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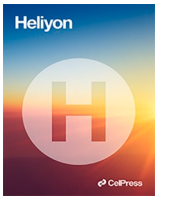

Research article

Knockdown of LncRNA-HAGLR restrains the viability and motility of pancreatic cancer via miR-625-5p/TAF15 axis *in vitro* and *in vivo*

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ABSTRACT

Dysregulation of long non-coding RNAs (lncRNAs) has been strongly involved to the development of pancreatic cancer (PC). However, the potential mechanisms by which lncRNA regulate PC development still need to be further explored. We attempted to elucidate the functional role and regulatory mechanism of lncRNA HAGLR on PC progression *in vitro* and *vivo*. RT-qPCR, Western blot, RNA pull-down, luciferase reporter assay, RNA immunoprecipitation assay, CCK-8 assay, EdU assay, flow cytometry, transwell assay and xenograft tumor experiment were performed in this study. We found that the expressions of HAGLR and TAF15 were increased in PC tissues and cells. HAGLR silencing restrained the PC cell growth and invasion, but induced cell apoptosis. Moreover, HAGLR targeted miR-625-5p to modulate the expression of TAF15. HAGLR overexpression partially eliminated the suppressive effect of TAF15 depletion on PC cell growth and the stimulative effect on apoptosis. *In vivo* assays showed that HAGLR knockdown inhibited PC cell growth by regulating the TAF15 expression. These findings suggest HAGLR could facilitate PC cell malignant behaviors through regulating the TAF15 expression, demonstrating that HAGLR might be a valuable target for the PC treatment.

1. Introduction

Pancreatic cancer (PC) is one of the most common malignant digestive system tumors, whose 5-year survival rate is only 9 % [1,2]. Despite remarkable progress in the diagnosis and treatment of PC, therapeutical approaches are still not very effective due to its atypical early symptoms and highly invasive properties. Therefore, novel oncogenic factors are still needed to be identified to provide further treatment strategies for PC.

Long non-coding RNAs (lncRNAs) are a new class of regulatory factors which lacks an open reading frame [3]. Growing evidence shows that lncRNAs are involved in regulating target gene expression, RNA transcription and post-transcriptional modification through complex molecular mechanisms [4]. Recently, lncRNAs have reported to mediate the proliferation and metastasis of many tumors including PC [5,6]. For instance, upregulation of lncRNA XIST is associated with a poor outcome in PC patients, and XIST facilitates PC cell proliferation via regulating the EGFR expression [7]. In addition, overexpression of lncRNA BX111 promotes the

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growth and metastasis of PC [8]. Lian et al. [9] have demonstrated that lncRNA HOX-AS1 enhances proliferation of PC cells through EZH2/LSD1 pathway. LncRNA HAGLR has been identified to be an important gene involved in regulating the growth of organisms [10]. Meanwhile, HAGLR exerts a significant functional role in various tumors, indicating that HAGLR has a strong potential as the prognostic marker of cancer patients [11]. However, no study has revealed the role of HAGLR in PC.

TBP-associated-factor 15 (TAF15), as a member of TET protein family, is responsible for regulating mRNA transcription, RNA splicing and transport [12]. It's important to note that TAF15 has lately been linked to numerous reports of lncRNA regulation influencing tumor growth. For instance, circ-CCT2 recruited and up-regulated the TAF15 expression to mediate the proliferation, mobility and invasion in hepatoblastoma [13]. LncRNA GMDS-AS1 regulated the TAF15 expression to participate in the growth, apoptosis mobility and invasion of lung adenocarcinoma [14]. LncRNA LINC00839 enhanced the expression of TAF15 to regulate the growth and metastasis of nasopharyngeal carcinoma [15]. Nevertheless, whether HAGLR modulates the TAF15 expression to affect the PC progression, as well as its possible mechanism remain unknown.

In this study, we uncovered the expression of HAGLR in PC for the first time, and whether HAGLR affected the PC progression through regulating the TAF15 expression. We hope the results can provide experimental and theoretical basis for the targeted therapy of HAGLR in PC.

2. Materials and methods

2.1. Human samples

40 pairs of tumor tissues and para-cancerous tissues (more than 2 cm from the tumor tissue edge) were removed from PC patients who admitted in our hospital from May 2020 to May 2021. Patients had no other tumors, and any preoperative chemotherapy or radiation before surgery. The tumor tissues were washed with saline and then stored into liquid nitrogen. The study was approved by the Ethics Committee of The First Affiliated Hospital of Harbin Medical University (2020XJSS13), and all patients signed the written informed consent.

2.2. Cell culture

The normal glandular epithelial cell line (HPDE) and PC cell lines (BxPC-3, SW1990, PANC-1, AsPC-1, Capan-2 and MIAPaCa-2) were purchased from the Shanghai Academy of Chinese Sciences (Shanghai, China). Cells were cultured in 90 % DMEM (Sigma-Aldrich, St. Louis, MO, USA) containing 10 % fetal bovine serum (FBS, HyClone, Logan, UT, USA) at 37 °C with 5 % CO_2.

2.3. Cell transfection

Three short hairpin RNAs (shRNAs) against HAGLR (sh-HAGLR#1, sh-HAGLR# 2 and sh-HAGLR#3), the corresponding negative control (sh-NC), miR-625-5p mimic (miR-625-5p), the corresponding negative control (miR-NC), miR-625-5p inhibitor (anti-miR-625-5p), the corresponding negative control (anti-NC), TAF15 overexpression vector (TAF15) and corresponding empty control vector (vector) were bought from Ribobio (Guangzhou, China), and transfected into PC cells by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The sequences of the shRNAs and plasmids were listed in Table S1. Cells were collected 48 h after transfection and used to assess the transfection efficiency by RT-qPCR or Western blot assays.

2.4. CCK-8 assay

Cells were inoculated into 96-well plates at a density of 1×10^4 cells/well for 0, 24, 48 and 72 h, respectively. 10 μl of CCK-8 solution (Sigma-Aldrich) was added and cultured for 4 h after incubation. The absorbance at 450 nm was measured for each well by using a microplate reader.

2.5. EdU staining

Cells were inoculated into 96-well plates at the density of 2×10^4 cells per well. After 24 h of culture, these cells were labeled in 50 μM of EdU medium (RiboBio, China) for 2 h. Subsequently, cells were subjected to the fixation and permeabilization for 20 min. Next, cells were treated with 100 μl of ApolloR reaction cocktail for 30 min. Cells were stained with Hoechest (5 μM) for 30 min and then observed under microscope.

2.6. Transwell assay

100 μl serum-free DMEM medium and 600 μl DMEM medium containing 10 % FBS were added into the upper and lower Transwell chambers (BD Biosciences), respectively. The upper chamber was pre-coated with Matrigel matrix (BD). A density of 1×10^5 cells/ml were inoculated to the upper chamber and incubated for 24 h at 37 °C. The invaded cells were stained with 0.1 % crystal violet and then counted under the inverted microscope.

