Long Non-Coding RNA HAGLROS Promotes the Malignant Progression of Bladder Cancer by Regulating the miR-330-5p/SPRR1B Axis

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Research Article

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Abstract

Background

Bladder cancer (BC) is the most common genitourinary malignancy worldwide, and its aetiology and pathogenesis remain unclear. Long noncoding RNAs can play vital roles in gene expression and diverse biological processes, especially in cancers. Accumulating evidence has shown that HAGLROS, a novel lncRNA, is closely related to the occurrence and progression of various cancers. However, the biological functions and underlying mechanisms of HAGLROS in BC remain unknown.

Methods

The relative expression of HAGLROS in BC was determined by bioinformatics analysis, transcriptome sequencing analysis and qRT–PCR. Gain- or loss-of-function assays were performed to study the biological roles of HAGLROS in BC. A CCK-8 assay was used to detect BC cell proliferation. BC cell invasion and migration were investigated by wound healing and Transwell assays. The cell cycle was analysed by flow cytometry assay. Western blot analysis and immunohistochemistry were performed to evaluate SPRR1B expression. The differential expression of candidate genes and their relationships were evaluated in data retrieved from the starBase database, the GEIPIA database, the Lnc2Cancer database and the LncBase database. FISH assays, subcellular fractionation assays and luciferase reporter assays were performed to explore the underlying molecular mechanisms of HAGLROS.

Results

HAGLROS expression is significantly upregulated in BC tissues and cells, and increasing HAGLROS expression was related to high pathologic grade. HAGLROS enhances the proliferation, migration and invasion of BC. Furthermore, SPRR1B is obviously upregulated and miR-330-5p is significantly downregulated in BC. Mechanistically, we found that HAGLROS is mainly located in the cytoplasm and positively regulates SPRR1B expression by sponging miR-330-5p, playing an oncogenic role in BC pathogenesis.

Conclusions

The present study demonstrates that HAGLROS is significantly overexpressed and plays an oncogenic role by regulating the miR-330-5p/SPRR1B axis in BC. HAGLROS may serve as a potential biomarker for the diagnosis and treatment of BC.

Background

Bladder cancer (BC) is the most common genitourinary malignancy worldwide. In the past decade, the morbidity and mortality of BC have increased significantly, showing a gradually younger trend [1]. Non-muscle-invasive bladder cancer (NMIBC) can be treated with transurethral resection of the bladder tumour and postoperative adjuvant chemotherapy/immunotherapy. Of note, approximately 50-70% of NMIBC will

relapse, and 10-20% will eventually progress to muscle-invasive bladder cancer (MIBC) or metastasize [2]. Although MIBC is mainly treated with radical cystectomy, there are many short-term and long-term complications, which cause great pain to patients [3, 4]. However, the aetiology and pathogenesis of BC remain unclear. Therefore, an in-depth understanding of the underlying mechanisms and identifying effective early detection biomarkers have great clinical significance for improving the diagnosis and therapeutic strategies of BC.

Long noncoding RNAs (lncRNAs) are noncoding RNAs greater than 200 nucleotides in length that were initially ignored as "transcription noises" due to a lack of protein-coding function [5]. However, the rapid development of RNA genomics technology highlights the notion that lncRNAs are key players in gene expression regulation and significantly contribute to human disease progression, especially in cancers [6]. In the past decade, growing evidence has shown that lncRNAs play a vital role in gene expression and diverse biological processes by regulating transcription, sponging miRNA, and modifying epigenetic regulation [6, 7]. Of note, a large number of lncRNAs have been found to play an important role in invasion, metastasis and drug resistance in BC [8–10]. For instance, lncRNA-SNHG1 promotes basal MIBC cell invasion by interacting with the PP2A catalytic subunit and inducing autophagy [11]. LncRNA-SLC16A1-AS1 induces metabolic reprogramming as a target and coactivator of E2F1 in BC progression [12]. HAGLR opposite strand lncRNA (HAGLROS), which is located on chromosome 2q31.1, is a 699-bp lncRNA that was first reported in 2018 [13]. This study revealed that HAGLROS was highly expressed in gastric cancer and promoted the proliferation, migration and invasion of gastric cancer. Moreover, high HAGLROS expression was associated with poor prognosis. Subsequently, accumulating evidence has shown that HAGLROS is closely related to the occurrence and progression of various cancers. However, the biological functions and underlying mechanisms of HAGLROS in BC remain unknown.

In the present study, the expression level, biological function and underlying mechanism of HAGLROS in BC were initially investigated. These results revealed that HAGLROS was significantly upregulated in BC tissues and cells compared with non-tumour tissues and cells, which was verified by in silico database analysis and our transcriptome sequencing dataset and qRT–PCR assay. Furthermore, knockdown of HAGLROS inhibited the proliferation, migration and invasion of BC and arrested T24 and 5637 cells at the G1 stage. Mechanistically, we found that HAGLROS was mostly distributed in the cytoplasm and could positively regulate small proline rich protein 1B (SPRR1B) expression by sponging miR-330-5p in a competing endogenous RNA (ceRNA)-dependent manner. Moreover, knockdown of miR-330-5p promotes SPRR1B expression and reverses the malignant phenotype inhibition of BC induced by silencing HAGLROS. Overall, our results suggest that the HAGLROS/miR-330-5p/SPRR1B axis is a promising novel biomarker that may serve as a powerful therapeutic and diagnostic target in BC.

Materials And Methods

Clinical sample collection and cell culture

Fresh BC tissue tissues and corresponding non-tumour normal tissues were collected from patients who underwent radical cystectomy. The samples were immediately cleaned with normal saline and snap-frozen in liquid nitrogen after resection. All samples were confirmed independently by two pathologists. The patients were informed of the study contents and signed informed consent forms. This study was approved and supported by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University.

Bladder cancer cell lines and cell culture

The BC cell lines T24 and 5637 and the normal bladder uroepithelium cell lines SV-HUC-1 and HEK-293T were purchased from the Institute of Cell Research, Chinese Academy of Sciences (Shanghai, China). T24 and SV-HUC-1 cells were cultured in DMEM (Gibco, USA) with 10% foetal bovine serum (Gibco, USA), and 5637 cells were cultured with RPMI-1640 medium (Gibco, USA) and 10% foetal bovine serum. All cells were cultured at 37 °C in a humidified 5% CO_2 atmosphere.

