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ORIGINAL ARTICLE

Long noncoding RNA plasmacytoma variant translocation 1 promotes progression of colorectal cancer by sponging microRNA-152-3p and regulating E2F3/MAPK8 signaling

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Abstract

The purpose of this study was to investigate the pathogenesis of colorectal cancer (CRC) and the effects of the long noncoding RNA plasmacytoma variant translocation 1 (PVT1) on CRC progression. Bioinformatics analysis verified PVT1 expression in tumor and normal tissues. Quantitative PCR and western blotting were used to measure mRNA and protein levels, respectively. The MTT, Transwell, colony formation, and in vivo assays were used to assess the effects of PVT1 on proliferation, migration, and invasion by CRC cells. Both PVT1 and microRNA (miR)-152-3p were shown to be colocalized in CRC cells using FISH assay. The target genes of miR-152-3p were predicted and verified by bioinformatics analysis, luciferase assay, and RNA pull-down assay. The ChIP assay revealed that E2F3 binds with the promoter of MAPK8. We found that PVT1 was overexpressed in CRC specimens, and its expression was higher in CRC cells than normal intestinal cells. Overexpression of PVT1 enhanced the proliferation, migration, and invasion of CRC cells, whereas PVT1 knockdown inhibited these processes. MicroRNA-152-3p was a target of PVT1, and E2F3 was a target of miR-152-3p. Rescue experiments confirmed the interaction between miR-152-3p and PVT1 and between miR-152-3p and E2F3. Luciferase and ChIP assay results confirmed that E2F3 modulates the transcriptional activation of MAPK8. Long noncoding RNA PVT1 activated E2F3 signaling by sponging miR-152-3p. The PVT1/miR-152-3p/E2F3/MAPK8 axis promoted CRC progression.

KEYWORDS
colorectal cancer, E2F3, lncRNA PVT1, miR-152-3p

1 | INTRODUCTION

Colorectal cancer (CRC) is a common malignant tumor.[1] In recent decades, diagnosis and treatment technology has made great progress; the 5-year survival rate for CRC in the localized stage is now 90.1%. However, only 39% of CRC cases are detected in the localized stage,[2,3] and the 5-year survival rate in distant metastasis cases is 69.2%. Therefore, recent research has focused on the pathogenesis of CRC and more effective strategies for the early diagnosis and treatment of CRC.[4-6]

Long noncoding RNAs (lncRNAs) are a family of noncoding RNAs with a length over 200 nt in the nucleus or cytoplasm. Long noncoding RNA plasmacytoma variant translocation 1 (PVT1) is a cancerous lncRNA with a transcript length of 1957 bases. Many studies have shown that PVT1 is overexpressed in malignant tumors in diseases such as non-small-cell lung cancer,[7] hepatocellular carcinoma,[8] gastric cancer,[9] CRC,[10] pancreatic cancer,[11] cervical cancer,[12] and ovarian cancer.[13] Thus, abnormal levels of PVT1 are closely associated with the progression of cancers, including CRC.[14,15] However, the role of PVT1 in CRC has not been fully elucidated.

MicroRNAs (miRNAs) are a class of single-stranded small RNAs that participate in the development of tumors by modulating biological processes such as tumor cell proliferation, invasion, and metastasis.[16] MicroRNA (miR)-152-3p is a member of the miR-148/152 cluster, which is involved in cellular activities such as proliferation, invasion, and angiogenesis.[17,18] MicroRNA-152 exerts a tumor-suppressive effect and is dysregulated in various malignant tumors. E2F transcriptional regulators regulate cell proliferation, differentiation, and apoptosis-related gene transcription.[19,20] E2F3, an important member of the E2F family, plays an important role in regulating cell proliferation, apoptosis, and differentiation.

In this study, we found that PVT1 is involved in the malignant progression of CRC. It promoted the proliferation, invasion, and migration of CRC cells by downregulating miR-152-3p. Additionally, E2F3 was a target of miR-152-3p. Moreover, E2F3 modulated the transcriptional activation of MAPK8. In short, PVT1 promoted CRC progression through the miR-152-3p/E2F3/MAPK8 signaling axis and thus could be used as a biomarker for CRC.

