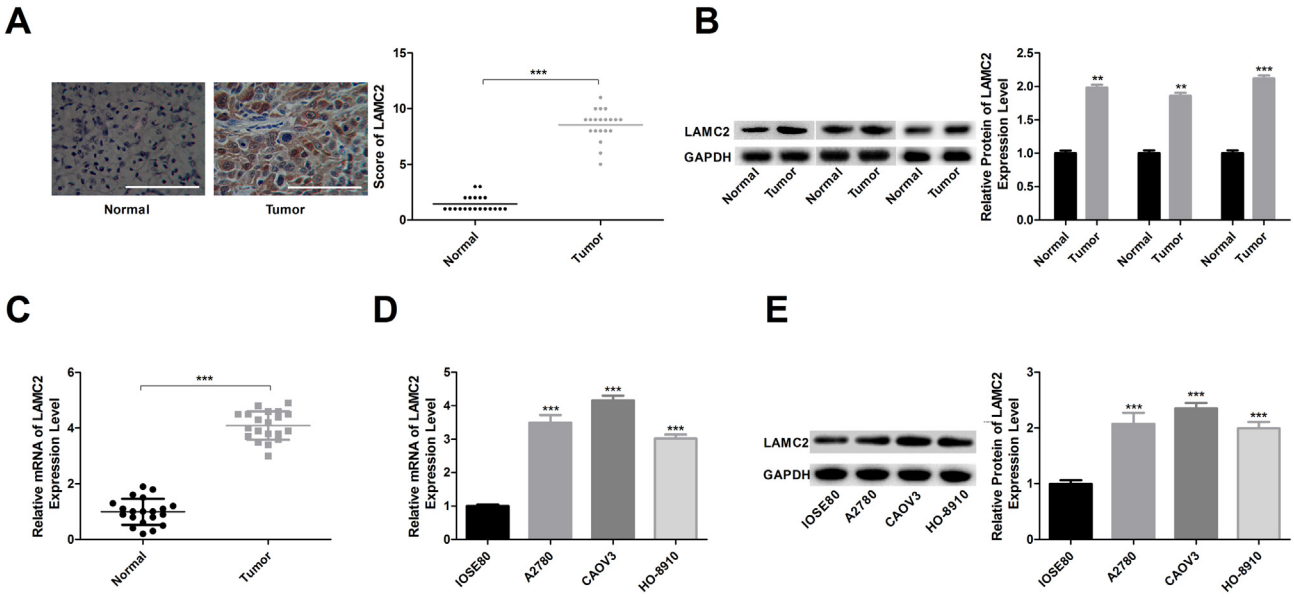

Fig. 1. High expression of LAMC2 was observed in ovarian cancer tissues and cells. A. Protein expression of LAMC2 was assessed by immunohistochemical staining with an antibody against LAMC2 in 20 paired ovarian cancer tissues and nearby para-carcinoma tissues (Scale bar = 500 μm). B. The expression patterns of LAMC2 protein in 3 matched ovarian cancer tissues and para-carcinoma tissues were examined by western blot. C. LAMC2 mRNA levels in 20 paired ovarian cancer tissues and para-carcinoma tissues were tested by qPCR. D–E. The mRNA and protein levels of LAMC2 were determined by qPCR and western blot in normal ovarian cell line (IOES80) and ovarian cancer cell lines (A2780, CAOV3 and HO-8910) (**P < 0.01, ***P < 0.001).

well plate overnight. Then, the cells were transfected with different vectors, followed by incubation with 10 μl of CCK-8 solution after 1, 2, 3, 4, or 5 days of the transfections. The culture medium was refreshed every day. The absorbance at 450 nm was detected using a spectrophotometer purchased from Shjingmi, Co., LTD (Shanghai, China).

2.8. Flow cytometry assay

Cell apoptosis was assessed by staining with an Annexin V-FITC/PI double-staining kit (YESEN Co., LTD, Shanghai, China) according to the manufacturer's instructions. After staining, cells were submitted to flow cytometry analysis (BD FACS Calibur, BD, USA) to determine the cell apoptosis rate.

2.9. Luciferase gene report assay

The 3′UTR sequence of LAMC2 mRNA (wild type, WT) and the mutated type (WT) of LAMC2 with binding sites mutated were inserted into the pmiR-GLO dual-luciferase vector (Promega, WI, USA). Subsequently, cells were treated with mimic, mimic-NC, inhibitor or inhibitor-NC together with WT or MT. After 48 h of treatment, cells were collected and submitted to a dual-luciferase reporter gene assay system (Promega, MI, USA). Renilla luciferase activity was used as an internal control.

2.10. Immunofluorescence technique

Cells seeded on coverslips were washed thrice with PBS and fixed with cold methanol and sealed with 5% goat serum (diluted with PBS) for 1 h. Next, the cells were probed with anti-p38 (1:100, No. ab31828, Abcam, MA, USA) antibody overnight at 4 °C and fluorescent second antibodies IgG AF568 (red) (Invitrogen, CA, USA) in the dark for 1 h. Eventually, cell nuclei were dyed with DAPI solution (1:10000 dilution) for 5 min and covered with anti-fluorescence quenching slides. The expression and subcellular location of p38 were measured by an Olympus Confocal FV100 Microscope.