RNA extraction and quantitative real-time PCR (qRT–PCR)

Total RNA was extracted from bladder tissue and cells using TRIzol reagent (Thermo Fisher, USA) according to the manufacturer's instructions. The primers for GAPDH were 5'-AAAGGGTCATCATCTCTG-3' (forward) and 5'-GCTGTT GTCATACTTCTC-3' (reverse). The primers for U6 were 5'-CTCGCTTCGGCAGC ACA-3' (forward) and 5'-AACGCTTCACGAATTTGCGT–3' (reverse). The primers for SPRR1B were 5'-CAAGGTTCCAGAGCCATG-3' (forward) and 5'-TACTTCTGCTTGGTCTTCT-3' (reverse). The primers for HAGLROS were 5'-GACGAATACACCTCTGAA-3' (forward) and 5'-AGTCTTAGCCTACTTCCT-3' (reverse). The miRNA primers were designed and synthesized by RiboBio Biotechnology (Guangzhou, China). qRT–PCR assays were performed using a qRT–PCR Starter Kit (RiboBio, Guangzhou, China) and a LightCycle 96 Fluorescent Quantitative PCR System (Roche, Switzerland) according to the manufacturer’s instructions. Expression was normalized by GAPDH and U6. Each experiment was repeated at least thrice.

RNA fluorescent in situ hybridization (FISH) and subcellular fractionation assay

FISH was performed to detect the subcellular colocalization of HAGLROS, miR-330-5p and SPRR1B. A FISH kit was purchased from Focofish Biotechnology (Guangzhou, China). The U6 probes were purchased from Sangon (Shanghai, China). Cell nuclei were counterstained with DAPI (Sangon, Shanghai, China). The cytoplasmic and nuclear fractions of T24 and 5637 cells were extracted using nuclear and cytoplasmic extraction reagents (Thermo Fisher, USA) according to the manufacturer’s protocol. Then, the RNA was extracted, and qRT–PCR was performed as previously described. Each experiment was repeated at least thrice.

Transcriptome sequencing

Briefly, total RNA was extracted from three pairs of fresh bladder cancer tissues and corresponding adjacent tissues. The eligible RNAs were sent to the sequencing company for quality control, library

construction and sequencing.

Cell transfection

Plasmids overexpressing HAGLROS (OE-HAGLROS), short hairpin (sh) HAGLROS and sh-SPRR1B were designed and synthesized by GenoMeditech Biotechnology (Shanghai, China). T24 and 5637 cells were transfected. The cells were harvested 48-72 hours. Purinomycin (Solarbio, Beijing, China) was used to screen stably transfected cells. The transfection efficiency was evaluated by qRT–PCR. The miR-330-5p mimics/inhibitors were purchased from RiboBio Biotechnology (Guangzhou, China). Each experiment was repeated at least thrice.

Western blot assay

Western blot assay was used to detect protein expression. The tissues and cells were lysed with ice-cold RIPA lysis buffer (Beyotime, Shanghai, China). The protein was quantified using a Bradford Protein Assay Kit (Beyotime, Beijing, China), separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Sigma, USA), transferred to a polyvinylidene fluoride (PVDF) membrane, blocked with bovine serum albumin (Solarbio, Beijing, China), hybridized with SPRR1B (Abcam, MA, USA) and GAPDH (Abmart, Shanghai, China), and incubated with a secondary antibody. The PVDF (Millipore, USA) membrane was treated with BeyoECL Plus reagent (Beyotime, China). Then, the bands were detected using a gel imager system (Bio–Rad, USA) and analysed by ImageJ software (NIH, Bethesda, MD, USA). Each experiment was repeated at least thrice.

Cell counting Kit-8 (CCK-8) assay

BC cell proliferation was observed using an Enhanced Cell Counting Kit-8 (Beyotime, Beijing, China) according to the manufacturer's instructions. Briefly, 5×10^3 cells were seeded onto 96–well plates per well. After 24 hours, 10 μl CCK-8 solution was added to each well, and the cells were incubated for 1 hour. Finally, the absorbance was measured at 450 nm using a microplate reader (Thermo Fisher, USA). Each experiment was repeated at least thrice.

Wounding healing assay

The migration capability of cells was tested by wound healing assay. The wounds were scratched using a 200-μl pipette tip in approximately 90% confluent cells. The migration of cells was acquired using a microscope (Olympus, Tokyo, Japan). Finally, wound healing areas were measured by ImageJ software (NIH, Bethesda, MD, USA). Each experiment was repeated at least thrice.

Transwell assay

The invasive capability of cells was observed using a Transwell assay. Briefly, 100 μl diluted Matrigel (Corning, USA) was added to the upper chamber of the Transwell (Corning, USA), and the Transwells were incubated at 37 °C for 1 hour. Then, 200 μl serum-free medium with 5×10^4 cells was seeded into each

upper chamber. Culture medium containing 10% FBS was added to the lower chamber. The cells in the upper chamber were wiped using cotton after 48 hours of incubation, fixed with paraformaldehyde, and stained with crystal violet. The number of invaded cells were recorded under a microscope. Each experiment was repeated at least thrice.

Flow cytometry assay

The cell cycle was determined by using a Cell cycle Analysis Kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. The cell cycle was assessed by flow cytometry. Each experiment was repeated at least thrice.

Immunohistochemistry (IHC)

Protein expression levels were detected by immunohistochemical staining. The bladder tissues were prepared into paraffin sections. Then, these sections were deparaffinized, used for antigen retrieval, and incubated with a primary antibody against SPRR1B (Proteintech, Wuhan, China, 1:150) overnight at 4 °C. A universal two-step assay kit (Zsboi, Beijing, China) was used for IHC. The immunostaining images were detected using an optical microscope. Each experiment was repeated at least thrice.

Dual-luciferase reporter assay

Luciferase reporter plasmids were designed and synthesized by GenoMeditech (Shanghai, China). HEK-293T cells were cotransfected with luciferase reporter plasmid and microRNAs. A dual-luciferase reporter assay system (Promega, USA) was used to determine the luciferase activity 48 h later according to the instructions. Each experiment was repeated at least thrice.

Tumour xenograft implantation in nude mice

T24 cells (2×10^6 cells) were subcutaneously inoculated into BALB/c nude mice (18-22 g). Then, tumour volumes were measured every 3 days. The animal experiments were performed at the Department of Experimental Animals, Kunming Medical University and approved and supported by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University. Each experiment was repeated at least thrice.

