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Original Article

Long non-coding RNA POC1B-AS1 depletion represses colorectal cancer progression through the microRNA-625-5p/FOXK1 axis

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Abstract: Objectives: Numerous long non-coding RNAs (lncRNAs) are aberrantly expressed in colorectal cancer (CRC), causing its high aggressiveness and uncontrolled growth. Therefore, exploring the regulatory activities of lncRNAs in CRC could facilitate the development of promising therapeutic targets. This study aimed to illustrate the role of POC1B antisense RNA 1 (POC1B-AS1) in CRC malignancy. Methods: POC1B-AS1 expression levels were quantified by quantitative real-time polymerase chain reaction. Its biological roles were investigated through functional experiments. Luciferase reporter and RNA immunoprecipitation assays were employed to illustrate the underlying mechanisms. Results: Highly expressed POC1B-AS1 in CRC was confirmed. Depletion of POC1B-AS1 resulted in reduced proliferative, colony formative, migratory and invasive capacities of CRC cells *in vitro*, and suppressed tumor growth *in vivo*. POC1B-AS1 functioned as a sponge for microRNA-625-5p (miR-625-5p), and consequently overexpressed forkhead box K1 (FOXK1) expression in CRC. Additionally, the repressing actions of POC1B-AS1 ablation on CRC cell aggressiveness were attenuated due to miR-625-5p downregulation or FOXK1 overexpression. Ablation of POC1B-AS1 inhibited the malignant progression of CRC cells by sequestering miR-625-5p and thereby up-regulating FOXK1. Conclusions: Our study suggest that the manipulation of POC1B-AS1/miR-625-5p/FOXK1 pathway-mediated aggressiveness in CRC may offer an effective therapeutic target.

Keywords: POC1B-AS1, long non-coding RNA, microRNA, ceRNA network

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related death worldwide [1]. Annually, about 1.2 million new cases of CRC are diagnosed, resulting in 860,000 mortalities globally [2]. Due to the absence of early symptoms and limitations in diagnostic strategies, many CRC cases are diagnosed at a late stage [3]. In recent years, with advances in surgical resection, radiochemotherapy, and the introduction of novel immunotherapies, the efficacy of treatment has improved significantly [4]; nevertheless, the prognosis for CRC patients remains unsatisfactory [5]. Therefore, exploring the molecular events underlying the oncogenesis and progression of CRC is crucial for the development of promising therapeutic strategies for patients with CRC.

Based on human transcriptome analyses, 98% of the human genome lacks protein-coding capacity and can be transcribed into non-coding RNAs (lncRNAs). lncRNAs are a group of noncoding transcripts comprising over 200 nucleotides [6]. Substantial evidence has revealed that lncRNAs are dysregulated in almost all types of human diseases and are involved in controlling many biological processes [7-9]. Furthermore, the modulatory roles of abnormally expressed lncRNAs in CRC progression have been intensively studied [10-12]. Given the importance of lncRNAs in CRC aggressiveness, it is crucial to investigate their

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contributions to colorectal carcinogenesis and progression.

In this study, we aimed to investigate the functional roles of POC1B-AS1 in CRC. Our findings may provide novel insight into the molecular mechanisms underlying CRC oncogenesis.

Materials and methods

Patient specimens

This study was approved by the Ethics Committee of The First Hospital of Jilin University. We enrolled thirty-nine CRC patients without other cancer diagnoses and collected both tumor and adjacent normal tissues. Written informed consent was obtained from all participants. None of the patients had received any anti-cancer treatments prior to surgery.

Cell culture and transfection

The normal human colon epithelial cell line FHC (ATCC; Manassas, VA, USA) was cultured in DMEM/F-12 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). HCT116 and HT29 cells were cultured in McCoy's 5A medium (Gibco). SW480 cells were cultured in L-15 medium (Gibco). All media were supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (Gibco).

All cells were cultured at 37°C in a humidified incubator with an atmosphere of 5% CO_2. Cells were transfected with siRNA targeting POC1B-AS1 (si-POC1B-AS1), non-specific siRNA, or the pcDNA3.1-FOXK1 plasmid (all from GenePharma, Shanghai, China) using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) when they reached 70-80% confluence.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen). To determine POC1B-AS1 expression, cDNA was synthesized using a PrimeScript™ RT reagent kit (Takara, Dalian, China). The cDNA was subjected to quantitative PCR with a TB Green® Premix Ex Taq™ II (Takara). GAPDH served as endogenous control for normalizing POC1B-AS1 and FOXK1 expression. To assess miR-625-5p expression, cDNA was generated using the miRcute miRNA First-Strand cDNA Synthesis Kit, followed by amplification with the miRcute miRNA qPCR Detection Kit (SYBR Green) (Tiangen, Beijing, China). U6 small nuclear RNA was used as the endogenous control for miR-625-5p.

Cell counting kit-8 (CCK-8) and colony formation assays

At 24 hours post-transfection, the cells were harvested using 0.25% trypsin. Then, 1×10^3 cells suspended in 100 μL of culture medium were seeded into each well of a 96-well plate. After culturing for the designated time periods, 10 μL of CCK-8 reagent (Dojindo, Rockville, MO, USA) was added to each well, and the plates were incubated for another 2 hours. The optical density was then measured for each well using a microplate reader.

For the colony formation assay, 500 cells were seeded in 2 mL of culture medium per well of a 6-well plate. After being cultured for two weeks, the colonies were stained with 0.1% crystal violet. The stained colonies were then visualized and imaged using a light microscope.

Transwell experiments

For the migration assay, cell suspensions was were prepared in serum-free medium. A total of 5×10^4 cells in 200 μL of serum-free medium were added to the upper chamber, while the lower chamber was filled with 600 μL of medium containing 20% FBS as a chemoattractant. After 24 hours of incubation, the cells on the lower surface of the membrane were fixed with 4% paraformaldehyde and then stained with 0.1% crystal violet. After extensive rising with PBS to remove excess stain, the migrated cells were photographed under a light microscope. For the invasion assay, the upper side of the membrane was pre-coated with Matrigel matrix to simulate the extracellular matrix; the remaining procedures were identical to those of the migration assay.