2.7. Flow cytometry analysis

Cells were digested with trypsin without EDTA, and cell concentration was adjusted to 5×10^4 cells/ml. Re-suspended cells were then incubated with Annexin V-FITC binding solution for 20 min, and stained with 5 μl Annexin V-FITC and 5 μl PI for 20 min at 4 °C. Apoptosis was detected by flow cytometry using 300 μl binding solution, and apoptosis analysis was performed with *FlowJo* software (Bethesda, USA).

2.8. RNA pull-down

With the Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific, Waltham, MA, USA), the biotinylated labeled HAGLR probes (Bio-HAGLR) or Bio-NC were incubated with magnetic beads for 2 h at room temperature, and then the mixtures were further hatched with BxPC-3 and SW1990 cells at 4 °C overnight. After the magnetic beads were eluted, the captured miRNAs were examined using RT-qPCR.

2.9. Luciferase reporter assay

The miNRAs targeted by HAGLR or TAF15 were predicted by Starbase online website (https://rnasysu.com/encori/). Wild type (WT)/mutant (Mut) sequences of HAGLR-3′UTR, or WT/Mut sequences of TAF15 were subcloned into pmiR-GLO dual-luciferase reporter gene expression vector. Then, miR-625-5p mimic (miR-625-5p) or miR-NC and wild-type or mutated-3′UTR of HAGLR/TAF15 were co-transfected into BxPC-3 and SW1990 cells. Using a dual-luciferase activity detection kit (Promega, USA), the relative luciferase activity of BxPC-3 and SW1990 cells was determined 48 h after transfection.

2.10. RNA immunoprecipitation (RIP) assay

A commercial RIP assay kit from Millipore (Billerica, MA, USA) was used to perform the RIP assay in order to confirm that miR-625-5p and TAF15, in addition to HAGLR, were involved. A magnetic bead-conjugated TAF15 antibody or IgG antibody was incubated with BxPC-3 and SW1990 cells lysed in RIPA buffer for 1 h. RNA complex was centrifuged and RNA was extracted by TRIzol solution. HAGLR expression or miR-625-5p level in protein-RNA complexes was detected through RT-qPCR.

2.11. RT-qPCR analysis

TRIzol reagent was used to isolate RNA from samples. RNA was synthesized into cDNA according to instructions of First-Strand cDNA Synthesis Kit (Cwbio, China). PCR reaction was performed on using SYBR Mixture PCR kit (Cwbio) on 7900HT system. The primer sequences were as follows: 5′-GGCTCTTCCCTAATGTGTGG-3′ (HAGLR forward) and 5′-CAGGTCCAGCATGAAACAGA-3′ (HAGLR reverse); 5′-CCGTCGTGATGTGAGTAG-3′ (TAF15 forward) and 5′-TCCTGTGTCCTTGTCTGT-3′ (TAF15 reverse); 5′-GGAGCGACATCCCTCCAAAAT-3′ (GAPDH forward) and 5′-GGCTGTTGTCATACTTCTCATGG-3′ (GAPDH reverse). GAPDH was used as the internal reference gene, and the relative expressions of HAGLR and TAF15 were calculated $2^{-\Delta\Delta Ct}$ method [16]. Besides, to examine the level of miR-625-5p, the reverse transcription experiment was conducted with the Bulge-Loop™ miRNA RT-qPCR Primer (RiboBio Co., Ltd., Guangzhou, China) at 42 °C for 1 h and at 70 °C for 10 min. The level of miR-625-5p was analyzed at 95 °C for 10 min, followed by 40 cycles at 95 °C for 5 s, 58 °C for 15 s and 70 °C for 20 s with U6 as the internal control. The primer sequences were shown as follows: miR-625-5p forward, 5′-GCCGAGAGGGGGAAAGTTCTA-3′, miR-625-5p reverse, 5′-CTCAACTGGTGTCGTGGAG-3′; U6 forward, 5′-CTCGCTTCGGCAGCACA-3′, and U6 reverse, 5′-AACGCTTCACGAATTTGCGT-3′.

2.12. Xenograft tumor experiment

Sh-NC, sh-HAGLR and pcDNA-TAF15 were inserted into pLV-cmv lentiviral vector (Hanbio, China) to construct stable transfected SW1990 cells. A total of 15 nude mice (5-weeks) were obtained from Qing Long Shan animal breeding grounds (Nanjing, China) and then acclimated for one week in pathogen-free animal room with the 12-h cycle of light-dark and the controlled temperature. Mice were then randomly divided into three groups: sh-NC group (n = 5), sh-HAGLR#1 group (n = 5) and sh-NHAGLR#1 + TAF15 group (n = 5). Mice were subcutaneously injected with SW1990 cells transfected with lentiviral vectors. The weight and volume of subcutaneous tumor were recorded every 7 d. Tumor volume was monitored five seven days for consecutive four weeks and quantified by the following formula: volume = $0.5 \times$ length $\times$ width^2. After 4 weeks, these nude mice were euthanized by intraperitoneally administrating with 100 mg/kg sodium pentobarbital and the tumor tissues were collected for next experiments. The study was approved by the Ethics Committee of The First Affiliated Hospital of Harbin Medical University (2020XJSS13).

2.13. Immunocytochemistry

The tissues were cut at 4 μm slices and dewaxed with xylene for 20 min. Then, the slices were incubated in citrate-phosphate buffer for 10 min, as well as 5 % H_2O_2 at room temperature. Subsequently, the slices were incubated with primary antibodies against Ki-67 (1:1000, ab833, Abcam, Cambridge, UK) at 4 °C overnight. Next, the slices were incubated with corresponding secondary antibody. Expression of related proteins was observed using the inverted microscope.

2.14. TUNEL staining

TUNEL staining was conducted by using the TUNEL staining kit (Beyotime, Shanghai, China). Briefly, the tumor tissues of mice were sliced into sections and then antigen repair was performed for 10 min. Next, sections were digested with proteinase K (20 mg/ml, Beyotime) and incubated with equilibration buffer for 40 min. Sections were rinsed with PBS and treated with alkaline phosphatase. Subsequently, proteins were visualized by NBT/BCIP chromogenic substrate and stained tissues were observed under fluorescence microscope. The normal nuclei were stained blue and the apoptotic nuclei were stained brown-yellow.