Bioinformatics

The DEGseq2 R package was used to identify differentially expressed lncRNAs and mRNAs (DE-lncRNAs and DE-mRNAs) according to the thresholds |log2 (FC) |>1.0 and *P* value < 0.05. A total of 408 BC samples and 19 normal samples were downloaded from TCGA database (https://portal.gdc.cancer.gov/). The DE-lncRNAs and DE-mRNAs overlapped between the RNA-sequencing data and TCGA data. The differential expression of candidate genes and the relationship of their expression levels were evaluated in data retrieved from the starBase database (https://starbase.sysu.edu.cn/), the GEIPIA database

(http://gepia.cancer-pku.cn/), the Lnc2Cancer database (http://bio-bigdata.hrbmu.edu.cn/lnc2cancer/) and the LncBase database (https://diana.e-ce.uth.gr/lncbase/).

Statistical analyses

All data were obtained from three independent experiments performed at least thrice. All results were presented as the mean ± SD. Statistical analysis was performed with one-way ANOVA and Student's t test using GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA, USA). In this study, $P < 0.05$ was considered statistically significant.

Results

HAGLROS expression is significantly upregulated in BC

HAGLROS expression in common tumours was analysed using the GEPIA database, and the results showed that HAGLROS was highly expressed in most tumours, including BC, lung squamous cell carcinoma, and cervical squamous cell carcinoma (Fig. 1a). To further study whether HAGLROS is significantly upregulated in BC, we retrieved and analysed data from the Lnc2Cancer database and the starBase database. HAGLROS was obviously elevated in BC (Fig. 1b, c, $P<0.05$). Subsequently, our transcriptome sequencing analysis identified 2210 DE-lncRNAs, including 1255 upregulated DE-lncRNAs and 955 downregulated DE-lncRNAs (Fig. 1d). There were 55 upregulated DE-lncRNAs in both the TCGA dataset and our RNA sequencing (RNA-seq) dataset (Fig. 1e). These data also confirmed that HAGLROS was significantly overexpressed (log2FoldChange=5.70, $P<0.01$) in BC tissues (Supplementary Table 1). Furthermore, qRT–PCR assays were used to HAGLROS expression levels in BC tissues and cells. HAGLROS was obviously upregulated in BC tissues and cells (Fig. 1f, g), and increasing HAGLROS expression was related to high pathologic grade (Fig. 1h). In conclusion, these results demonstrate that HAGLROS expression is significantly upregulated in BC.

HAGLROS plays an oncogenic role in BC

To further study the biological functions of HAGLROS in BC, we constructed and screened HAGLROS overexpression and knockdown vectors, which were transfected into T24 and 5637 BC cells for subsequent experiments. The CCK-8 assay results indicated that HAGLROS overexpression promoted BC cell proliferation and that HAGLROS knockdown inhibited BC cell proliferation (Fig. 2a). To investigate the role of HAGLROS in BC, wound healing assays and transwell assays were performed. The results revealed that upregulated HAGLROS expression strengthened BC cell migration and invasion, while downregulated HAGLROS expression decreased BC cell migration and invasion (Fig. 2b-e). Moreover, the flow cytometry data showed that knockdown of HAGLROS induced G1-stage arrest in T24 and 5637 cells (Fig. 2f-g). To further study whether HAGLROS regulates BC tumorigenesis in vivo, subcutaneous xenograft mouse models, including the OE-HAGLROS group and sh-HAGLROS group, were established using transfected T24 BC cells. The results suggested that HAGLROS overexpression significantly

promoted BC growth compared with downregulation of HAGLROS (Fig. 2h-i). Collectively, these results reveal that HAGLROS promotes proliferation, migration, and invasion and plays an oncogenic role in BC.

HAGLROS positively regulates SPRR1B expression

To explore the underlying mechanism of HAGLROS in BC cells, the cytoplasmic and nuclear fractions of T24 and 5637 cells were extracted for qRT–PCR. The results showed that HAGLROS was mostly located in the cytoplasm of BC cells (Fig. 3a). Therefore, we hypothesized that HAGLROS functioned as a ceRNA in BC. Then, TCGA dataset and our transcriptional sequencing dataset were used to identify the DE-mRNAs in BC. Our transcriptome sequencing analysis identified 689 differentially upregulated mRNAs in BC (Fig. 3b). These mRNAs were overlapped, and the top 10 mRNAs in the two datasets were intersected (Supplementary Fig. 1a-b, Supplementary Table 2). The data showed that six mRNAs were highly expressed in both groups, and SPRR1B was the most highly expressed in our sequencing dataset (log2FoldChange=11.83, $P<0.01$). Furthermore, qRT–PCR assay, western blot assay and IHC were used to detect SPRR1B expression levels in BC. The results showed that SPRR1B was highly overexpressed at both the transcriptional and protein levels in BC (Fig. 3c-f). In addition, increasing HAGLROS expression obviously promoted SPRR1B expression, whereas decreasing HAGLROS expression inhibited SPRR1B expression in BC cells (Fig. 3g-h). Together, these results suggest that HAGLROS positively regulates SPRR1B expression.

HAGLROS regulates SPRR1B expression by sponging miR-330-5p

To study the regulatory mechanism of HAGLROS on SPRR1B, the potential miRNAs that shared putative binding sites with HAGLROS and the SPRR1B cluster were predicted using the Lncbase database and the StarBase database. MiR-330-5p shared putative binding sites with HAGLROS and the SPRR1B cluster (Fig. 4a). In addition, qRT–PCR was used to evaluate miR-330-5p expression levels in BC, and the results suggested that miR-330-5p was significantly downregulated in BC (Fig. 4b, c). Furthermore, HAGLROS overexpression inhibited the expression of miR-330-5p, and HAGLROS knockdown increased the expression of miR-330-5p (Fig. 4d). To further explore the relationship among HAGLROS, miR-330-5p and SPRR1B, FISH assays were performed to confirm their subcellular localizations in T24 and 5637 BC cells. The results indicate that all the above three genes are mainly located in the cytoplasm. In addition, HAGLROS may interact with miR-330-5p, and miR-330-5p could interact with SPRR1B (Fig. 4e, f). Moreover, the dual-luciferase reporter assay data showed that HEK-293T cells cotransfected with wild-type HAGLROS and miR-330-5p mimics obviously decreased luciferase activity, but mut-type HAGLROS did not induce this change (Fig. 4g). Additionally, HEK-293T cells cotransfected with wild-type SPRR1B and miR-330-5p mimics, but not mut-type SPRR1B, significantly inhibited the relative luciferase activity (Fig. 4h). In summary, these results demonstrate that HAGLROS regulates the expression of SPRR1B by sponging miR-330-5p.