Tumor xenograft models

All animal experiments were conducted in accordance with the guidelines approved by

the Animal Ethics Committee of The First Hospital of Jilin University. The pLKO.1 vector (Addgene, Inc.; Cambridge, MA, USA) was used to construct lentiviruses expressing either short-hairpin RNA targeting POC1B-AS1 (sh-POC1B-AS1) or a non-targeting control (sh-NC; GenePharma). These constructs were then transfected into 293T cells. The lentiviruses were harvested by ultracentrifugation and used to infect HCT116 cells. Subsequently, HCT116 cells stably expressing sh-POC1B-AS1 or sh-NC were selected and subcutaneously inoculated into the flanks of nude BALB/c mice (GemPharmatech, Jiangsu, China). Tumor size was measured weekly using a vernier caliper, and the volume was calculated using the formula: Volume = 0.5 × length × width^2. After 5 weeks, all mice were euthanized. The xenograft tumors were excised, photographed, and weighed.

Subcellular fractionation assay

The nuclear and cytosolic fractions of CRC cells were separated using a Cytoplasmic and Nuclear RNA Purification Kit (Norgen, Thorold, ON, Canada). Following RNA from these fractions, qRT-PCR was performed to determine the subcellular distribution POC1B-AS1.

Bioinformatic analysis

Potential miRNAs that could be sponged by POC1B-AS1 were predicted using Starbase 3.0. The downstream targets of miR-625-5p were predicted using two databases: TargetScan and miRDB.

Luciferase reporter assay

DNA fragments of wild-type (wt) or mutant (mut) POC1B-AS1 and FOXK1, containing the putative miR-625-5p binding sites, were synthesized by GenePharma. These fragments were then cloned into the psiCHECK™-2 vector (Promega) to generate the wt-POC1B-AS1, mut-POC1B-AS1, wt-FOXK1, and mut-FOXK1 reporter constructs. For the luciferase assay, cells were co-transfected with either the wt or mut reporter constructs, along with the miR-625-5p mimic or a negative control mimic. After 48 hours, luciferase activities were measured using the Dual-Luciferase® Reporter Assay System (Promega, Madison, WI, USA).

RNA immunoprecipitation (RIP) assay

CRC cells were lysed in RIP lysis buffer from the EZ-Magna RIP Kit (Merck-Millipore). The lysates were immunoprecipitated with an anti-Ago2 antibody and then treated with proteinase K. Following this, RNA was extracted from the immunoprecipitates, and the enrichment for POC1B-AS1 and miR-625-5p was evaluated by qRT-PCR.

Western blot analysis

Total protein was extracted utilizing RIPA buffer (Beyotime, Shanghai, China). After separation via 10% SDS-PAGE, the proteins were transferred onto a PVDF membrane. The membrane was then blocked with 5% skim milk at room temperature and subsequently incubated with primary and secondary antibodies following standard protocols. The primary antibodies used were against FOXK1 (cat. no. sc-373810; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and GAPDH (ab8245; Abcam, Cambridge, UK). Finally, protein signals were visualized using an ECL detection reagent (Pierce).

Statistical analysis

All data are shown as mean ± standard deviation and were analyzed using SPSS 19.0 (IBM Corp., Armonk, NY, USA). Comparisons between two groups were performed using Student's t-test. Differences among multiple groups were examined by one-way analysis of variance followed by Tukey's post hoc test. A *P* value of less than 0.05 was considered significant.

Results

POC1B-AS1 is overexpressed in CRC

We first examined the expression levels of POC1B-AS1 in colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ) using data from The Cancer Genome Atlas (TCGA). As shown in **Figure 1A**, POC1B-AS1 was significantly overexpressed in both COAD and READ tissues compared to normal tissues. Consistent with this finding, an elevated level of POC1B-AS1 was also confirmed in CRC tissues from our own cohort (**Figure 1B**). Furthermore, we detected POC1B-AS1 expression in a panel of CRC cell lines by qRT-PCR. The results demonstrated that POC1B-AS1 was upregulated in

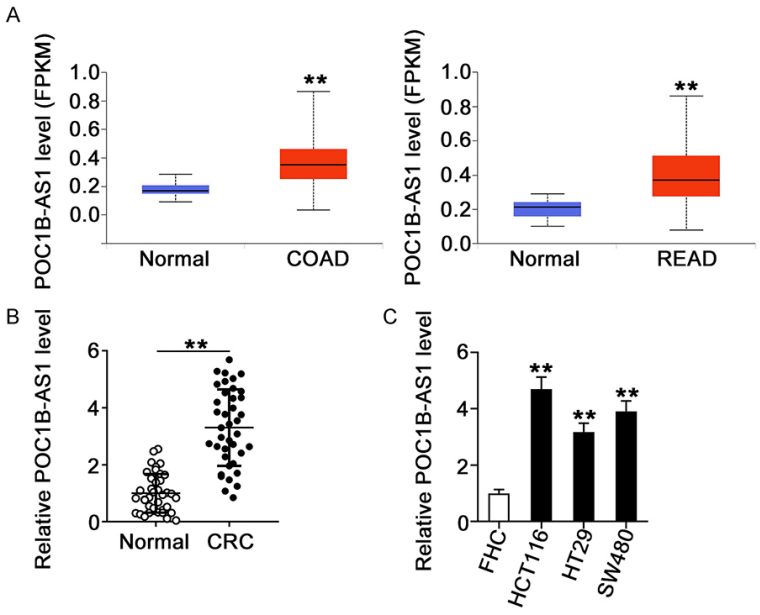

Figure 1. POC1B-AS1 is highly expressed in CRC. A. POC1B-AS1 expression levels in CORD and READ from TCGA cohorts. B. POC1B-AS1 expression in CRC tissues from our own cohort. C. POC1B-AS1 expression in CRC cell lines. **P < 0.01. COAD, colonic adenocarcinoma; READ, rectal adenocarcinoma; CRC, colorectal cancer; FPKM, Fragments Per Kilobase of exon model per Million mapped fragments; POC1B-AS1, POC1B antisense RNA 1.

all examined CRC cell lines compared to a normal colonic epithelial cell line (**Figure 1C**).