2.11. Tumour formation assay

The animal assay was performed in accordance with institutional

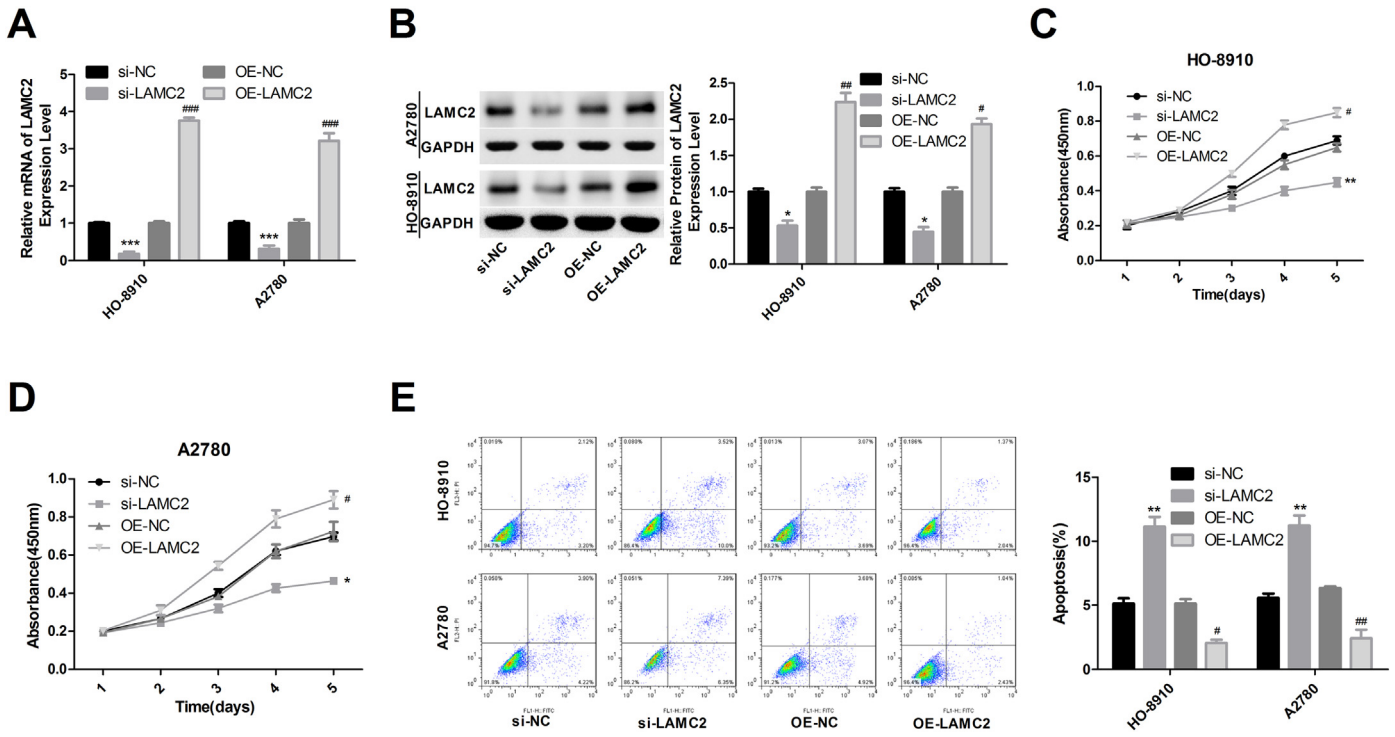

Fig. 2. LAMC2 upregulation promoted cell proliferation and inhibited cell apoptosis in ovarian cancer cells. HO-8910 and A2780 cells were respectively treated with si-/OE-LAMC2 and si-/OE-NC; examples are shown in A and B, respectively. QPCR and western blot were used to assess cell transfected efficiencies after 24 or 48 h of transfection. C–D. CCK-8 was used to measure cell proliferation. E. Flow cytometry was performed to determine cell apoptosis after 48 h of cell transfection with Annexin V/PI staining (**P < 0.01, ***P < 0.001, si-LAMC2 group vs si-NC group; #P < 0.05, ###P < 0.001, OE-LAMC2 group vs OE-NC group).