Decreasing miR-330-5p expression reverses malignant phenotypes inhabited by silencing HAGLROS in BC cells

To further verify the regulatory mechanism of the HAGLROS/miR-330-5p/SPRR1B molecular axis, a series of gain- and loss-of-function assays were performed. The results showed that knockdown of miR-330-5p reversed the inhibitory effects on the proliferation, invasion and migration induced by silencing HAGLROS in BC cells (Fig. 5a-e). In addition, the miR-330-5p inhibitor also reversed the expression of SPRR1B induced by silencing HAGLROS in BC cells (Fig. 5f). Furthermore, the miR-330-5p mimic inhibited SPRR1B expression, whereas the miR-330-5p inhibitor promoted SPRR1B expression (Fig. 5g). Moreover, SPRR1B knockdown reversed the malignant phenotypes of BC cells promoted by decreasing miR-330-5p (Supplementary Fig. 2). These results suggest that HAGLROS plays an important role in regulating the biological behaviour of BC by regulating the miR-330-5p/SPRR1B axis.

Discussion

BC is the tenth most common malignant tumour and the fourteenth leading cause of cancer mortality worldwide and is characterized by a high recurrence rate, rapid progression and poor prognosis [1, 14]. At present, mechanisms involved in the pathogenesis and progression of BC remain unclear, and effective targets for early diagnosis and treatment of BC are lacking. In recent years, accumulating evidence has demonstrated that lncRNAs play an important regulatory role in cancer proliferation, invasion, metastasis, apoptosis, drug resistance and cancer stem cell stemness [6, 15, 16]. Importantly, abnormal expression of lncRNAs is associated with increasing cancer-related mortality. For example, lncRNA CDKN2B-AS1 regulates progression and metastasis through the Cyclin-D pathway by interacting with miR-141 in renal cell carcinoma [17]. LncRNA030, a novel lncRNA, maintains breast cancer stem cell stemness by stabilizing SQLE mRNA and increasing cholesterol synthesis [18]. In addition, an increasing number of studies have found that lncRNAs promote chemoresistance by regulating certain phase enzymes, altering drug efflux, repairing damaged DNA and inhibiting apoptosis in tumours [15]. In the past decade, some of the significant lncRNAs were found to be related to BC incidence and development. For instance, UCA1 is specific and sensitive for the diagnosis of BC [19]. Elevated H19 expression is associated with poor prognosis in BC [20]. As a novel lncRNA, HAGLROS was first reported to be significantly overexpressed in gastric cancer in 2018 [13]. This study revealed that HAGLROS, a direct target of the transcription factor STAT3, was associated with rapid progression and poor prognosis by sponging miR-100-5p to increase mTOR expression and interacting with mTORC1 components to activate the mTORC1 signalling pathway. Subsequently, numerous studies were performed to investigate the correlation between HAGLROS and cancers. In hepatocellular carcinoma, HAGLROS promotes proliferation, inhibits apoptosis and enhances autophagy by regulating the miR-5095/ATG12 signalling pathway [21]. Yang et al. reported that HAGLROS was also significantly upregulated in ovarian cancer and positively correlated with pathological grades and clinical stages [22]. Notably, HAGLROS regulates lipid metabolism reprogramming in intrahepatic cholangiocarcinoma via the mTOR signalling pathway [23]. In brief, growing evidence indicates that HAGLROS is closely related to apoptosis, autophagy, drug resistance and metabolic reprogramming and plays an oncogenic role in cancers [24–26]. However, the biological functions and mechanisms of HAGLROS in BC remain unknown. In the present study, HAGLROS expression levels in BC were initially investigated using an in silico database, our transcriptome

sequencing database, and qRT–PCR assays. These results demonstrated that HAGLROS is obviously increased in BC. Moreover, a series of gain- and loss-of-function studies showed that HAGLROS can significantly promote proliferation, migration and invasion in BC.

The regulatory mechanism of lncRNAs depends in part on their subcellular localizations. Our research shows that HAGLROS is mainly distributed in the cytoplasm of BC cells via subcellular fractionation assay and FISH assay. Therefore, we hypothesized that HAGLROS regulates BC growth and metastasis in a ceRNA-dependent manner, which is the most important and extensive regulatory mechanism of lncRNAs, especially in cancers. Thus, according to the analysis of TCGA dataset and our transcriptome dataset, we found that SPRR1B was significantly overexpressed, which was further confirmed in BC tissues and cells. SPRR1B is a member of the SPRR family. Relative studies have revealed that the SPRR family is closely associated with carcinoma by regulating the epithelial-mesenchymal transition (EMT). Abundant evidence suggests that SPRR1B is closely associated with the progression and prognosis of lung cancer [27–30]. Furthermore, DGUOK-AS1 promotes the progression of cervical squamous cell carcinoma by sponging miR-499a-5p and increasing SPRR1B expression [31]. In cutaneous melanoma, abnormally overexpressed SPRR1B facilitates proliferation, invasion and migration and leads to poor prognosis [32]. Moreover, SPRR1B may promote entry into the G0 phase in non-squamous cell carcinoma [33]. To our knowledge, the present study is the first to initially explore the correlation between SPRR1B and bladder tumours. The results showed that SPRR1B was significantly upregulated and that aberrantly elevated SPRR1B was related to malignant phenotypes in BC.

Numerous studies have shown that upregulated miR-330-5p can inhibit progression by interacting with proteins in a variety of tumours, including lung cancer [34], colorectal cancer [35], pancreatic cancer [36], and prostate cancer [37]. Chen et al. found that miR-330-5p can suppress the progression of bladder cancer by inhibiting MTGR1 expression and the downstream Notch signalling pathway in BC [38]. Our study also showed that miR-330-5p was downregulated and could inhibit progression by interacting with SPRR1B in BC.