Depleted POC1B-AS1 hinders CRC cell growth and motility

Based on POC1B-AS1 expression levels, HCT-116 and SW480 cell lines were selected for subsequent experiments. Specific siRNAs were designed to knock down POC1B-AS1. Among them, si-POC1B-AS1#1 demonstrated the highest silencing efficiency (**Figure 2A**) and was therefore chosen for all subsequent loss-of-function assays. Depletion of POC1B-AS1 significantly suppressed the proliferation of CRC cells (**Figure 2B** and **2C**). Furthermore, POC1B-AS1 ablation also cell motility (**Figure 2D** and **2E**). Collectively, these findings indicate that POC1B-AS1 plays an oncogenic role in CRC.

POC1B-AS1 functions as a miR-625-5p sponge

To investigate the molecular mechanism of POC1B-AS1 in CRC, we first predicted its subcellular localization using the lncLocator software. The prediction indicated that POC1B-AS1 is predominantly localized in the cytoplasm (**Figure 3A**). We then verified this prediction experimentally through subcellular fractionation assays, which confirmed that POC1B-AS1 is primarily distributed in the cytoplasm of CRC cells (**Figure 3B**). Since previous studies have demonstrated that cytoplasmic lncRNAs can function as molecular decoys for specific miRNAs, thereby attenuating the miRNAs' suppressive effects on their target genes [13], we hypothesized that POC1B-AS1 may act as a ceRNA in CRC cells.

Using Starbase 3.0, we identified 25 candidate miRNAs (**Table 1**) that contain potential binding sequences for POC1B-AS1. We then analyzed the expression patterns of these candidates in the TCGA dataset. Among them, five miRNAs (miR-323a-3p, miR-323b-3p, miR-455-5p, miR-625-5p, and miR-766-5p) were found to be downregulated in both COAD and READ (**Figure 3C-G**), and were therefore selected for further validation. We first detected their expression in CRC cells following POC1B-AS1 knockdown. The results showed that only miR-625-5p was upregulated in si-POC1B-AS1-transfected cells, while the other four miRNAs remained unchanged (**Figure 3H**). We next performed a luciferase reporter assay to determine whether miR-625-5p (**Figure 3I**) directly interacts with POC1B-AS1. As shown in **Figure 3J**, transfection with an miR-625-5p mimic markedly reduced the luciferase activity of the wt-POC1B-AS1 reporter, but had no effect on the mut-POC1B-AS1 construct. Furthermore, both POC1B-AS1 and miR-625-5p were significantly enriched in immunoprecipitates obtained with an anti-Ago2 antibody compared to the control IgG (**Figure 3K**). Collectively, these results demonstrate that POC1B-AS1 acts as a molecular sponge for miR-625-5p.

POC1B-AS1 regulates FOXK1 expression by sequestering miR-625-5p

FOXK1, an important driver of CRC progression [14, 15], was predicted to contain potential

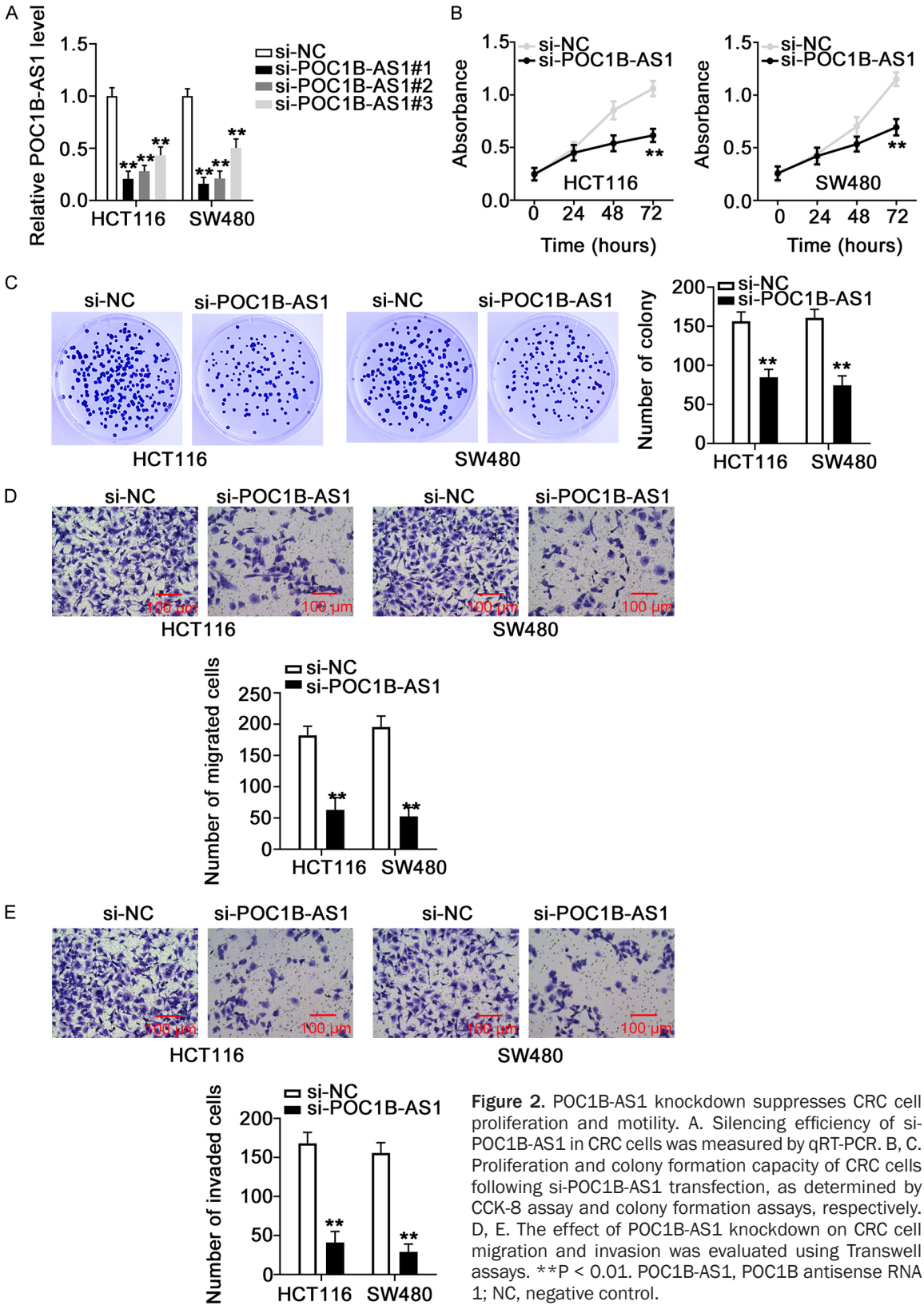

Figure 2. POC1B-AS1 knockdown suppresses CRC cell proliferation and motility. A. Silencing efficiency of si-POC1B-AS1 in CRC cells was measured by qRT-PCR. B, C. Proliferation and colony formation capacity of CRC cells following si-POC1B-AS1 transfection, as determined by CCK-8 assay and colony formation assays, respectively. D, E. The effect of POC1B-AS1 knockdown on CRC cell migration and invasion was evaluated using Transwell assays. **P < 0.01. POC1B-AS1, POC1B antisense RNA 1; NC, negative control.