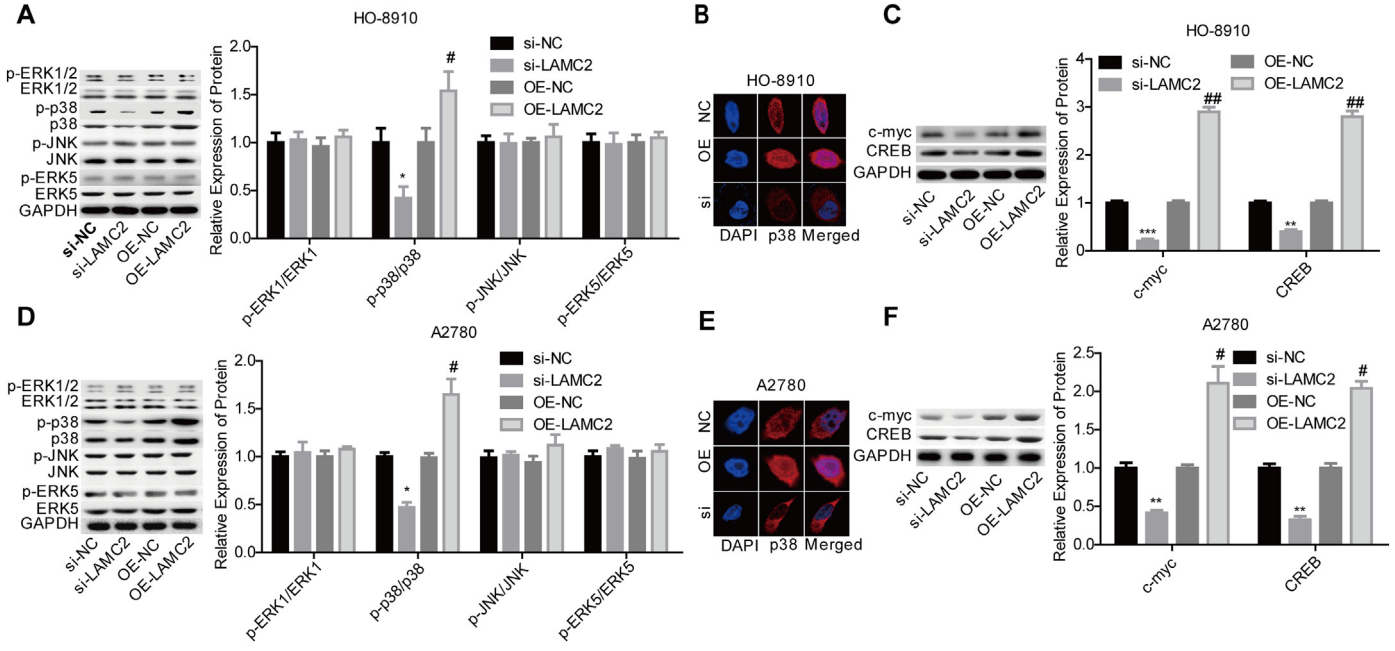

Fig. 3. Upregulation of LAMC2 increased p38 protein expression and stability in ovarian cancer cells. A. The protein expression levels of ERK1/2, p38, ERK5 and JNK, and their phosphorylation (p-ERK1/2, p-p38, p-ERK5, p-JNK) were detected by western blot after HO-8910 cells were transfected with si-LAMC2, si-NC, OE-LAMC2 or OE-LAMC2. B. The subcellular location of p38 protein was evaluated by immunofluorescence staining with p38 antibody after HO-8910 cells were modulated by LAMC2 expression (Scale bar = 50 μm). C. Western blot analysis was used to test the effects of LAMC2 over-expression or depletion on c-myc and CREB expression in HO-8910 cells. D. The protein expression levels of ERK1/2, p-ERK1/2, p38, p-p38, ERK5, p-ERK5, JNK and p-JNK were detected by western blot after A2780 cells were transfected with si-LAMC2, si-NC, OE-LAMC2 or OE-LAMC2. E. The subcellular location of p38 protein was evaluated by immunofluorescence staining with p38 antibody after A2780 cells were modulated by LAMC2 expression (Scale bar = 50 μm). F. Western blot analysis was used to test the effects of LAMC2 over-expression or depletion on c-myc and CREB expression in A2780 cells (***P < 0.001, si-LAMC2 group vs si-NC group; ##P < 0.01, OE-LAMC2 group vs OE-NC group).