2.15. Western blot

Total cellular protein was extracted with RIPA reagent (San Jose, CA, USA) and then measured with BCA protein detection kit (Solarbio, Beijing, China). Equal amounts of protein lysates (40 μg per lane) were separated by 12 % SDS-PAGE and transferred to PVDF membrane. The protein membranes were sealed with 5 % nonfat dry milk and incubated at 4 °C overnight with the corresponding primary antibodies against TAF15 (1:1000, ab134916, Abcam), Cleaved caspase-3 (1:500, ab2302, Abcam), E-cadherin (1:1000, ab241677, Abcam), Vimentin (1:1000, ab8978, Abcam) and GAPDH (1:1000, ab9485, Abcam). Subsequently, these membranes were incubated with the matched secondary antibody (1:10000, Amersham Biosciences) for 2 h at room temperature. Besides, to detect the expression of TAF15 in the cell nucleus, tumor cells were treated for the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime) based on the operation instruction to collect the cell nucleus. Lamin B1 (1:1000, ab16048, Abcam) was served as the control protein. Protein bands were exposed by ECL developing solution (GE) and visualized using Gel imaging system. The gray value of target protein band was detected by ImageJ software.

2.16. Immunofluorescence assay

BxPC-3 and SW1990 cells were sowed on glassy plates and cultured at 37 °C with 5 % CO_2. After being washed with PBS for three times, BxPC-3 and SW1990 cells were fixed with 4 % paraformaldehyde (Solarbio) at room temperature for 15 min and then washed with PBS thrice. Next, cells were incubated with BSA blocking buffer (Solarbio), 0.2 % Triton X-100 (Solarbio), and the primary antibodies against TAF15 (1:500, ab272864, Abcam) at 4 °C overnight. Subsequently, cells were washed with PBS for three times and treated with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (1:1000, ab150077, Abcam) for 1 h at room temperature. Next, cells were stained with Mounting Medium, antifading (with DAPI) (Solarbio) and photographed using a fluorescence microscopy.

2.17. Statistical analysis

SPSS software version 21.0 was used for the data analysis, and measurement data were presented as mean ± standard deviation.

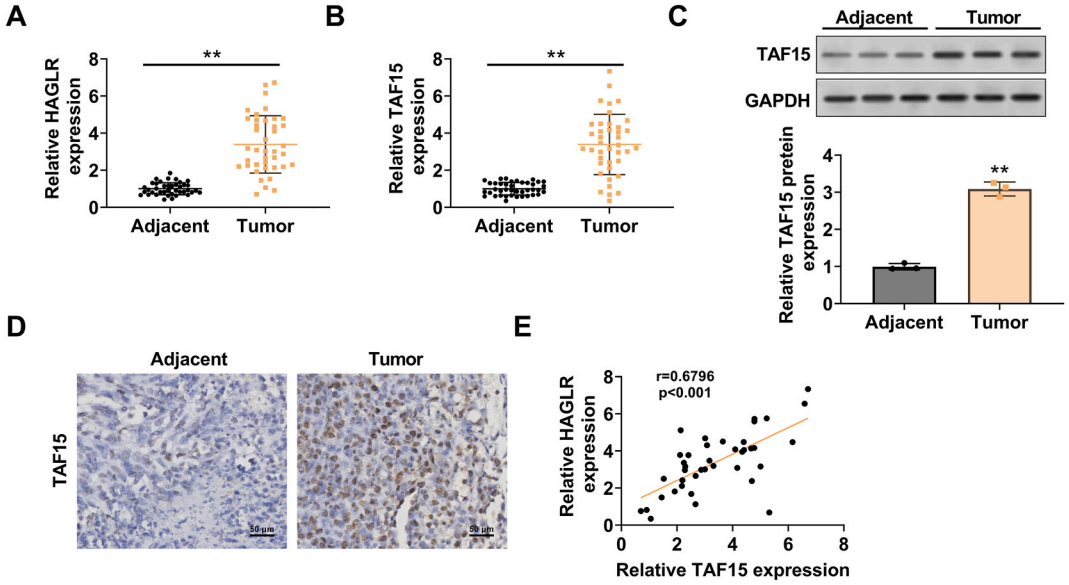

Fig. 1. HAGLR and TAF15 were upregulated in PC tissues. (A) The expression of HAGLR in PC tissues and normal adjacent tissues was detected by RT-qPCR. (B) The relative mRNA expression of TAF15 in PC tissues and normal adjacent tissues was examined by RT-qPCR. (C) The relative protein expression of TAF15 in PC tissues and normal adjacent tissues was determined by Western blot. The full and non-adjusted images for blots have been provided as supplementary material. (D) The level of TAF15 PC tissues as well as adjacent normal tissues was detected by immunohistochemistry. (E) *Spermans* correlation test was performed to determine the correlation between HAGLR and TAF15. *P < 0.05 *vs.* Adjacent.

The student t-test was used to compare the difference between the two groups, and the analysis of variance with post-hoc Tukey test was used for comparison among groups. *Spearmans* correlation coefficient was used to determine the correlation between HAGLR expression and TAF15 expression. Statistically significant differences were defined when $P < 0.05$.

3. Results

3.1. HAGLR and TAF15 were upregulated in PC tissues

Firstly, 40 pairs of PC tissues and corresponding para-PC tissues were collected to detect the expression of HAGLR and TAF15. HAGLR expression was upregulated in PC tissues (Fig. 1A). Similarly, both the transcriptional and translational expressions of TAF15 were increased in PC tissues (Fig. 1B and C). Besides, results of immunohistochemical staining revealed that TAF15 was overexpressed in PC tissues (Fig. 1D). TAF15 expression was positively correlated with the expression of HAGLR (Fig. 1E). Additionally, the association between HAGLR expression and clinicopathological features were investigated. Distant metastasis, histological grade and TNM stage were correlated with HAGLR expression, however, age, gender and tumor size were not (Table 1). Collectively, the aberrant expression of HAGLR and TAF15 in PC indicates that both genes may be involved in regulating the biological function of PC.

3.2. HAGLR knockdown inhibited PC cell proliferation and invasion but induced apoptosis

Next, we addressed the role of HAGLR on the biological function of PC. Compared with normal glandular epithelial cell, HAGLR was generally upregulated in PC cells (Fig. 2A). Among these PC cells, BxPC-3 and SW1990 cells exhibited the highest expression of HAGLR, thus these two cells were chosen for the follow-up experimental studies. Subsequently, shRNAs targeted HAGLR were used to downregulate the expression of HAGLR in both BxPC-3 and SW1990 cells. The expression of HAGLR in sh-HAGLR group was significantly reduced, while HAGLR expression in sh-HAGLR#1 group was the lowest (Fig. 2B). Therefore, sh-HAGLR#1 was selected for cell functional verification in the subsequent experiments. Based on CCK-8 results, HAGLR knockdown significantly reduced BxPC-3 and SW1990 cell viability (Fig. 2C). Edu proliferation assay further confirmed that HAGLR silencing prominently suppressed proliferation of BxPC-3 and SW1990 cells, in which the numbers of EdU positive cells in sh-HAGLR#1 group were significantly decreased compared with sh-NC group (Fig. 2D). Transwell assay showed that HAGLR downregulation inhibited the invasion capability of PC cells (Fig. 2E and F). An analysis of flow cytometry showed that HAGLR knockdown significantly increased apoptosis of BxPC-3 and SW1990 cells (Fig. 2G and H). Western blot analysis showed that interference of HAGLR significantly increased expression of cleaved caspase-3 and E-cadherin, but reduced the Vimentin expression (Fig. 2I). Taken together, these results suggested that HAGLR played a pro-cancerous role by modulating proliferation, invasion, and apoptosis in PC.