Conclusion

In summary, our study demonstrates that HAGLROS is significantly more highly expressed and plays an oncogenic role in BC. Mechanistically, HAGLROS promotes the proliferation, migration and invasion of BC by positively regulating SPRR1B expression by sponging miR-330-5p. These data provide a novel basis for investigating the mechanism of the incidence and development of BC. Collectively, our findings suggest that HAGLROS is a potential tumour biomarker that may function as a powerful diagnostic and therapeutic target of BC.

Abbreviations

BC: Bladder cancer; NMIBC: Non-muscle-invasive bladder cancer; MIBC: Muscle-invasive bladder cancer; lncRNA: Long non-coding RNA; HAGLROS: HAGLR opposite strand lncRNA; SPRR1B: Small proline rich

protein 1B; OE: Overexpressed; sh: Short hairpin; qRT-PCR: quantitative realtime PCR; FISH: fluorescent in situ hybridization; CCK-8: Cell counting Kit-8; IHC: Immunohistochemistry; EMT: Epithelial-mesenchymal transition; CeRNA: Competing endogenous RNA; FBS: Fetal bovine serum; ANOVA: One-way analysis of variance; UCA1:urothelial cancer-associated 1.

Declarations

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Authors' contributions

Conceptualization ideas: JSW, SWX, HFW.

Formal analysis: SWX, SF, DBY.

Investigation: SWX, YNL, XHQ.

Methodology: SWX, YGZ, YLH.

Project administration: JSW, HFW.

Resources: JSW, HFW.

Supervision: JSW, HFW.

Verification: SWX, HFW.

Writing - original draft: SWX.

Writing - review & editing: SWX, JSW, HFW.

All authors read and approved this final manuscript.

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Availability of data and materials

All data in our study are available upon request.

Ethics approval and consent to participate

All patients were known the purpose of this study and signed the inform consents. All experiments performances were approved by the Medical Ethics Committee of the Second Affiliated Hospital of

Kunming Medical University.

Consent for publication

All authors agree to publish.

Competing interests

The authors declare that they have no competing interests.

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Figures

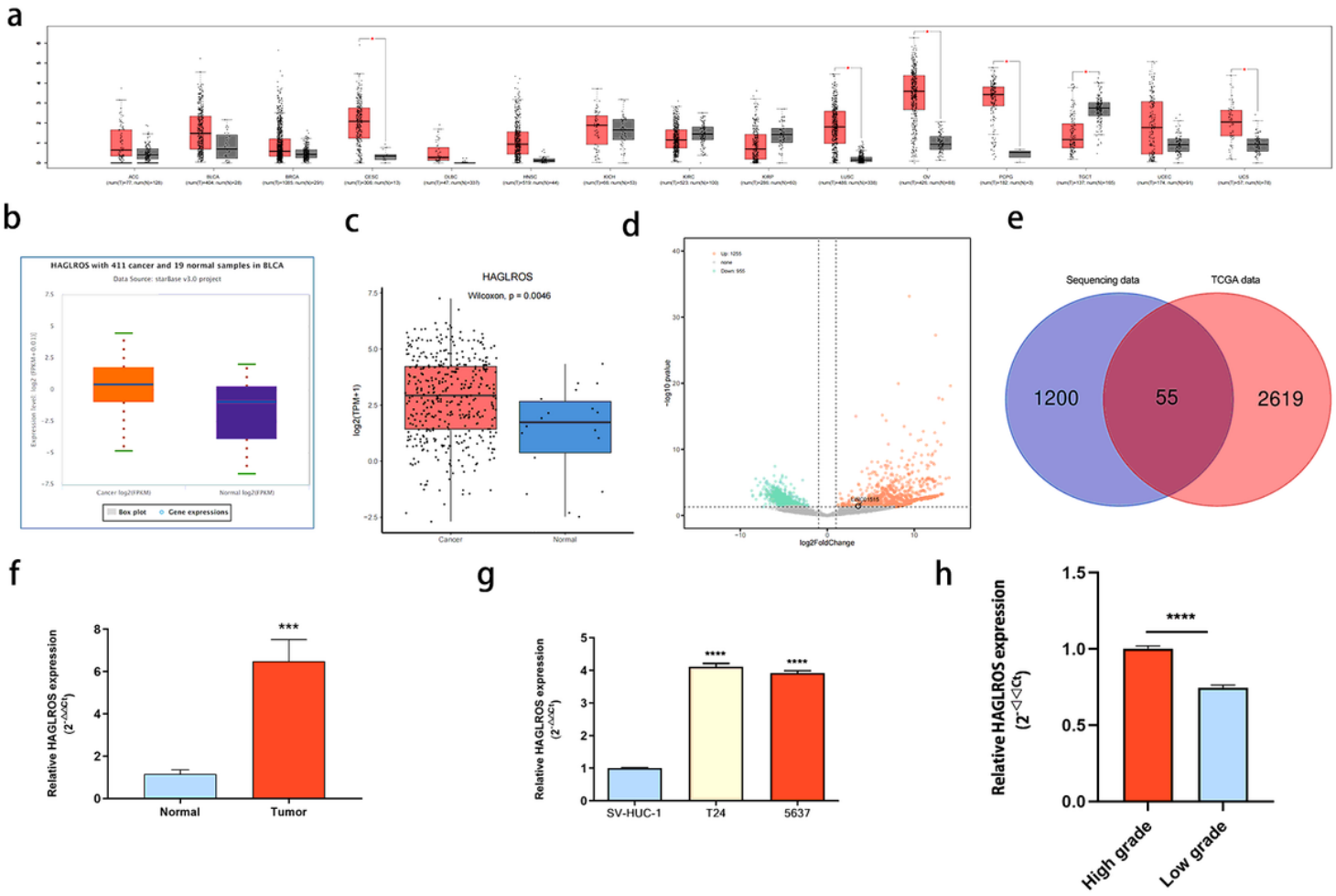

Figure 1

HAGLROS expression is significantly upregulated in BC. (**a**) Expression of HAGLROS in common tumour samples and paired normal tissues. (**b, c**) The HAGLROS expression level of BC was explored using the Lnc2Cancer database (*P*<0.001) and the starBase database (*P*<0.001). (d) Volcano plot of DE-lncRNAs in our transcriptome sequencing dataset. Each point in the plot represents a gene. Orange dots represent upregulated genes, whereas green dots represent downregulated genes. (**e**) Venn diagram of upregulated DE-lncRNAs based on the overlap between TCGA dataset and our RNA-sequencing dataset. (**f, g**) HAGLROS expression is upregulated in BC samples compared with corresponding nontumour tissues and cells. (**h**) HAGLROS expression is increased in high pathologic grade. Each experiment was repeated at least thrice. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