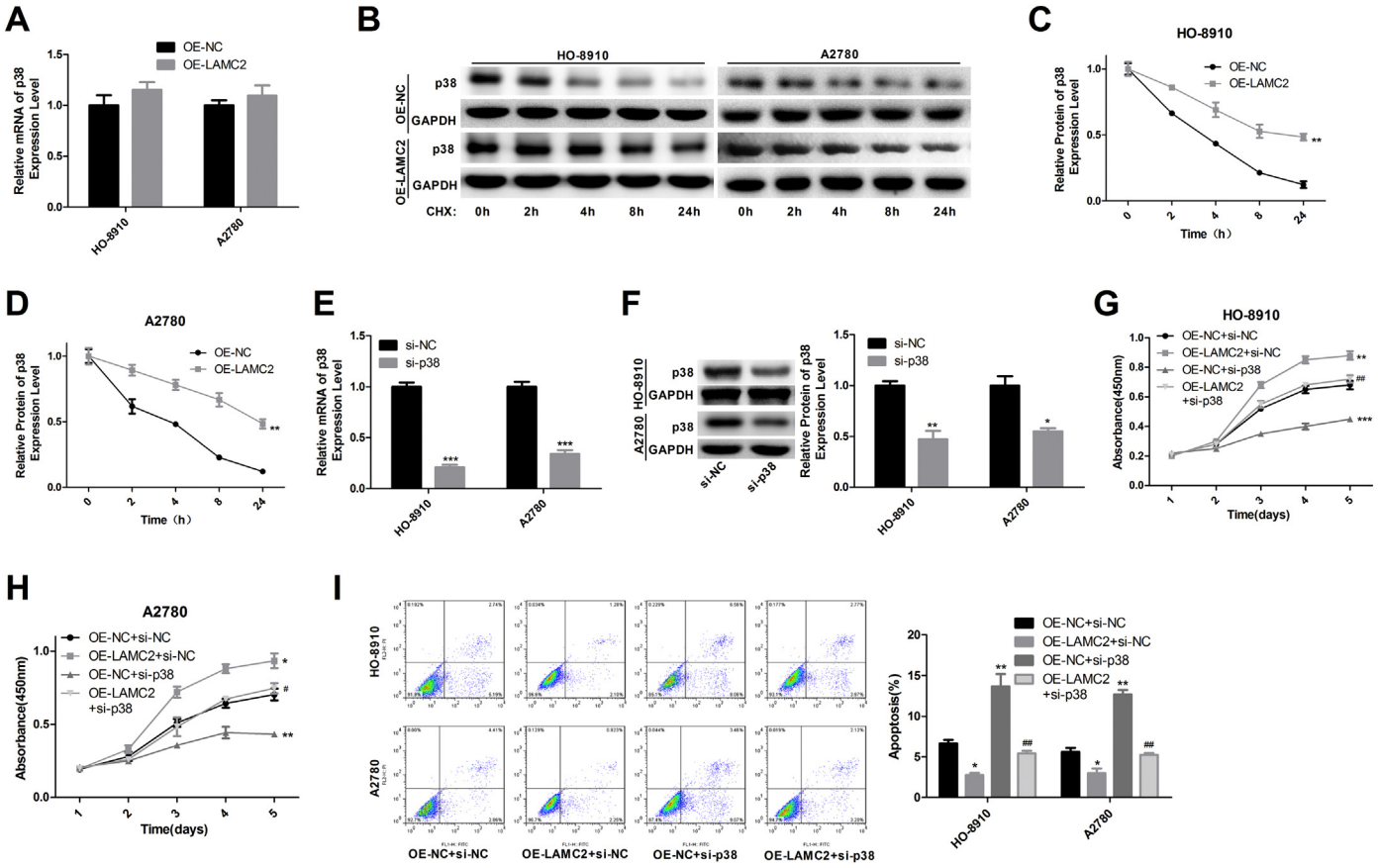

Fig. 4. LAMC2 promoted ovarian cancer progression by upregulating p38 expression. A. QPCR analysis of the mRNA level of p38 after upregulation of LAMC2 in A2780 and HO-8910 cells. B. Cycloheximide (CHX; 100 μg/ml) was added to A2780 and HO-8910 cells' culture medium after cells were infected with OE-LAMC2 or OE-NC to incubate for 0, 2, 4, 8 and 24 h to determine the effect of LAMC2 on p38 protein stability. C–D. Statistical diagram of p38 protein expression for CHX treatment. E–F. QPCR and western blot assays were used to assess the knockdown efficiency of si-p38 (***P < 0.001). G–H. Cell proliferation was detected with CCK-8 assay in A2780 and HO-8910 cells with various treatments. I. Cell apoptosis was assessed by flow cytometry assay in different treated A2780 and HO-8910 cells (**P < 0.01, ***P < 0.001, OE-LAMC2 + si-NC group/OE-NC + si-p38 group vs OE-NC + si-NC group; $^{\#\#}$P < 0.01, OE-LAMC2 + si-p38 group vs OE-LAMC2 + si-NC group).

guidelines and was approved by the Experimental Animal Center of the First Affiliated Hospital of Zhengzhou University. Four-week-old female BALB/c-nude mice were used for the tumour formation assay. Mice were subcutaneously injected in the armpit area with one million of A2780 cells with mimic-NC + OE-NC, mimic + OE-NC, mimic-NC + OE-LAMC2 or mimic + OE-LAMC2 stable expression. Mice were observed over 21 days, and then the tumours were dissected and weighed.

2.12. Data analysis

Experiments were performed at least three times, and the results are presented as the mean ± SD (standard deviation). Statistical analysis was performed using Student's *t*-test or one-way ANOVA. A *P*-value < 0.05 was identified as statistically significant.

3. Results

3.1. LAMC2 is over-expressed in ovarian cancer tissues and cells

To determine the effects of LAMC2 on the progression of ovarian cancer, we first assessed its expression profiles in ovarian cancer tissues and cells. Compared with the adjacent normal tissue samples, the LAMC2 expression level was obviously elevated in ovarian cancer tissue samples (Fig. 1A). Western blot and qPCR analysis also demonstrated that LAMC2 was highly expressed in ovarian cancer tissues at both the protein and mRNA levels compared to the nearby normal tissues (Fig. 1B–C). Furthermore, LAMC2 expression was also elevated in ovarian cancer cell lines such as A2780, CAOV3 and HO-8910 compared with the transformed ovarian cell line IOSE80 (Fig. 1D–E). All findings suggest that LAMC2 was over-expressed in ovarian cancer.