3.3. HAGLR targeted miR-625-5p to modulate the level of TAF15

Based on the Starbase, there were two miRNAs targeted by both HAGLR and TAF15, including miR-625-5p and miR-296-3p (Fig. 3A). Besides, the potential downstream targets of TAF15 have been predicted by GTRD (Gene Transcription Regulation Database; http://gtrd20-06.biouml.org/), and the relevant results have been exhibited in Table S2. As demonstrated by Fig. 3B, the biotinylated labeled HAGLR probes enriched more miR-625-5p but not miR-296-3p. Thus, miR-625-5p was chosen for the target and

Table 1
The correlation between the HAGLR expression and clinical-pathological characteristics of forty patients with PC.

Characteristic	All cases	HAGLR expression		P-value
		High (n = 20)	Low (n = 20)	
Gender				0.749
male	23	12	11	
female	17	8	9	
Age (years)				0.519
< 60	16	7	9	
≥ 60	24	13	11	
Tumor size (cm)				0.113
< 2	21	8	13	
≥ 2	19	12	7	
Distant metastasis				0.027*
Positive	19	6	13	
Negative	21	14	7	
Histological grade				0.025*
High/moderate	23	15	8	
Low	17	5	12	
TNM Stages				0.004**
I/II	21	6	15	
III/IV	19	14	5	

A chi-square test was employed to compare groups with differing levels of HAGLR expression. *P < 0.05, **P < 0.01.

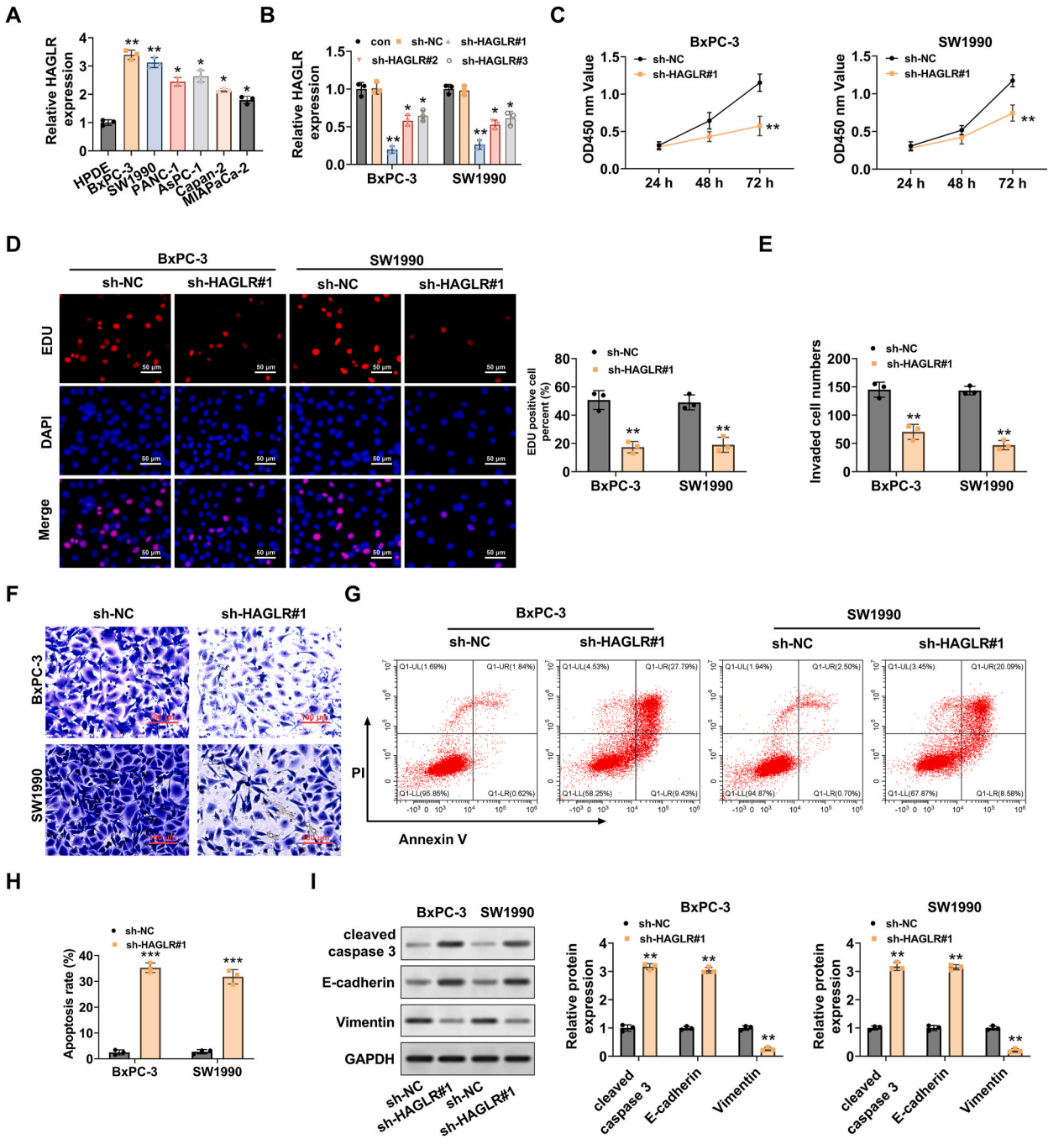

Fig. 2. HAGLR knockdown inhibited PC cell proliferation and invasion but induced apoptosis. (A) The relative mRNA expression of HAGLR in one normal glandular epithelial cell line (HPDE) and six PC cell lines (BxPC-3, SW1990, PANC-1, AsPC-1, Capan-2 and MIAPaCa-2) was measured by RT-qPCR. *P < 0.05 and **P < 0.01 *vs.* HPDE. (B) RT-qPCR was used to evaluate the efficiency of shRNA-mediated knockdown of HAGLR in SW1990 and BxPC-3 cells. *P < 0.05 and **P < 0.01 *vs.* sh-NC. (C) The cell viability of SW1990 and BxPC-3 cells was detected by CCK-8 assay. *P < 0.05 *vs.* sh-NC. (D) Effect of HAGLR knockdown on the proliferation of SW1990 and BxPC-3 cells was detected by EdU staining. *P < 0.05 *vs.* sh-NC. (E and F) Effect of HAGLR knockdown on the invasive ability of SW1990 and BxPC-3 cells was detected by transwell assay. *P < 0.05 *vs.* sh-NC. (G and H) The effect of HAGLR knockdown on apoptosis in SW1990 and BxPC-3 cells was measured by flow cytometry. *P < 0.05 *vs.* sh-NC. (I) Effect of HAGLR knockdown on the expression of cleaved caspase-3, E-cadherin and Vimentin of SW1990 and BxPC-3 cells was measured by western blotting. The full and non-adjusted images for blots have been provided as supplementary material. *P < 0.05 *vs.* sh-NC.