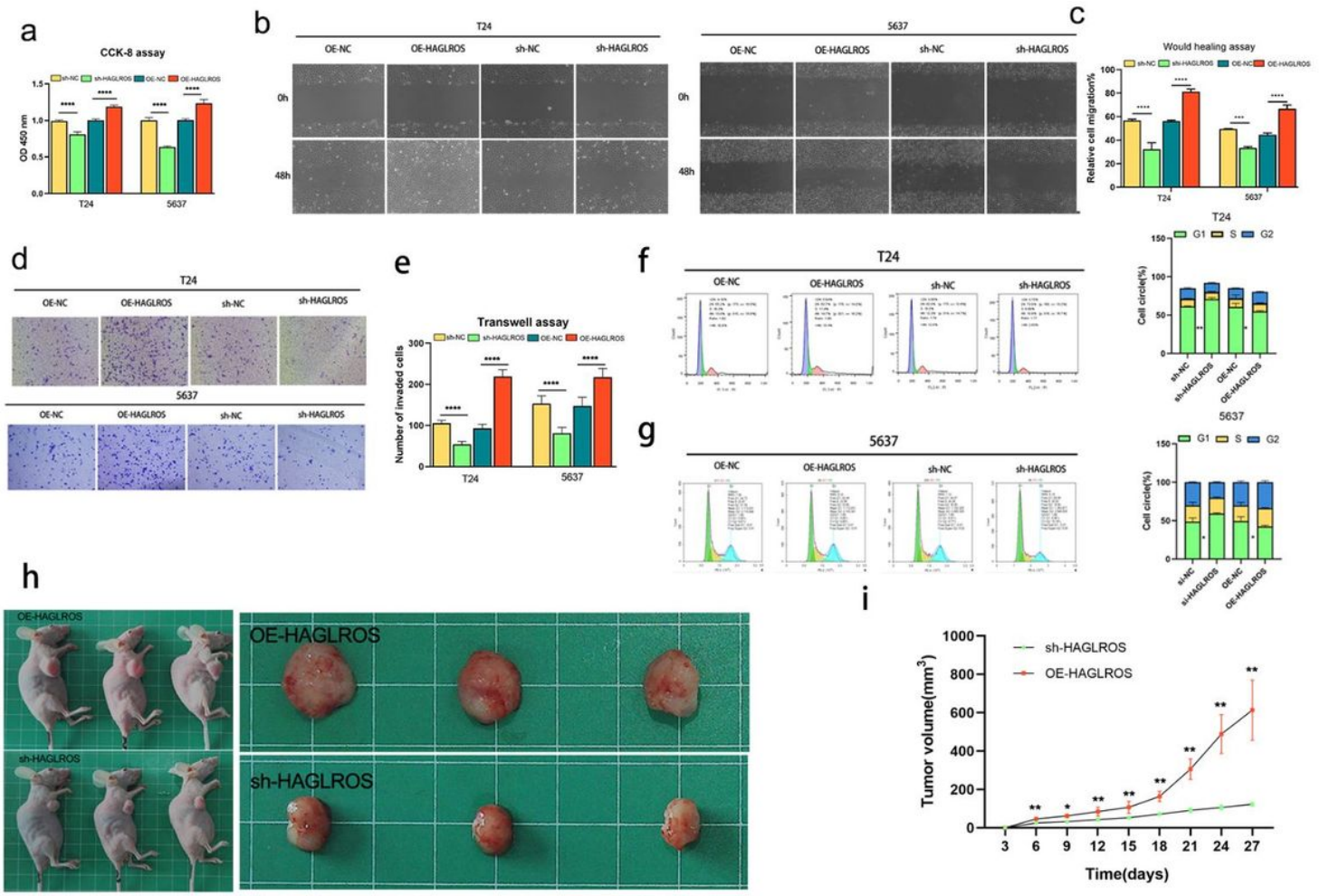

Figure 2

HAGLROS promotes the proliferation, migration and invasion of BC in vitro and in vivo. (**a**) Proliferation levels were assessed by CCK-8 assays in T24 and 5637 BC cells. (**b, c**) Cell migration was determined by wound healing assays. (**d, e**) Cell invasion was monitored by Transwell assay in T24 and 5637 BC cells. (**f, g)** The cell cycle of T24 and 5637 BC cells. (**h**) Nude mice were subcutaneously injected with T24 cells transfected with OE-HAGLROS or sh-HAGLROS. (**i**) Tumour volumes were calculated every 3 days after 5 days of injection. Each experiment was repeated at least thrice. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