3.2. Upregulation of LAMC2 promotes cell proliferation and inhibits cell apoptosis in ovarian cancer cells

Then, we investigated the function of LAMC2 in the proliferation and apoptosis of ovarian cancer cells through loss/gain-of-function assays. Transfection of OE-LAMC2 significantly promoted LAMC2 expression at the mRNA and protein levels, while si-LAMC2 decreased LAMC2 expression in A2780 and HO-8910 cells (Fig. 2A–B), suggesting that OE-LAMC2 and si-LAMC2 were successfully constructed. In addition, upregulation of LAMC2 significantly enhanced the proliferation of A2780 (Fig. 2C) and HO-8910 cells (Fig. 2D) and repressed cell apoptosis (Fig. 2E), whereas knockdown of LAMC2 with si-LAMC2 transfection showed the opposite result (Fig. 2C–E), indicating that LAMC2 served as an oncogene in ovarian cancer.

3.3. LAMC2 promotes ovarian cancer progression by upregulating p38 expression

Next, to probe the mechanism underlying LAMC2 in ovarian cancer, we explored whether ERKs are under the modulation of LAMC2 in

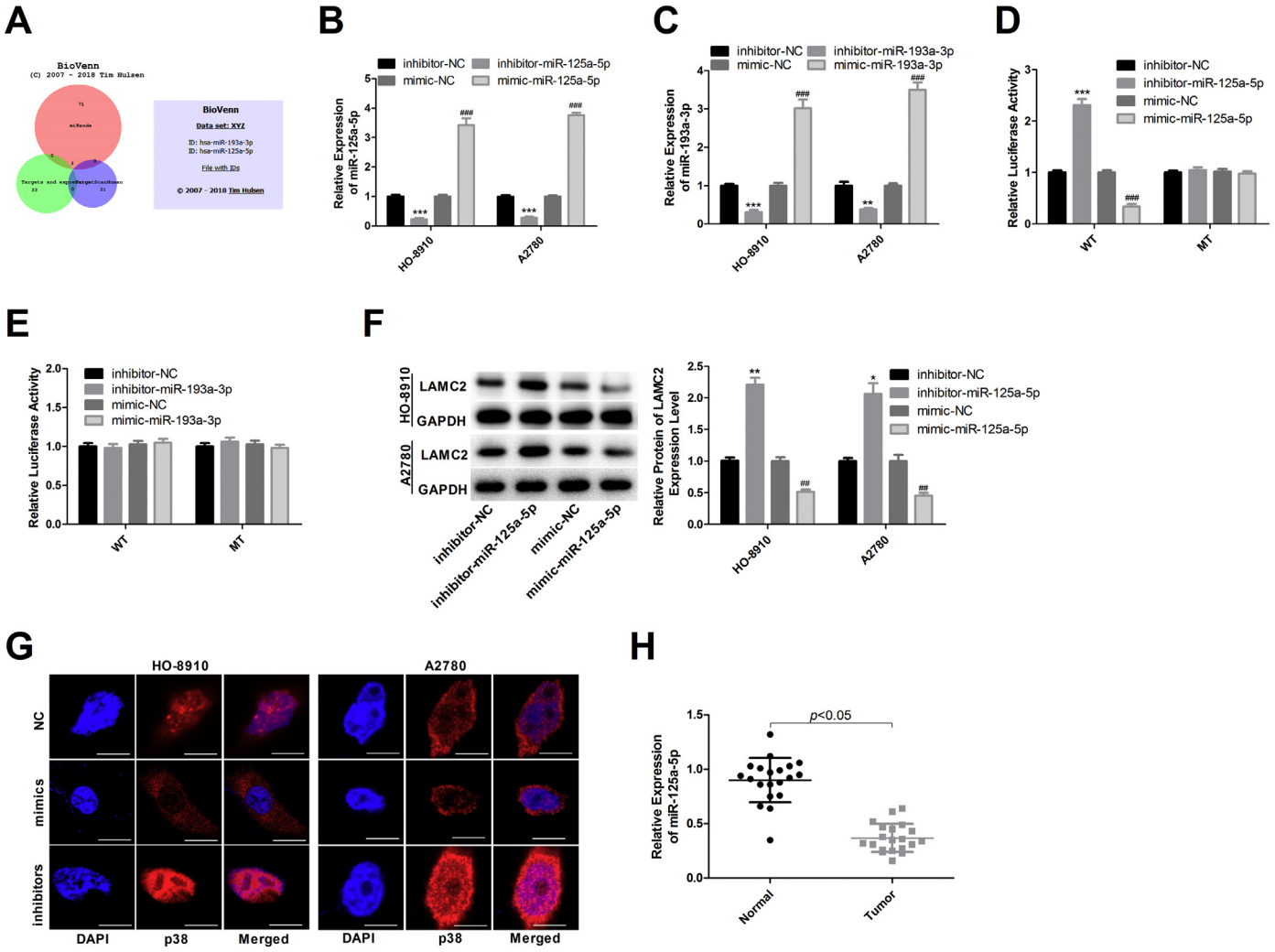

Fig. 5. miR-125-5p negatively regulated LAMC2 expression in A2780 and HO-8910 cells. A. Biovenn showed the predicted miRNAs of LAMC2. B–C. RT-PCR was used to test the mRNA levels of miR-125-5p and miR-193-3p after A2780 and HO-8910 cells were transfected with mimic, mimic-NC, inhibitor or inhibitor-NC lentivirus vectors of miR-125-5p and miR-193-3p for 48 h. D–E. A luciferase reporter gene assay was used to detect the effects of miR-125-5p or miR-193-3p on the post-transcriptional activity of LAMC2. F. Western blot analysis of LAMC2 expression after A2780 and HO-8910 cells were transfected with mimic-miR-125-5p for 48 h. G. Immunofluorescence was used to detect the location of p38 protein following up/downregulation of miR-125-5p in A2780 and HO-8910 cells 48 h after treatment (Scale bar = 50 μm). H. QPCR analysis of miR-125-5p expression levels in 20 paired ovarian cancer tissues and adjacent normal tissues (***P < 0.001, inhibitor group vs inhibitor-NC group; $^{###}$P < 0.001, mimic group vs mimic-NC group).