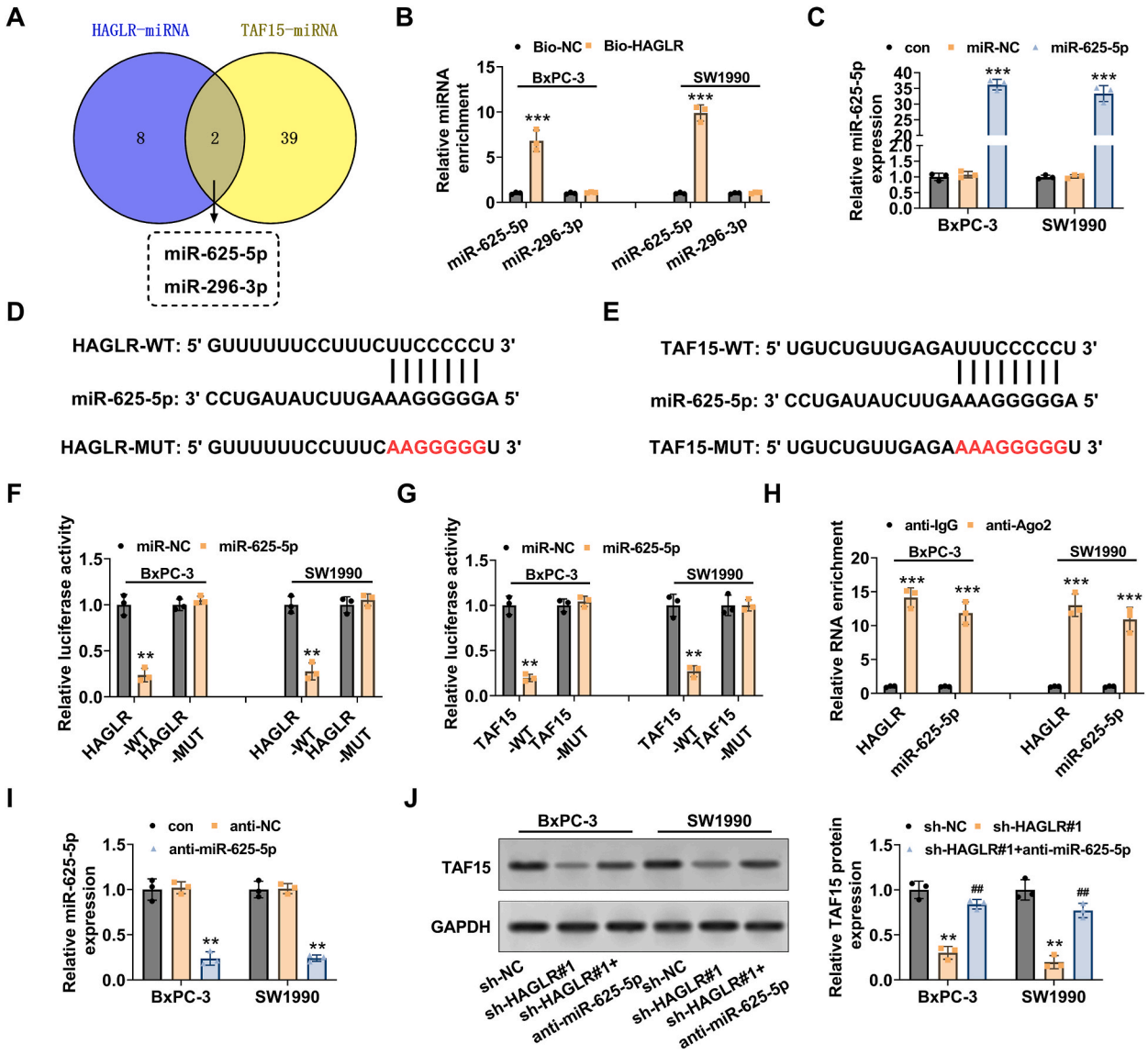

Fig. 3. HAGLR targeted miR-625-5p to regulate the expression of TAF15. (A) MiRNAs targeted by both HAGLR and TAF15 were predicted by Starbase. (B) RNA pull-down examined the enrichment of HAGLR and miR-625-5p, as well as HAGLR and miR-296-3p. ***P < 0.001 *vs.* Bio-NC. (C) MiR-625-5p overexpression was detected by PCR. ***P < 0.001 *vs.* miR-NC. (D) The binding sites between HAGLR and miR-625-5p, as well as their mutant sequences. (E) The binding sites between miR-625-5p and TAF15, as well as their mutant sequences. (F) The relationship between HAGLR and miR-625-5p was validated by dual-luciferase reporter assay. **P < 0.01 *vs.* miR-NC. (G) The relationship between miR-625-5p and TAF15 was validated by dual-luciferase reporter assay. **P < 0.01 *vs.* miR-NC. (H) The interaction between HAGLR and TAF15, as well as miR-625-5p and TAF15 was confirmed by RIP assay. ***P < 0.001 *vs.* anti-IgG. (I) PCR was performed to determine the effect of miR-625-5p silencing on miR-625-5p expression. **P < 0.01 *vs.* anti-IgG. (J) West blotting was used to detect the effect of HAGLR and miR-625-5p silencing on TAF15 expression. The full and non-adjusted images for blots have been provided as supplementary material. **P < 0.01 *vs.* sh-NC; $^{\#\#}$P < 0.01 *vs.* sh-HAGLR#1.

overexpressed in both BxPC-3 and SW1990 cells for the further study (Fig. 3C). Starbase predicted the binding sites between miR-625-5p and TAF15 as well as between HAGLR and miR-625-5p, and the mutant sequences of HAGLR and TAF15 were created for the following study (Fig. 3D and E). Subsequently, luciferase report assay revealed that overexpression of miR-625-5p markedly reduced the relative luciferase activity in WT-HAGLR and WT-TAF15, while it had no effect on the luciferase activity in Mut-HAGLR and Mut-TAF15 (Fig. 3F and G). RIP results identified that HAGLR and miR-625-5p were enriched for TAF15 (Fig. 3H). Moreover, down-regulation of miR-625-5p (Fig. 3I) rescued the decrease in the expression of TAF15 by transfection of sh-HAGLR#1 in both cells (Fig. 3J). Taken together, HAGLR targeted miR-625-5p to modulate the expression of TAF15.