binding sites for miR-625-5p (**Figure 4A**). Transfection with an miR-625-5p mimic significantly reduced the luciferase activity of the wt-FOXK1, but had no notable effect on the mut-

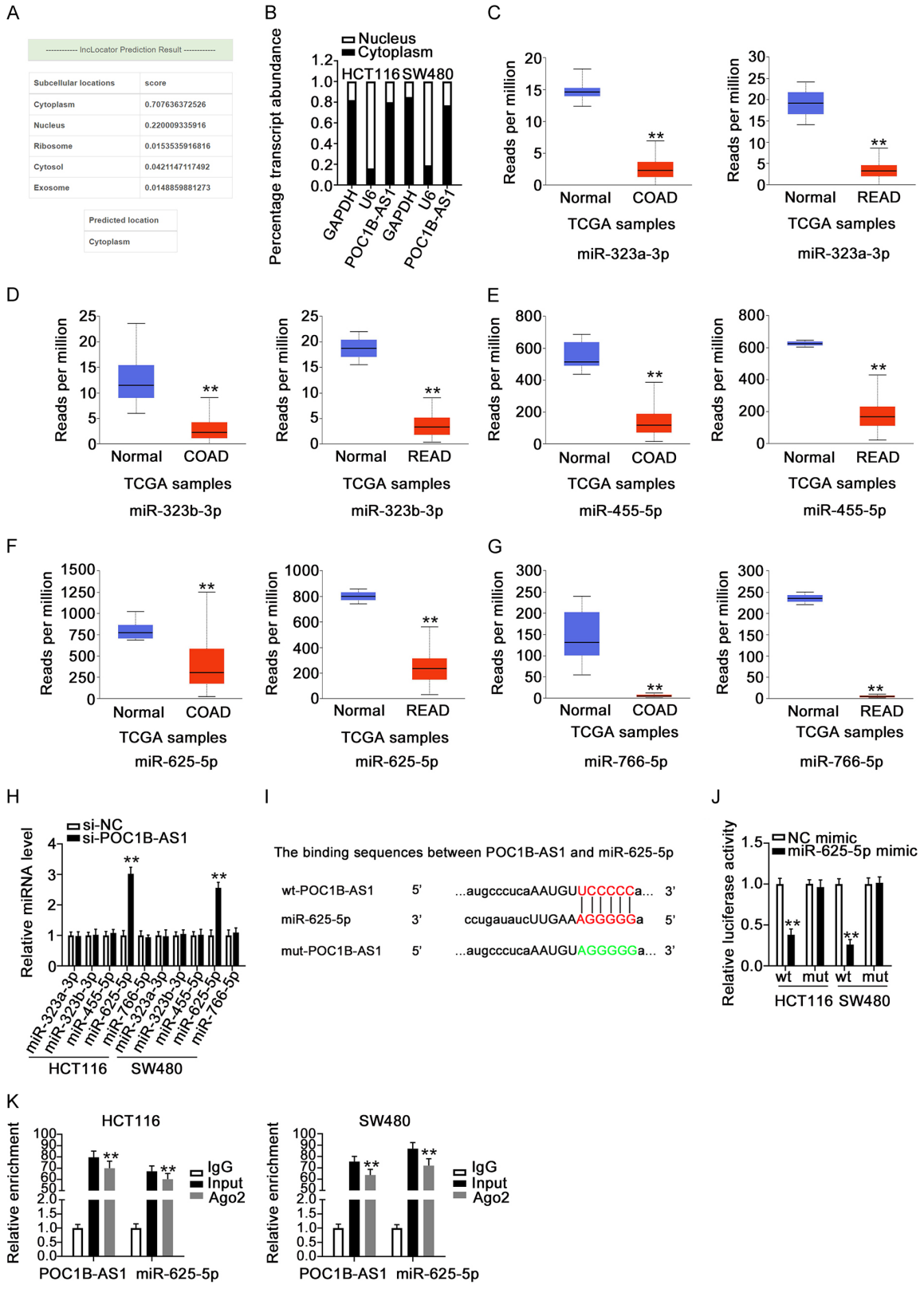

Figure 3. POC1B-AS1 functioned as a molecular sponge for miR-625-5p in CRC. A. Subcellular localization of POC1B-AS1 predicted by lncLocator. B. Experimental validation of POC1B-AS1 localization in CRC cells by subcellular fractionation assay. C-G. Expression levels of miR-323a-3p, miR-323b-3p, miR-455-5p, miR-625-5p, and miR-766-5p in CORD and READ from TCGA cohorts. H. Relative expression of the five candidate miRNAs in CRC

cells following transfection with si-POC1B-AS1. I. Schematic diagram of the predicted binding site between POC1B-AS1 and miR-625-5p. J. Activity in CRC cells co-transfected with either wt-POC1B-AS1 or mut-POC1B-AS1 reporter constructs and miR-625-5p mimic or NC mimic. K. RIP assay using an anti-Ago2 antibody to confirm the direct interaction between POC1B-AS1 and miR-625-5p. **P < 0.01. POC1B-AS1, POC1B antisense RNA 1; COAD, colon adenocarcinoma; READ, rectum adenocarcinoma; CRC, colorectal cancer; FPKM, Fragments Per Kilobase of exon model per Million mapped fragments; TCGA, The Cancer Genome Atlas; NC, negatice control; miR, microRNA; wt, wild-type; mut, mutant.