ovarian cancer A2780 and HO-8910 cells. Among the four members of ERKs, ERK1/2, p38, JNK and EKK5, only p38 expression and its phosphorylated level (p-p38) were significantly increased when LAMC2 was upregulated in HO-8910 cells and vice versa (Fig. 3A). In addition, upregulation of LAMC2 in HO-8910 cells also translocated p38 protein from the cytoplasm to the nucleus compared to the OE-NC group (Fig. 3B). In addition, the expression of p38 down-stream proteins, such as c-myc and CREB, were all increased in the OE-LAMC2 group, whereas they were decreased si-LAMC2 group (Fig. 3C). These results also applied to the A2780 cells (Fig. 3D–F).

In addition, LAMC2 upregulation significantly increased the stability of p38 protein but had no obvious influence on p38 mRNA levels in both A2780 and HO-8910 cells (Fig. 4A–D), indicating that p38 might take part in the process in which LAMC2 facilitates ovarian cancer progression. To this end, we assessed cell proliferation and apoptosis after A2780 and HO-8910 cells were transfected with OE-LAMC2 and si-p38 simultaneously. Si-p38 transfection significantly decreased the expression of p38 at both the mRNA (Fig. 4E) and protein levels (Fig. 4F) in A2780 and HO-8910 cells. In addition, knockdown of p38 expression rescued the effects of LAMC2 upregulation on cell proliferation promotion (Fig. 4G–H) and apoptosis repression (Fig. 4I). These findings clarify that LAMC2 promoted the progression of ovarian cancer via a p38 upregulation-dependent mechanism.

3.4. LAMC2 is negatively regulated by miR-125-5p in ovarian cancer cells

To further explore the molecular mechanism of LAMC2 in ovarian cancer, we searched the potential miRNAs that regulate LAMC2 expression using bioinformatics analysis. The results revealed that miR-125-5p and miR-193a-3p could bind to the 3′UTR of LAMC2 mRNA through analysis using 3 websites (miRanda, TargetScan, and Targets and Expression) (Fig. 5A). To further explore their effects on LAMC2 expression, we performed gain/loss-of-function assays in both A2780 and HO-8910 cells. Transfection with mimic significantly increased the expression of miR-125-5p and miR-193-3p, while inhibitor transfection decreased their expression levels (Fig. 5B–C). Luciferase reporter gene assay showed that miR-125-5p negatively regulated the activity of LAMC2 (Fig. 5D), whereas miR-193-3p showed no obvious effect (Fig. 5E). The upregulation of miR-125-5p with mimic significantly reduced LAMC2 protein expression and vice versa (Fig. 5F). MiR-125-