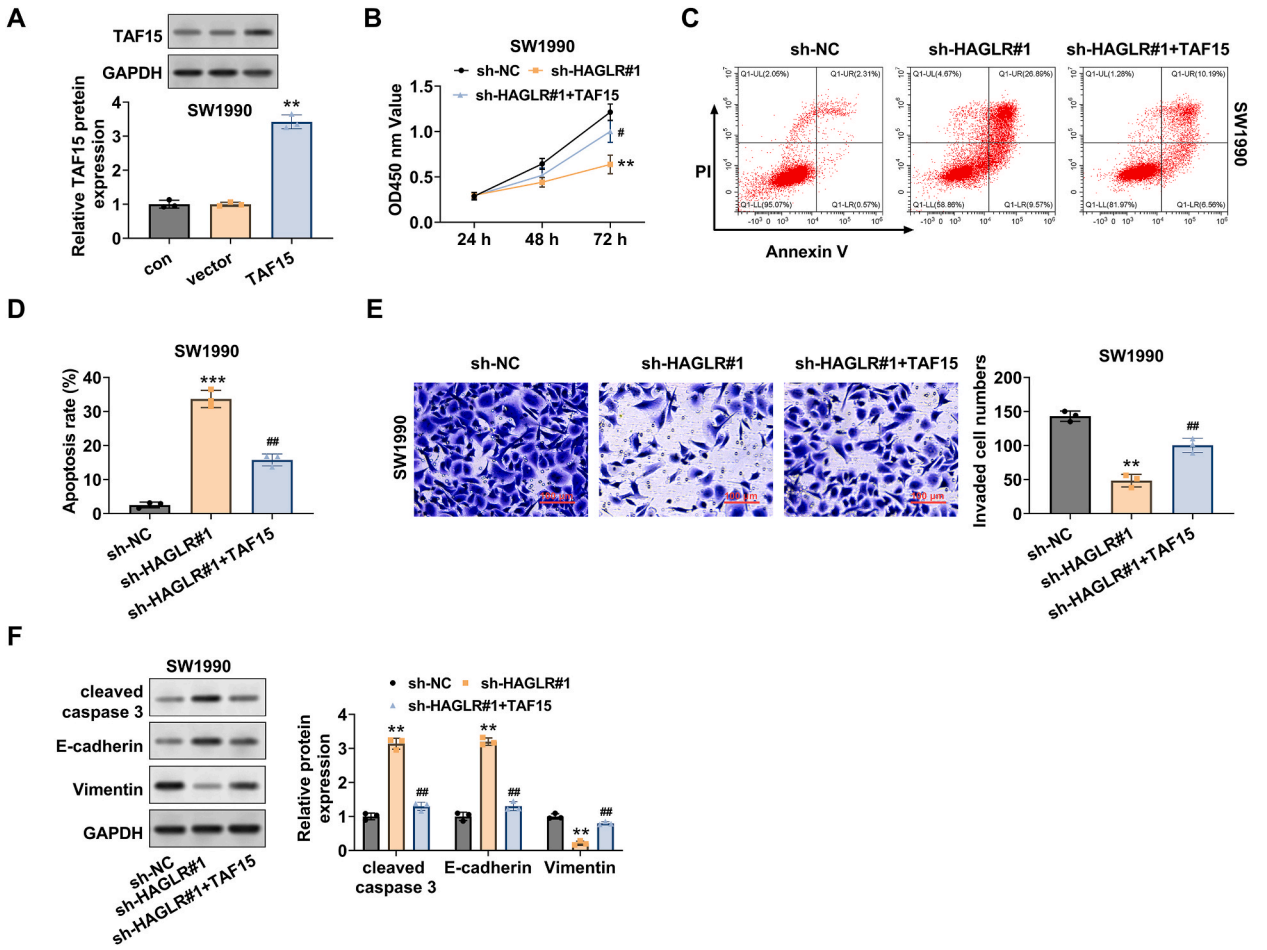

Fig. 4. HAGLR promoted PC cell proliferation and invasion but inhibited apoptosis by upregulating TAF15. (A) Western blot was used to detect the overexpression of TAF15 in SW1990 cells. The full and non-adjusted images for blots have been provided as supplementary material. $^{**}P < 0.01$ *vs.* con. (B) Effect of TAF15 overexpression on the proliferation of SW1990 cells transfected with sh-HAGLR#1 was detected by CCK-8 assay. $^{**}P < 0.01$ *vs.* sh-NC; $^{\#}P < 0.05$ *vs.* sh-HAGLR#1. (C and D) Flow cytometry was used to detect the effect of TAF15 overexpression on the apoptosis of SW1990 cells transfected with sh-HAGLR#1. $^{***}P < 0.001$ *vs.* sh-NC; $^{\#\#}P < 0.01$ *vs.* sh-HAGLR#1. (E) Transwell assays were used to measure the effect of TAF15 overexpression on the invasive ability of SW1990 cells that had been transfected with sh-HAGLR#1. $^{**}P < 0.01$ *vs.* sh-NC; $^{\#\#}P < 0.01$ *vs.* sh-HAGLR#1. (F) Western blotting was used to determine the effect of TAF15 overexpression on the expression of cleaved caspase-3, E-cadherin, and Vimentin of SW1990 cells transfected with sh-HAGLR#1. The full and non-adjusted images for blots have been provided as supplementary material. $^{**}P < 0.01$ *vs.* sh-NC; $^{\#\#}P < 0.01$ *vs.* sh-HAGLR#1.

3.4. HAGLR promoted PC cell proliferation and invasion but repressed apoptosis by upregulating TAF15

To further confirm the role of HAGLR/miR-625-5p/TAF15 axis in the progression of PC, TAF15 was overexpressed in SW1990 cells (Fig. 4A). As indicated by cell function assays, downregulation of HAGLR inhibited SW1990 cell proliferation and invasion, but induced apoptosis (Fig. 4B–E). These above biological function changes could be reversed by overexpression of TAF15 (Fig. 4B–E). Additionally, western blotting showed that overexpression of TAF15 inverted the promoting effect of sh-HAGLR#1 on the levels of cleaved caspase-3 and E-cadherin, and the inhibitory effect on Vimentin expression in SW1990 cells (Fig. 4F). Besides, since TAF15 is a member of the TET family of proteins and primarily localizes to the cell nucleus, we investigate whether HAGLR impacts TAF15 nuclear translocation. As shown in Fig. S1, knockdown of HAGLR decreased the relative protein expression of TAF15 in the cell nucleus (Fig. S1A), as well as the nuclear translocation of TAF15 (Fig. S1B) in both BxPC-3 and SW1990 cells. Together, HAGLR enhanced PC cell proliferation and invasion but inhibited apoptosis by upregulating TAF15.