Table 1. The targets of POC1B-AS1 predicted by Starbase 3.0

Target rank	miRNA name	Target rank	miRNA name
1	hsa-miR-155-5p	14	hsa-miR-4701-5p
2	hsa-miR-216b-5p	15	hsa-miR-4761-3p
3	hsa-miR-3194-3p	16	hsa-miR-510-5p
4	hsa-miR-320a	17	hsa-miR-513a-5p
5	hsa-miR-320b	18	hsa-miR-519a-3p
6	hsa-miR-320c	19	hsa-miR-519b-3p
7	hsa-miR-320d	20	hsa-miR-519c-3p
8	hsa-miR-323a-3p	21	hsa-miR-588
9	hsa-miR-323b-3p	22	hsa-miR-625-5p
10	hsa-miR-3611	23	hsa-miR-760
11	hsa-miR-374a-3p	24	hsa-miR-766-5p
12	hsa-miR-4429	25	hsa-miR-892c-5p
13	hsa-miR-455-5p		

hsa, human; miR, microRNA.

FOXK1 group (**Figure 4B**). Furthermore, ectopic expression of miR-625-5p downregulated FOXK1 at the protein level (**Figure 4C**). Given that POC1B-AS1 sequesters miR-625-5p, which in turn directly targets FOXK1, we hypothesized that POC1B-AS1 regulates FOXK1 expression by acting as a miR-625-5p decoy. Consistent with this, knockdown of POC1B-AS1 suppressed FOXK1 expression, and this suppression was reversed by co-treatment with an miR-625-5p inhibitor (**Figure 4D**). In summary, these results demonstrate that POC1B-AS1 upregulates FOXK1 expression by sponging miR-625-5p in CRC.

The tumor-promoting role of POC1B-AS1 is mediated through the miR-625-5p/FOXK1 axis

To determine whether the miR-625-5p/FOXK1 axis functions as the downstream effector of POC1B-AS1 in CRC, we performed a series of rescue experiments. We first confirmed the transfection efficiency of the miR-625-5p inhibitor and pcDNA3.1-FOXK1 construct. Transfection with the miR-625-5p inhibitor effectively reduced miR-625-5p levels in CRC cells (**Figure 5A**), while pcDNA3.1-FOXK1 transfection resulted in marked FOXK1 overexpression (**Figure 5B**). The impaired proliferative capacity of CRC cells induced by si-POC1B-AS1 was substantially restored by either miR-625-5p inhibition or pcDNA3.1-FOXK1 overexpression (**Figure 5C** and **5D**). Similarly, the suppressive effects of si-POC1B-AS1 on CRC cell migration and invasion were also reversed by miR-625-5p downregulation or FOXK1 upregulation (**Figure 6A** and **6B**). In conclusion, these rescue experiments demonstrate that POC1B-AS1 promotes CRC aggressiveness by regulating the miR-625-5p/FOXK1 axis.

Inhibition of POC1B-AS1 restrains in vivo tumor growth

To verify whether POC1B-AS1 modulates the growth of CRC cells *in vivo*, we employed tumor xenograft models. As shown in **Figure 7A-C**, POC1B-AS1 silencing significantly suppressed both tumor volume and weight. Furthermore, analysis of the xenograft tissues revealed that the expression levels of POC1B-AS1 and FOXK1 were decreased (**Figure 7D** and **7E**), while the level of miR-625-5p was increased (**Figure 7F**) in the sh-POC1B-AS1 group compared to the control. In summary, these results demonstrate that silencing POC1B-AS1 inhibits CRC tumor growth *in vivo*.

Discussion

Accumulating evidence has demonstrated that the dysregulation of numerous lncRNAs contributes to the high aggressiveness, uncontrolled growth, and early metastatic potential of CRC [16-19]. These molecules are increasingly recognized as critical regulators of CRC initiation and progression [20, 21]. A deeper understanding of their regulatory mechanisms could therefore provide valuable insights for identifying novel therapeutic targets. In this study, we aimed to elucidate the specific role