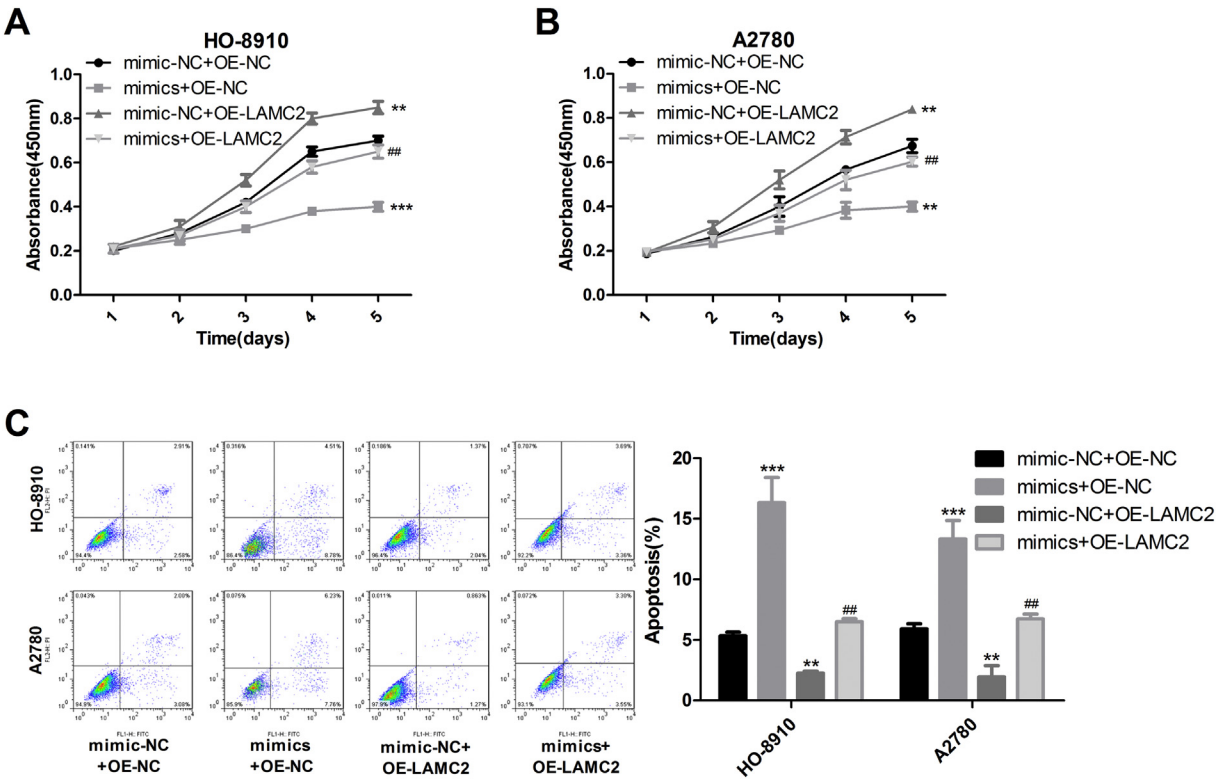

Fig. 6. miR-125-5p inhibited cell proliferation and promoted cell apoptosis by downregulating LAMC2 expression in A2780 and HO-8910 cells. A2780 and HO-8910 cells were transfected with mimic-NC + OE-NC, mimic + OE-NC, mimic-NC + OE-LAMC2 and mimic + OE-LAMC2, and examples cells are respectively shown in A and B. A CCK-8 assay was performed to determine cell viability. C. Flow cytometry with Annexin V/PI staining was used to assess early and late cell apoptosis rates (**P < 0.01, ***P < 0.001, mimic + OE-NC group/mimic-NC + OE-LAMC2 group vs mimic-NC + OE-NC group; ##P < 0.01, ###P < 0.001, mimic + OE-LAMC2 group vs mimic + OE-NC group).

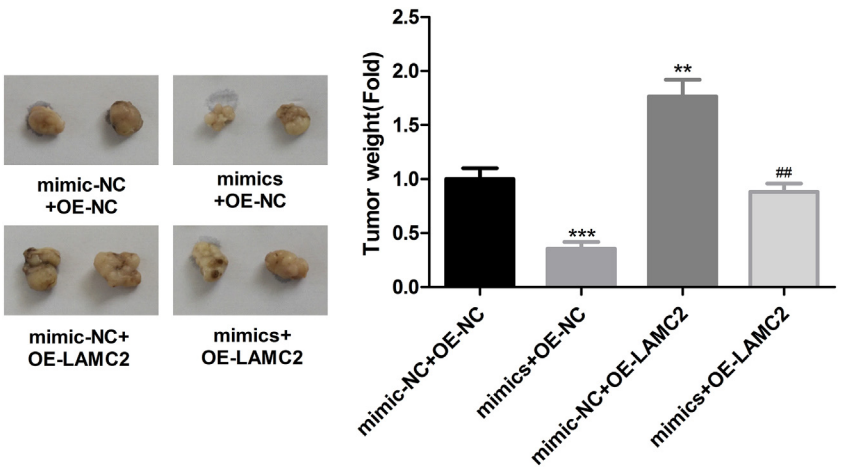

Fig. 7. miR-125-5p reduced the tumourigenesis of A2780 cells by downregulating LAMC2 expression. An in vivo tumour-burdened experiment was carried out to explore the effects of miR-125-5p/LAMC2 on the tumourigenesis of A2780 cells (**P < 0.01, ***P < 0.001, mimic + OE-NC group/mimic-NC + OE-LAMC2 group vs mimic-NC + OE-NC group; ##P < 0.01, mimic + OE-LAMC2 group vs mimic + OE-NC group).