3.5. HAGLR promoted tumor growth in vivo by regulating TAF15

Finally, we verified the effect of HAGLR on tumor growth *in vivo* by regulating TAF15. As shown in Fig. 5A and B, HAGLR knockdown led to smaller tumor size and volume, while overexpression of TAF15 reversed the inhibitory effect of HAGLR knockdown

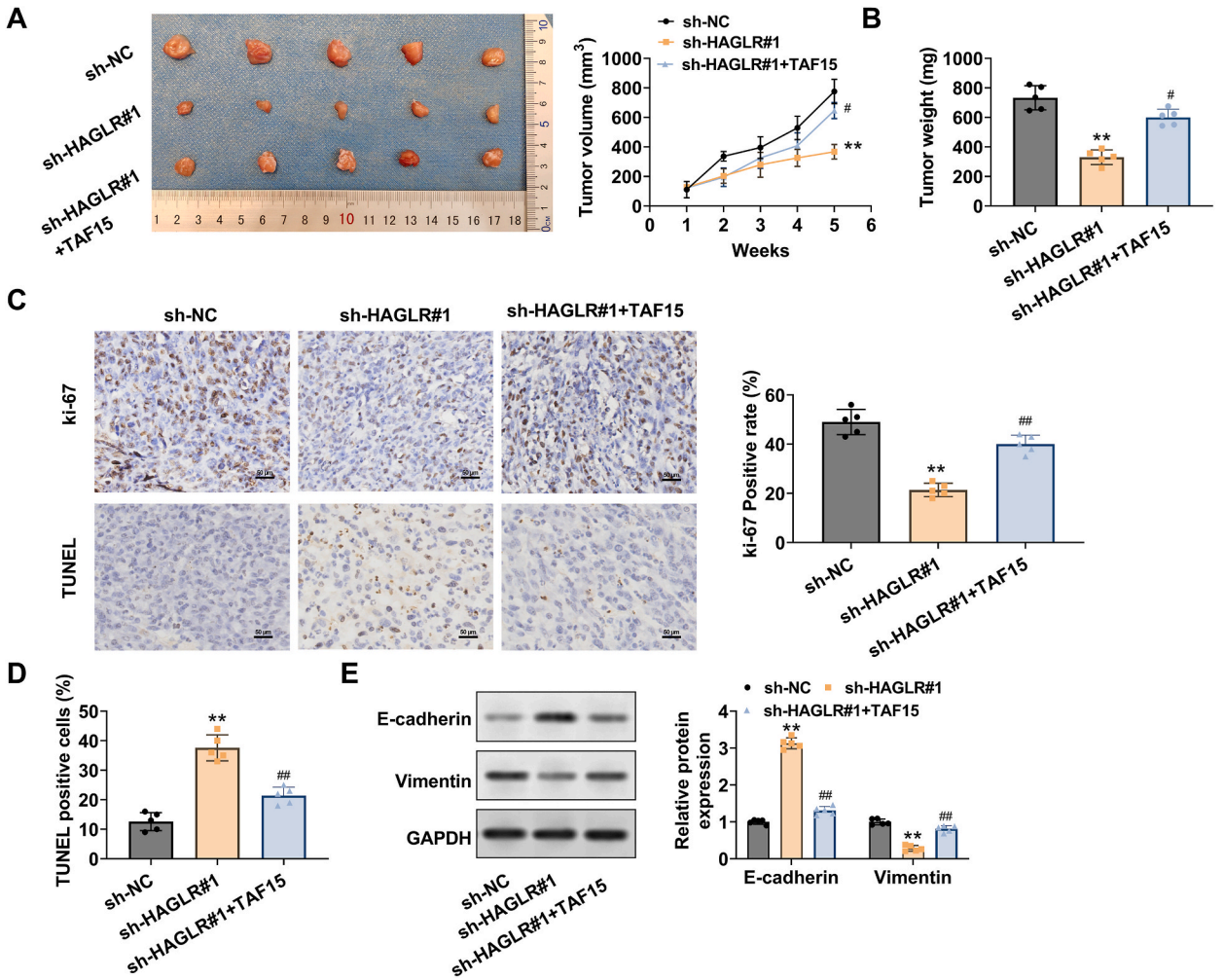

Fig. 5. HAGLR and TAF15 were upregulated in PC tissues. (A) Photographs of tumor tissues derived from xenografted mice injected with SW1990 cells stably expressing shRNA-HAGLR and/or TAF15, and the tumor volume changes of xenografted mice. (B) Changes in tumor weight of mice injected with SW1990 cells stably expressing shRNA-HAGLR and/or TAF15. (C and D) Ki-67 expression and apoptosis in tumor tissues were detected by immunohistochemistry staining and TUNEL staining, respectively. (D) Vimentin and E-cadherin were detected by western blotting in tumor tissues. The full and non-adjusted images for blots have been provided as supplementary material. $^{**}P < 0.01$ *vs.* sh-NC; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs.* sh-HAGLR#1.

on tumor growth. Immunohistochemical staining and TUNEL results showed that HAGLR knockdown inhibited the expression of Ki-67 in tumor tissues and increased the proportion of TUNEL-positive cells, while overexpression of TAF15 could alleviate these above effects (Fig. 5C and D). Western blotting revealed that TAF15 overexpression reversed the promoting effect of HAGLR knockdown on E-cadherin expression and inhibitory effect on the Vimentin expression in tumor tissues (Fig. 5E). Altogether, HAGLR promoted PC progression through positively regulating the expression of TAF15 *in vivo*.

4. Discussion

Due to its extremely high malignant potential in the digestive system, PC has had a major detrimental effect on the overall prognosis of patients. It has been established that high invasiveness and metastasis are predictive factors that impact the prognosis of PC patients. Thus, it is of great significance to find sensitive genes to guide the early diagnosis and treatment of patients [17]. LncRNA has recently been demonstrated to play an important role in tumor development, invasion and metastasis [18,19]. Our study mainly discovered that lncRNA HAGLR was highly expressed in PC. Downregulation of HAGLR could effectively reduce PC cell growth and invasion. Furthermore, HAGLR participated in the PC cell growth *in vivo* and *in vitro* through miR-625-5p/TAF15 axis.