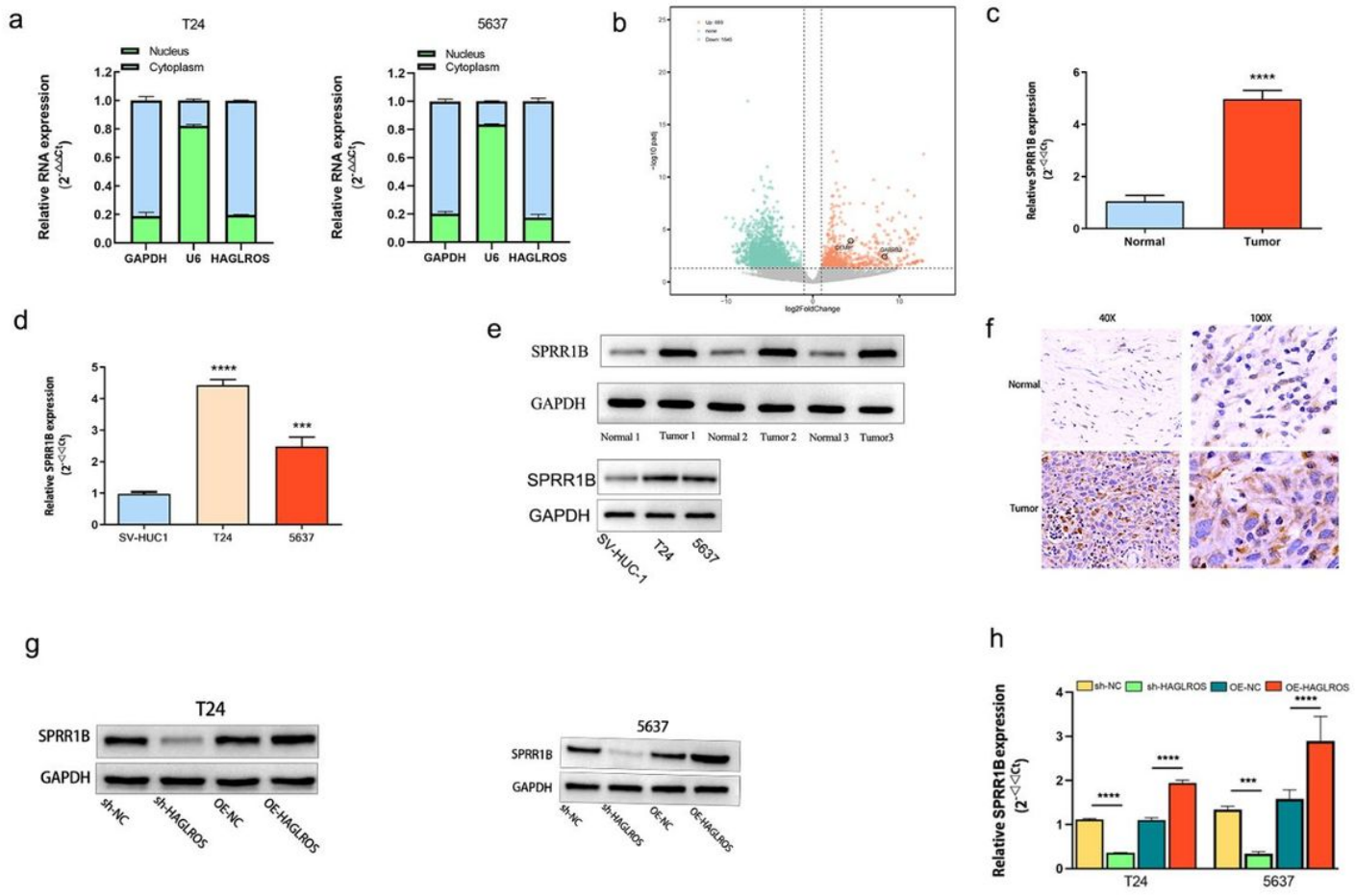

Figure 3

HAGLROS positively regulates SPRR1B expression. (**a**) The subcellular localization of HAGLROS was determined by subcellular fractionation assay in T24 and 5637 BC cells. (**b**) Volcano plot of DE-mRNAs in our transcriptome sequencing dataset. Each point in the plot represents a gene. Orange dots represent upregulated genes, whereas green dots represent downregulated genes. (**c-e**) The expression of SPRR1B was determined by qRT–PCR and Western blot assay in bladder tissues and cells. (**f**) SPRR1B expression was determined by IHC in BC tissues and corresponding non-tumour samples. (**g, h**) SPRR1B levels were evaluated by qRT–PCR and Western blot assay in BC cells transfected with OE-HAGLROS and sh-HAGLROS. Each experiment was repeated at least thrice. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