5p upregulation significantly decreased p38 protein nuclear accumulation, while knockdown of miR-125-5p promoted the accumulation of p38 protein in the nucleus (Fig. 5G). Moreover, the expression of miR-125-5p was significantly decreased in ovarian cancer tissues compared with the adjacent normal tissues (Fig. 5H). These results suggest that miR-125-5p negatively regulated LAMC2 expression and promoted p38 nuclear export in ovarian cancer cells.

3.5. miR-125-5p inhibits ovarian cancer progression through downregulation of LAMC2 expression

Finally, we explored the effects of miR-125-5p/LAMC2 on ovarian cancer progression. Upregulation of miR-125-5p apparently repressed cell proliferation (Fig. 6A–B) and induced cell apoptosis (Fig. 6C); these effects were all weakened when LAMC2 was upregulated in A2780 and HO-8910 cells. Furthermore, LAMC2 upregulation rescued the tumourigenesis inhibition induced by miR-125-5p upregulation in A2780 cells (Fig. 7). These findings make clear that miR-125-5p suppressed the malignant phenotype transformation of ovarian cancer in a LAMC2-dependent manner.

4. Discussion

LAMC2, a key component of laminin-332 that is a component of the epithelial basement membrane [18], is reported to be over-expressed in many types of human cancers, including ovarian cancer, and

contributes to cancer invasion and migration. Notably, LAMC2 expression is associated with budding cancer cells located at the tip of invading malignant epithelium [19]. To further characterize the role and mechanism of LAMC2 in the occurrence and development of ovarian cancer, we carried out both in vivo and in vitro experiments. Our results showed that LAMC2 was over-expressed in ovarian cancer tissues and cell lines such as A2780, CAOV3 and HO-8910 compared to the corresponding normal paracancerous tissues and human transformed ovarian epithelial cell line IOSE80. Furthermore, we found that the upregulation of LAMC2 significantly enhanced ovarian cancer cell viability and repressed cell apoptosis, further confirming that LAMC2 served as an oncogene in ovarian cancer.

Conventional mitogen-activated protein kinases (MAPKs) mainly contain four major groups: ERK1/2, JNK, p38 and ERK5, which can be activated by a variety of growth factors or cytokines and subsequently regulate various cell functions, especially for cell survival, apoptosis and differentiation [20]. MAPKs are frequently over-activated in multiple kinds of cancers, including ovarian cancer [21], and play a crucial role in their malignant progression [22,23]. For example, ERK1/2/p38 MAPK activation plays an important role in the nuclear factor of activated T cells (NFATC1)-induced cell growth and tumourigenesis enhancement in ovarian cancer [24]. Moreover, inhibition of p38 MAPK was identified to improve cisplatin sensitivity in cisplatin-resistant ovarian cancer [25]. In the present study, we demonstrated that overexpression of LAMC2 increased the protein expression of p38 and facilitated its nuclear accumulation, while it showed no obvious influence on ERK1/2, JNK or ERK5. The downregulation of p38 impaired the oncogenic role of LAMC2 in ovarian cancer, suggesting that p38 was strongly implicated in LAMC2-induced ovarian cancer progression. In a previous study [26], LAMC2 expression was shown to be correlated with the phosphorylated form of MAPK/ERK1/2 protein. Degen et al. [27] found that the phosphorylation of ERK (p-ERK) was significantly elevated in all LAMC2-over-expressing neoplastic cells compared with normal cells, and inhibition of ERK signalling with U0126 [28] or AZD62444 [29] decreased LAMC2 expression, suggesting that MAPKs and LAMC2 could interact with and positively regulate each other's expression.

We also demonstrated that LAMC2 expression was controlled by miR-125a-5p in ovarian cancer. Upregulation of miR-125a-5p decreased LAMC2 expression, and downregulation of miR-125a-5P increased LAMC2 expression. Human miR-125a-5p, a member of the mature miR-125a family, is located at 19q13.41 [30]. Recently, miR-125a-5p was reported to be expressed at low levels and to function as a tumour repressive gene in various kinds of cancers, such as gastric [31], prostate [32], cervical [33], lung [34], hepatocellular [35] and ovarian [36] carcinomas. Conformably, the current study also revealed that the expression of miR-125-5p was significantly reduced in ovarian cancer tissues and that the upregulation of miR-125a-5p inhibited cell proliferation and tumourigenesis and induced cell apoptosis by targeting the LAMC2 gene, emphasizing that miR-125a-5p serves as a tumour suppressor in ovarian cancer. Evidences have demonstrated that VEGFA, FUT4, BAP1 and BRMS1 negatively regulated by miR-125a-5p promotes in colorectal cancer, bladder cancer, breast cancer and gastric cancer [37–40], respectively. However, whether these targeted genes are involved in miR-125a-5p-mediated ovarian cancer progression remains unclear.

In conclusion, this study makes clear that the over-expression of LAMC2 in ovarian cancer significantly promotes the malignant phenotype conversion of ovarian cancer cells through upregulating p38 expression while under the negative regulation of miR-125a-5p. Overall, our results provide the potential of highlighting the miR-125a-5p/LAMC2/p38 axis as a therapeutic target for ovarian cancer.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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