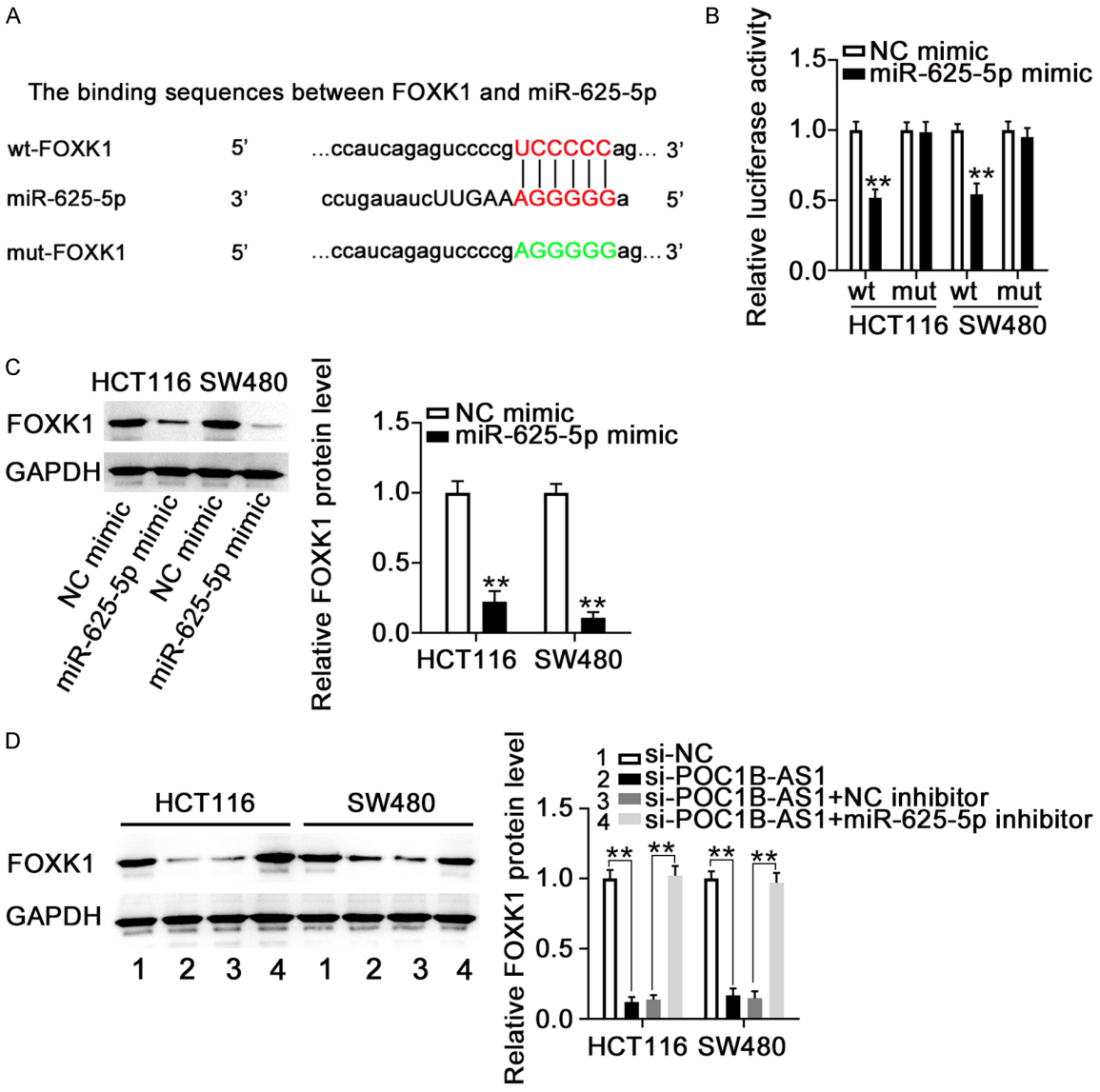

Figure 4. POC1B-AS1 controls FOXK1 expression in CRC cells by sequestering miR-625-5p. A. The predicted binding site of FOXK1 and miR-625-5p. B. Luciferase reporter assay was conducted in CRC cells after co-transfection of wt-FOXK1 or mut-FOXK1 and miR-625-5p mimic or NC mimic. C. FOXK1 protein expression in miR-625-5p-overexpressed CRC cells was measured by western blotting. D. POC1B-AS1-silenced CRC cells were further treated with miR-625-5p inhibitor or NC inhibitor, followed by the detection of FOXK1 protein expression. **P < 0.01. POC1B-AS1, POC1B antisense RNA 1; miR, microRNA; wt, wild-type; mut, mutant; FOXK1, forkhead box K1.

and mechanism of the lncRNA POC1B-AS1 in CRC pathogenesis.

Recent studies have increasingly linked the dysregulation of lncRNAs to CRC tumorigenesis. For instance, lncRNAs such as SPINT1-AS1 [20], FAM201A [22], and SNHG16 [23] are overexpressed in CRC and have been shown to promote malignant behavior. Conversely, other lncRNAs including GAS5 [24], CKMT2-AS1 [25], and MIR4435-2HG [26] are downregulated in CRC, where their loss of function contributes to disease progression. In line with these findings, our study identified POC1B-AS1 as a highly expressed lncRNA in CRC. Functional experiments demonstrated that POC1B-AS1 depletion attenuated CRC cell proliferation, colony formation, migration, and invasion. Moreover, inhibition of POC1B-AS suppressed tumor growth *in vivo*. Collectively, these findings