This study confirmed for the first time the oncogenic role of HAGLR in PC, suggesting that HAGLR played a vital regulatory role in PC. As a new type of lncRNA molecule discovered in 2017, HAGLR has shown tumor-promoting or anti-tumor activity in various tumors. For instance, HAGLR expression is increased in thyroid carcinoma, and HAGLR overexpression promotes the proliferation,

migration, and invasion of thyroid cancer cells [20]. In breast cancer, high HAGLR expression is positively correlated with poor prognosis in patients, HAGLR silencing inhibits breast cancer cell growth [21]. HAGLR is lowly expressed in gastric cancer that suppresses the proliferation and invasion of gastric cancer cells [22]. In lung adenocarcinoma, the decreased expression of HAGLR is related to a shorter survival time of patients, and HAGLR directly interferes the progression of lung adenocarcinoma via modulating E2F1 expression [23]. In general, HAGLR may have distinct effects on different tumor types, and the regulatory role of HAGLR in more types of tumors needs to be further explored. HAGLR has not yet been reported in any research pertaining to PC. HAGLR was upregulated in PCs in the present study. Downregulation of HAGLR repressed the PC cell proliferation and invasion, but enhanced apoptosis. Additionally, due to strong relation between PC and invasion, invasion-related proteins, including E-cadherin and Vimentin were also detected in this study. HAGLR knockdown promoted the expression of E-cadherin and suppressed the expression of Vimentin. Besides, a slight difference in these indicators exerted between BxPC-3 and SW1990 cells, which might be due to the different expression levels of HAGLR in different PC cell lines. Taken together, HAGLR knockdown inhibited PC cell proliferation and invasion but induced apoptosis.

To further investigate the downstream regulatory pathway of HAGLR in PC, it is found that HAGLR could regulate the TAF15 expression through targeting miR-625-5p. LncRNAs have been demonstrated to be always the targets of miRNAs in the indirect modulation of gene expression [18]. HAGLR/miR-326/CDKN2A axis is reported to enhance proliferation and reduce apoptosis of acute myeloblastic leukemia cells [24]. HAGLR promotes proliferation and migration but inhibits apoptosis of melanoma cells through miR-4644/ASB11 pathway [25]. HAGLR enhances 5-Fu resistance of gastric cancer cells by the regulation of LDHA via sponging miR-338-3p [26]. HAGLR sponges miR-335-3p to accelerate the progression of triple negative breast cancer via modulating WNT2 [21]. In this study, HAGLR targeted miR-625-5p to modulate the TAF15 expression. Moreover, the inhibitory effect of HAGLR knockdown on proliferation and invasion and the promoting effect on apoptosis of PC cells *in vitro* were counteracted, at least partly by the overexpression of TAF15. Thus, these results indicated that HAGLR targeted miR-625-5p to modulate the expression of TAF15, which at least partly regulated the cell proliferation, invasion and apoptosis in PC. This might be due to the different effects of HAGLR knockdown and overexpression on TAF15 expression. Besides, plenty of researches have been shown that the role of lncRNAs in the malignant progressions of PC can be partly rescued with the downstream target genes. For instance, the inhibitory role of siRNA against lncRNA HEIH-1 (si-HEIH-1) in cell proliferation, migration and invasion of PC is partly restored by the overexpression of CDK8 [27]. Knockdown of lncRNA CTBP1-AS2 inhibits proliferation, migration and invasion but enhances apoptosis of PC cells, which is partly recovered by the overexpression of USP22 [28]. Thus, our results revealed that the role of HAGLR in PC cell progressions was partly not completely canceled out via miR-625-5p/TAF15 axis, indicating that there might be additional mechanisms by which HAGLR promoted PC progression. Lu et al. [11] report that HAGLR promotes non-small cell lung cancer proliferation and invasion via enhanced de novo lipogenesis, which makes us speculate HAGLR may facilitate the malignant progressions of PC through other mechanisms, such as lipogenesis, but not just through classical miRNA/mRNA pathways. Secondly, HAGLR may sponge other miRNAs to foster the PC progression, since HAGLR has been shown to target a series of different miRNAs to regulate the progression and development of other cancers, such as miR-143-5p in esophageal cancer [29], miR-185-5p in colon cancer [30], miR-6785-5p in hepatocellular carcinoma [31], miR-335-3p in triple negative breast cancer [21], miR-338-3p in gastric cancer [26], miR-4644 in melanoma [25], and miR-326 in acute myeloblastic leukemia [24]. Thirdly, HAGLR expression is revealed to be significantly correlated with gene promoter methylation [32], and HAGLR can be regulated by IGF2BP2 in an m6A-dependent manner in thyroid cancer [20]. Thus, other epigenetic regulation on HAGLR may contribute to its role in the PC progression. Fourthly, Zhang and colleagues [33] prove that HAGLR boosts triple-negative breast cancer progression by activating Wnt signaling pathway. Therefore, HAGLR may also accelerate the PC progression through the signaling pathway, such as Wnt signaling pathway. In addition, miR-625-5p may be sponged by other lncRNAs or circRNAs to participate in the PC progression. For instance, miR-625-5p can be targeted by circ_0007534 to regulate cell proliferation, apoptosis, and invasion in pancreatic ductal adenocarcinoma [34]. Thus, more investigations are needed in the following study to verify these suppositions. Altogether, HAGLR modulated growth, invasion and apoptosis of PC cells via modulating the TAF15 level.

However, this study still has some limitations. Firstly, the sample sizes were small, and the normal controls were lack in this study. Secondly, only one PC cell line was used for the *in vivo* experiment. Thirdly, we did not verify the classical signal pathway related to the development of PC. Fourthly, a relationship between TAF15 and the clinicopathological characteristics of PC patients has not been established. Additionally, although we predicted the potential downstream targets of TAF15, a more in-depth investigation of the downstream effects of TAF15 should be explored in the following study. These above limitations will be addressed in further work.

5. Conclusion

In summary, our work revealed that HAGLR was overexpressed in PC. HAGLR silencing effectively restrained PC cell proliferation and invasion but facilitated apoptosis. In addition, HAGLR targeted miR-625-5p to modulate the TAF15 expression. HAGLR promoted PC cell growth through the regulation of the expression of TAF15. Based on these results, synergistic application of HAGLR and TAF15 might be a new strategy for targeted therapy of PC.

Ethical statement

The study was approved by the Ethics Committee of The First Affiliated Hospital of Harbin Medical University (2020XJSS13).

Data availability statement

All data generated or analyzed during this study are included in this published article.

CRediT authorship contribution statement

Jiafu Wang: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Huiqi Gao:** Validation, Methodology, Investigation. **Peng Fu:** Software, Resources, Methodology, Formal analysis. **Lin Lin:** Visualization, Supervision, Software, Formal analysis. **Lifan Wang:** Visualization, Validation, Supervision, Resources. **Yue Han:** Writing – review & editing, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37254.

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