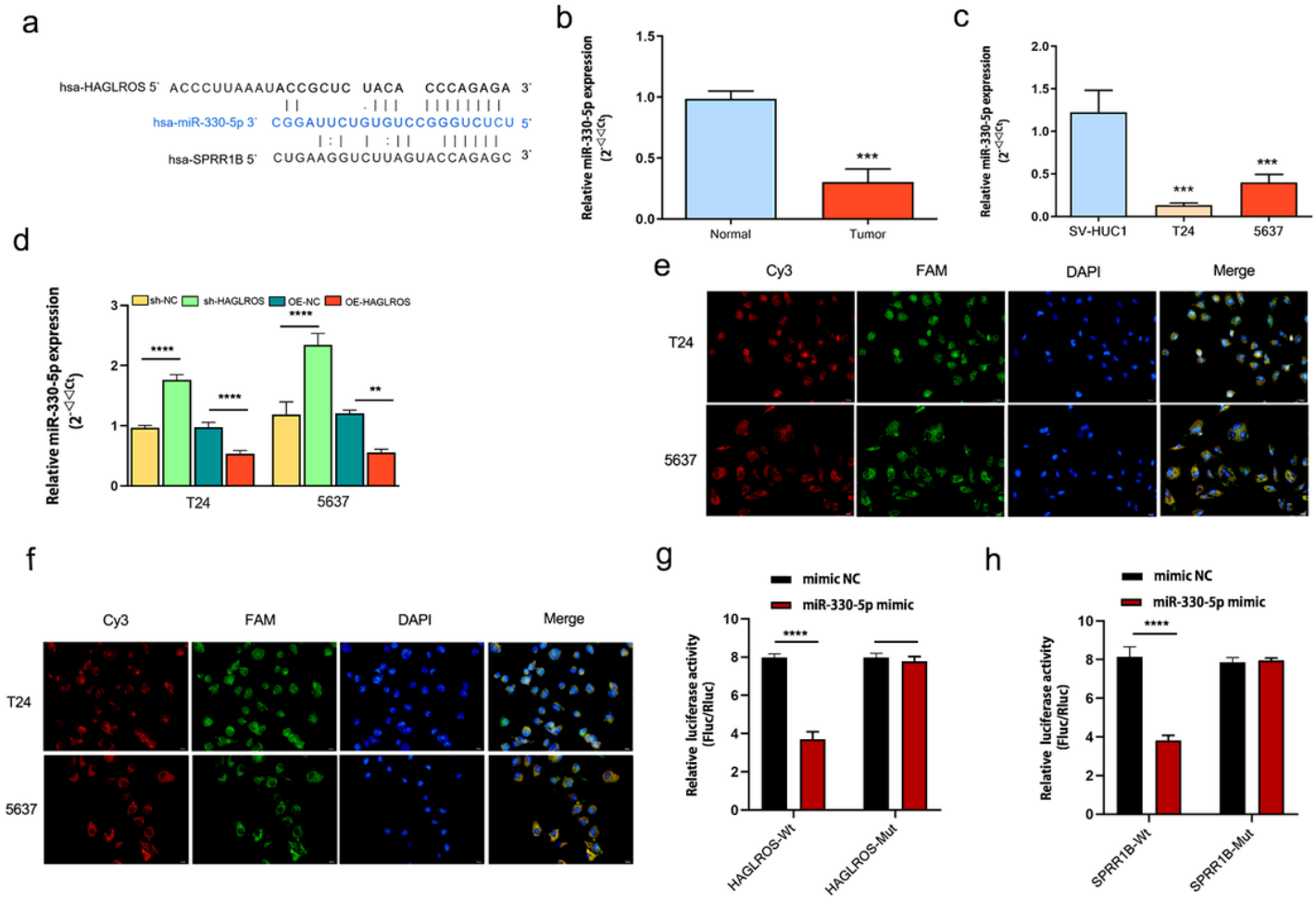

Figure 4

HAGLROS regulates SPRR1B expression by sponging miR-330-5p. (**a**) The specific binding sites among HAGGLROS, miR-330-5p and SPRR1B were predicted by the Lncbase database and the starBase database. (**b, c**) miR-330-5p expression was examined in bladder tissues and cells using qRT–PCR. (**d**) The level of miR-330-5p was determined using qRT–PCR. (**e**) The FISH assay was used to confirm the subcellular colocalization between HAGLROS and miR-330-5p in BC cells. HAGLROS (Cy3, red), miR-330-5p (FAM, green), and cell nuclei (DAPI, blue)

(**f**) The FISH assay was used to confirm the subcellular colocalization between miR-330-5p and SPRR1B in BC cells. SPRR1B (Cy3, red), miR-330-5p (FAM, green), cell nuclei (DAPI, blue) (**g, h**) The relative luciferase activities were measured to determine the level of interaction between miR-300-5p and HAGLROS and between miR-300-5p and SPRR1B. Each experiment was repeated at least thrice. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

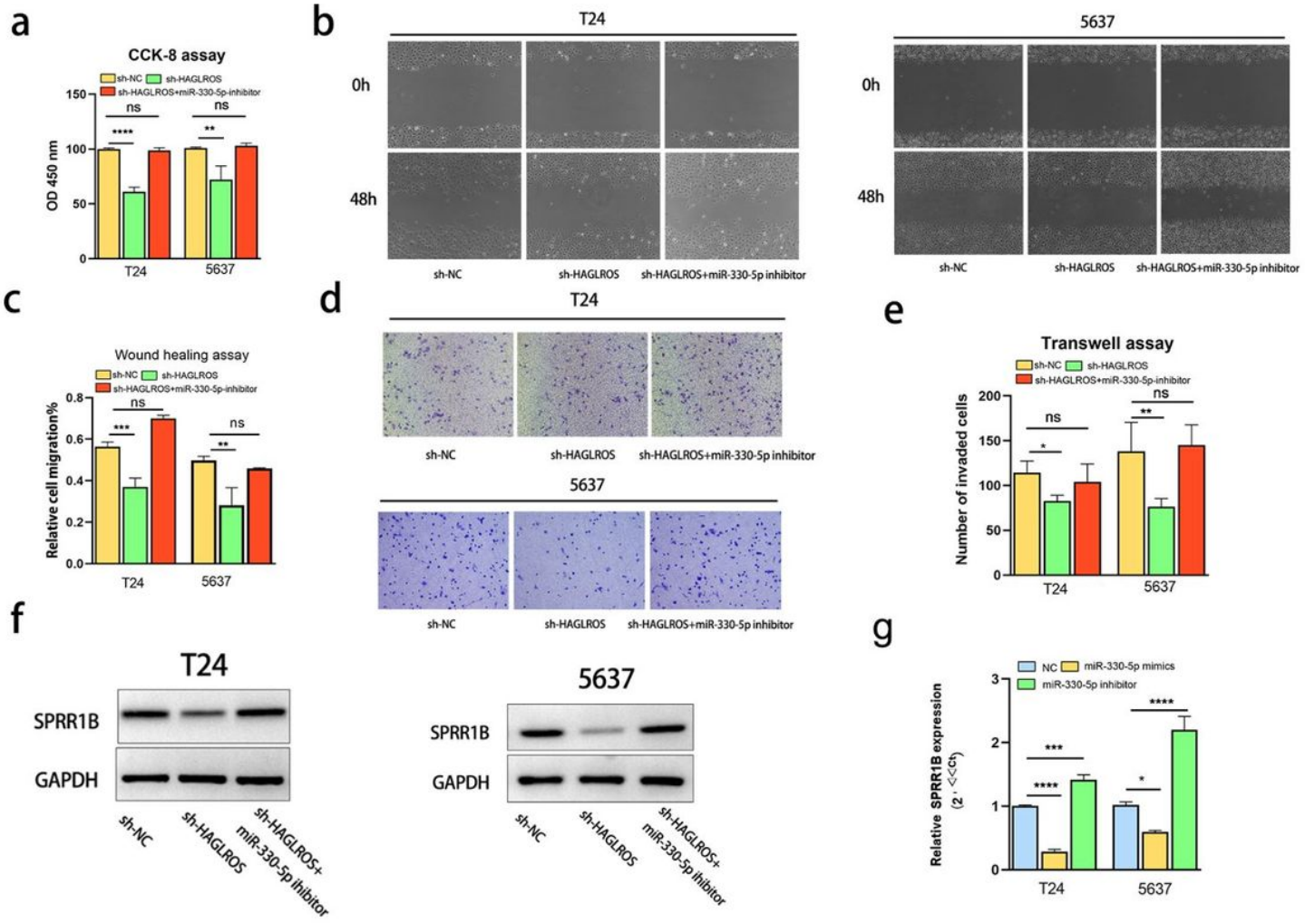

Figure 5

Decreased miR-330-5p reverses malignant phenotypes induced by silencing HAGLROS in BC cells. (**a**) Downregulated miR-330-5p significantly reverses cell proliferation inhibition by silencing HAGLROS in T24 and 5637 BC cells. (**b, c**) Downregulated miR-330-5p obviously reverses cell migration inhibition by silencing HAGLROS in T24 and 5637 BC cells. (**d, e**) Downregulated miR-330-5p obviously reverses cell invasion inhibition by silencing HAGLROS in T24 and 5637 BC cells. **(f)** Downregulated miR-330-5p obviously reverses SPRR1B expression reduced by silencing HAGLROS in T24 and 5637 BC cells. (**g**) SPRR1B expression levels were measured using qRT–PCR assay in the miR-330-5p mimic and inhibitor groups. Each experiment was repeated at least thrice. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Supplementary Files

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