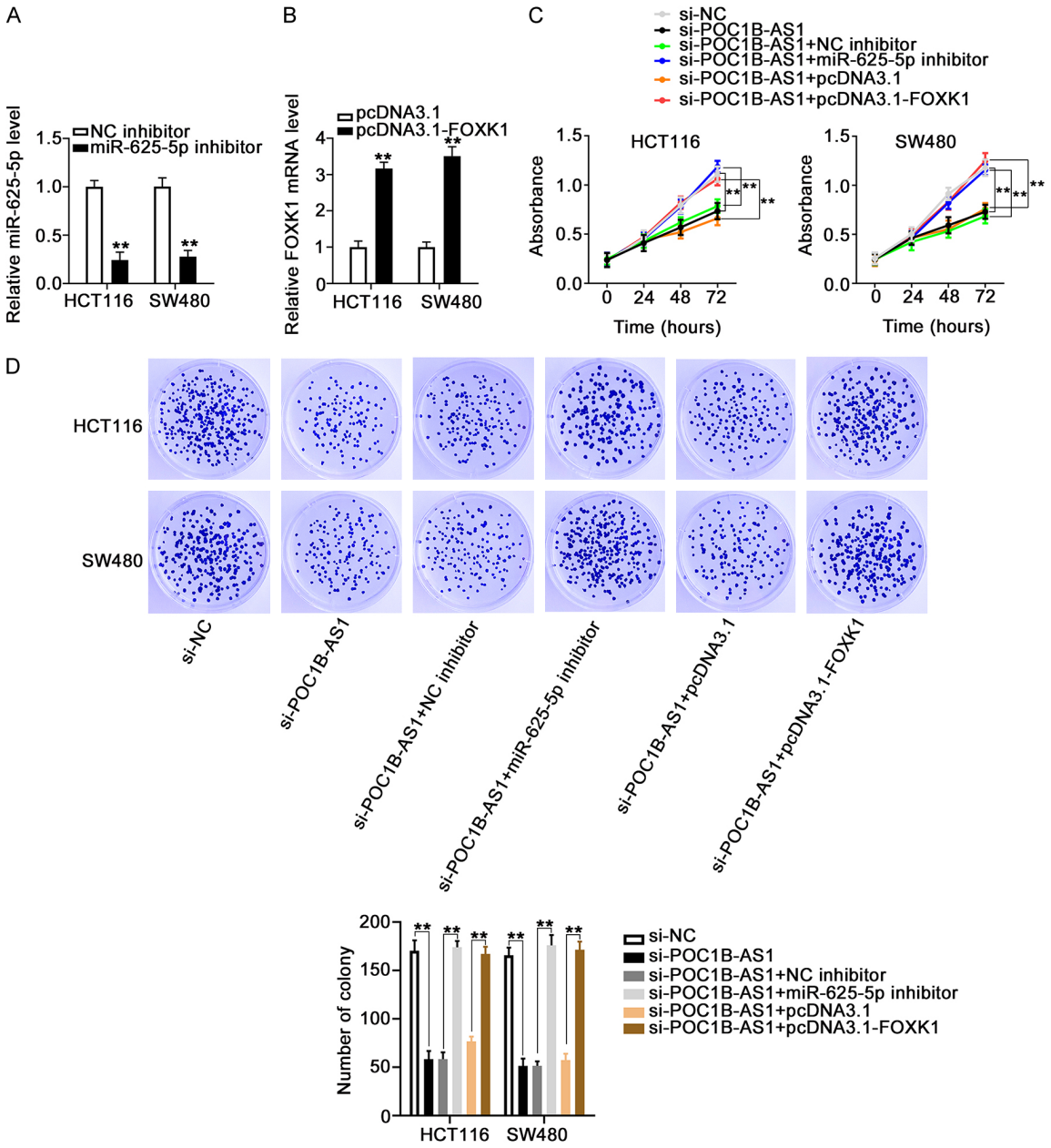

Figure 5. POC1B-AS1 affects the growth of CRC cells *in vitro* by targeting miR-625-5p/FOXK1 axis. A. The transfection efficiency of miR-625-5p inhibitor in CRC cells. B. The transfection efficiency of pcDNA3.1-FOXK1 in CRC cells. C, D. POC1B-AS1-silenced CRC cells were cotransfected with miR-625-5p inhibitor or pcDNA3.1-FOXK1. After transfection, cell proliferation and colony formation was analysed by CCK-8 and colony formation assays. **P < 0.01. POC1B-AS1, POC1B antisense RNA 1; miR, microRNA; NC, negative control; FOXK1, forkhead box K1; miR, microRNA.

advance our understanding of the complex molecular mechanisms underlying CRC initiation and progression.

The regulatory mechanisms of lncRNAs are closely associated with their subcellular localization [27]. LncRNAs typically modulate gene expression through direct interactions with proteins or chromatin [28]. In contrast, cytoplasmic lncRNAs often function as ceRNAs, a widely recognized mechanism for their post-transcriptional regulation [29]. These ceRNAs can sequester specific miRNAs by binding to miRNA recognition elements, thereby preventing the

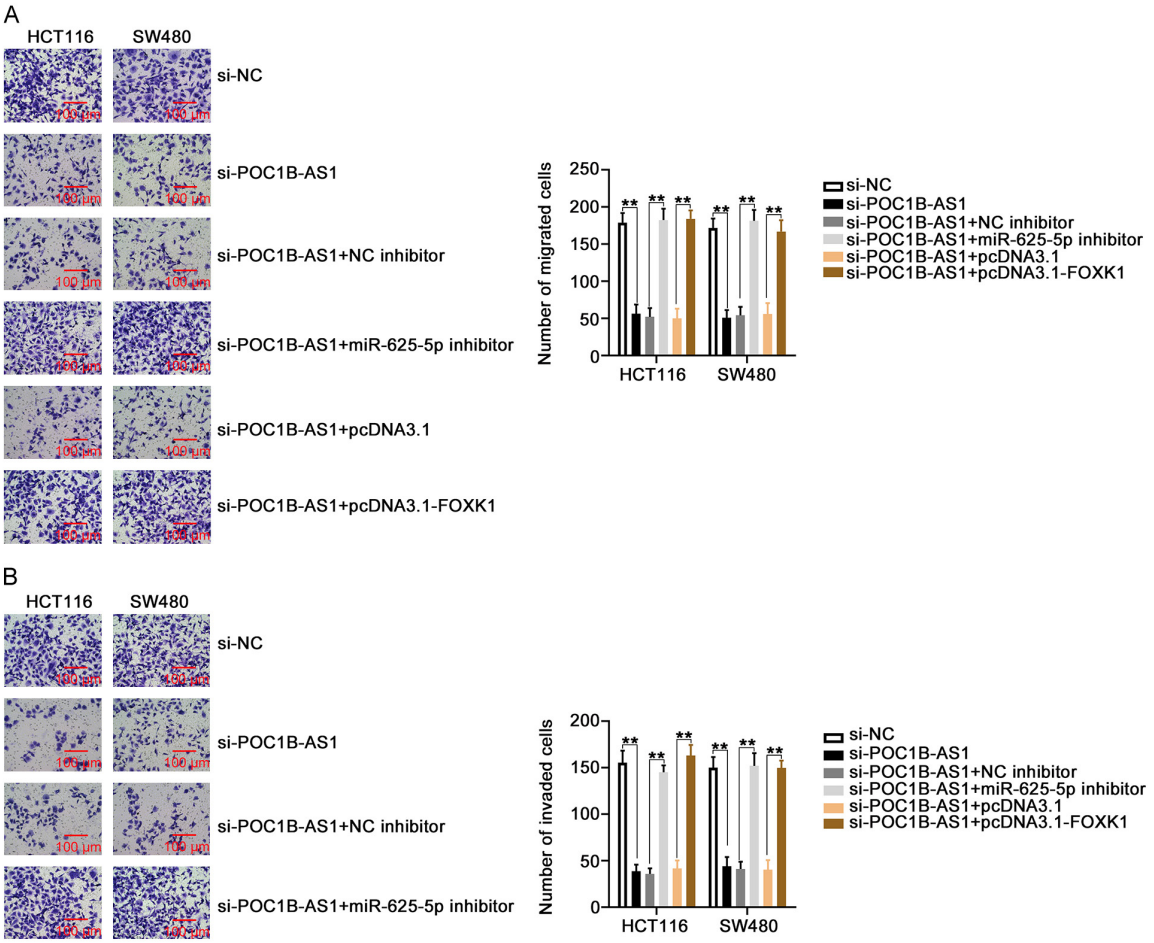

Figure 6. POC1B-AS1 regulates the motility of CRC cells by targeting miR-625-5p/FOXK1 axis. A, B. The motility of abovementioned cells was determined by Transwell migration and invasion experiments. **P < 0.01. POC1B-AS1, POC1B antisense RNA 1; miR, microRNA; NC, negative control; FOXK1, forkhead box K1; miR, microRNA.

miRNAs from repressing their target mRNAs. Given this context, we sought to determine the subcellular localization of POC1B-AS1 in CRC cells and to decipher its precise molecular mechanisms.

Based on predictions from lncLocator and experimental validation by subcellular fractionation, we identified POC1B-AS1 as a cytoplasmic lncRNA in CRC. This localization suggested that POC1B-AS1 may exert its tumor-promoting functions by acting as a ceRNA. To test this hypothesis, we performed bioinformatics analysis, which identified miR-625-5p as a binding partner of POC1B-AS1. The direct interaction between POC1B-AS1 and miR-625-5p was subsequently confirmed through luciferase reporter and RIP assays. Further investigation revealed FOXK1 as a direct target of miR-625-5p in CRC. We also demonstrated that POC1B-AS1 positively regulates FOXK1 expression, and that this regulation depends on its ability to sequester miR-625-5p. Collectively, these results establish a novel ceRNA network in CRC, in which POC1B-AS1 upregulates FOXK1 by competitively binding to miR-625-5p.

The dysregulation of miR-625-5p has been documented in various human cancers. In the context of CRC, its downregulation has been associated with lymph node metastasis and liver metastasis [30]. Additionally, a low miR-625-5p level has been identified as an indicator of unfavorable prognosis [30]. Functionally,

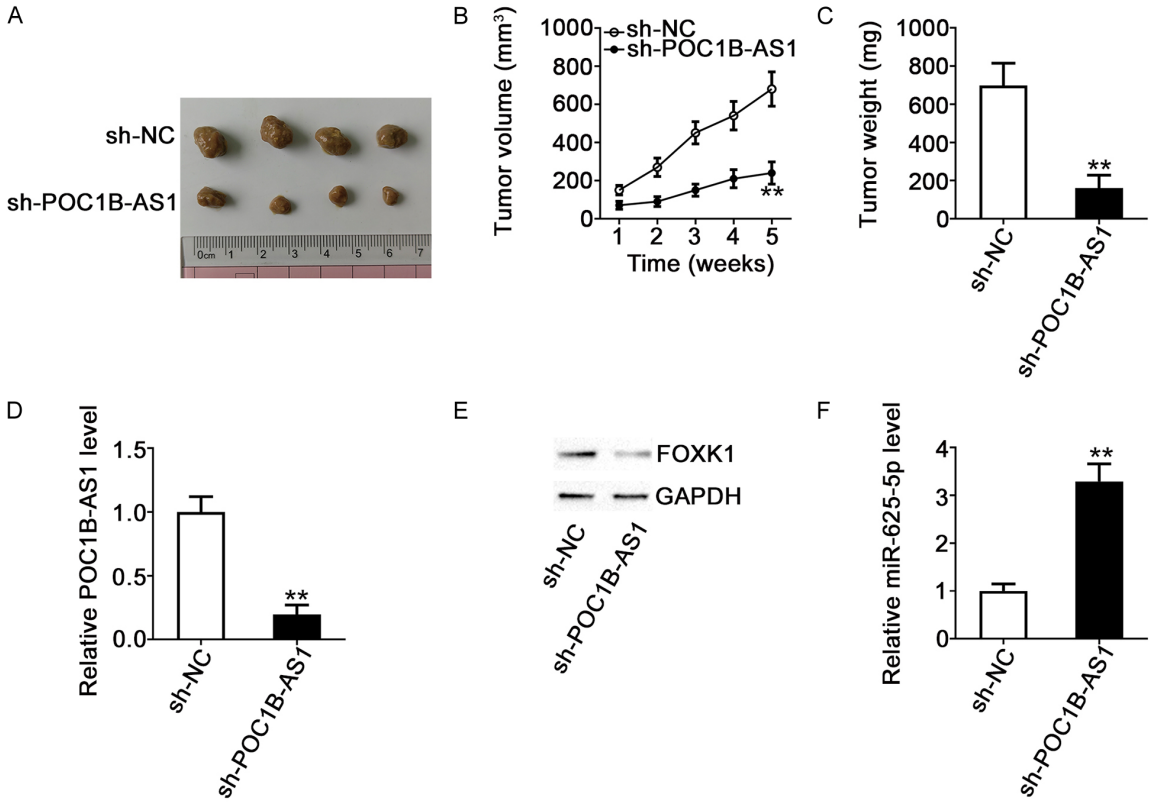

Figure 7. POC1B-AS1 knockdown suppresses tumor growth *in vivo*. A. Representative photographs of tumor xenografts. B. Growth curve of tumor xenografts. C. The weight of tumor xenografts. D-F. POC1B-AS1, FOXK1 and miR-625-5p levels in tumor xenografts. **P < 0.01. POC1B-AS1, POC1B antisense RNA 1; NC, negative control; FOXK1, forkhead box K1; sh, short-hairpin RNA.

miR-625-5p has been shown to suppress tumorigenic processes in CRC [30-32]. FOXK1, a member of the forkhead family of transcription factors, is known to promote oncogenesis by regulating multiple aggressive cancer phenotypes [14, 15, 33]. Our experimental data further substantiate this regulatory axis, as the anti-tumor effects resulting from POC1B-AS1 knockdown were significantly attenuated by either miR-625-5p inhibition or FOXK1 overexpression. These rescue experiments confirm that the miR-625-5p/FOXK1 axis serves as a critical downstream effector pathway through which POC1B-AS1 facilitates CRC progression.

This study has certain limitations. First, while we have delineated the POC1B-AS1/miR-625-5p/FOXK1 regulatory axis, the specific functional roles of miR-625-5p in CRC were not independently investigated. Second, our experiments focused on the effects of POC1B-AS1 on proliferation, colony formation, migration, and invasion; other malignant behaviors such as immune evasion, anti-apoptotic capacity, altered energy metabolism, and angiogenesis remain to be explored. Addressing these aspects will be an important focus of our future research.

Conclusions

POC1B-AS1 is upregulated in CRC and functions as a tumor promoter. Mechanistically, POC1B-AS1 promotes CRC progression by acting as a molecular sponge for miR-625-5p, which leads to the subsequent upregulation of FOXK1. Our findings suggest that targeting the POC1B-AS1/miR-625-5p/FOXK1 axis may represent a promising therapeutic strategy for colorectal cancer.

Disclosure of conflict of interest

None.

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