2 | MATERIALS AND METHODS

2.1 | Specimens

Twenty-five pairs of tumor tissues and adjacent normal tissues were collected from CRC patients hospitalized at the Affiliated Hospital of Hebei University of Engineering and stored at −80°C. Ten patients were in stage II, nine were in stage III, and six were in stage IV. Not all patients underwent chemotherapy or radiotherapy before the study. This study was approved by the Ethics Committee of the Affiliated Hospital of Hebei University of Engineering, and all participants provided signed informed consent. All procedures were carried out in accordance with the Declaration of Helsinki.

2.2 | Cell cultures

Human CRC cell lines SW620, SW480, LOVO, HCT116, HT-29, DLD-1, and M5 and normal intestinal epithelial cells (HFC) were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. HCT116, LOVO, and SW620 cells were incubated with McCoy's 5A, F12k, and L15 media, respectively, each containing 10% FBS. HT-29, SW480, M5, and HFC were cultured with DMEM (Gibco) supplemented with 10% FBS (Gibco) and 100 U/mL penicillin-streptomycin (Beyotime) at 37°C in a humidified atmosphere with 5% CO_2. DLD1 cells were incubated in RPMI-1640 medium containing 10% FBS.

2.3 | Cell transfection

An miR-152-3p mimic (forward [F], 5′-UCAGUGCAACUGACAGAACUUGG-3′; reverse [R], 5′-UAGCCACGGUUGUGUAAAGUCUG-3′), an miR-152-3p inhibitor (F, 5′-CGCGCUAGCAGCACGUAAAU-3′; R, 5-GUGCAGGGUCCGAGGUCAUC-3′), and their negative controls (F, 5′-CAGUACUUUUGUGUAGUACAA-3′; R, 5′-CAGUACUUUUGUGUAGUACAA-3′), as well as a si-E2F3 (GCTTCCAAAGACTTGGCTT) were obtained from GenePharma. The pSilencer (shPVT1-1, 5′-GCUUGGAGGCUGAGGAGUUTT-3′; shPVT1-2, 5′-CCCAACAGGAGGACAGCUUTT-3′) and pcDNA3.1 plasmids for silencing or overexpressing PVT1 were obtained from General Biocompany. The antagomir-152-3p was purchased from RiboBio. Colorectal cancer cells were seeded into a 24-well plate. Twenty-four hours later, the cells were transfected with mimic, inhibitor, negative controls, or specific plasmids for 48 hours using Lipofectamine 2000 following the manufacturer's instructions.

2.4 | Cell viability assay

After 48 hours of transfection, 1×10^4 cells were seeded into a 96-well plate and incubated at room temperature. At 12, 24, 48, and 72 hours, the culture medium was replaced with new medium containing 10% MTT solution. After 4 hours, 100 μL DMSO was added into each well. The absorbance of each well was measured with a microplate reader (Sunrise Tecan's Magellan 2) at a wavelength of 450 nm, and the cell viability curve was drawn.

2.5 | Wound healing assay

After transfection, cells were seeded into a 24-well plate (2×10^5 cells/mL) and cultured in an incubator at 37°C in 5% CO_2. A cultured cell scratch model was established by using a sterile pipette tip. Cells were then rinsed with D-Hank's solution to remove the falling cells, and serum-free culture medium was added. Subsequently, cells were observed under a microscope and imaged (Leica).

2.6 | Transwell cell migration assay

First, the upper chamber was precoated with Matrigel (BD). Transfected SW620 and LOVO cells were seeded into the upper chamber (Millipore) and cultured in 200 μL serum-free medium. Medium containing 12% FBS was added to the lower chamber. Twenty-four hours later, cells that had invaded the lower chamber

were fixed with methanol and stained with 0.1% crystal violet at room temperature for 20 minutes. Finally, the stained cells were observed and imaged under an optical microscope (Nikon).

2.7 | Colony formation experiment

After transfection, the SW620 and LOVO cells (2×10^2 cells/well) were seeded into a 12-well plate and cultured with medium containing 10% FBS. The cells were cultured at 37°C in an atmosphere humidified with 5% CO_2 for 2 weeks. Finally, the cell colonies were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet at room temperature for 15 minutes. The colonies were imaged and counted using ImageJ software (Bio-Rad).

2.8 | Quantitative real-time PCR

After extracting total RNA with TRIzol reagent (Invitrogen), 5 μg RNA was reverse transcribed into cDNA using a reverse transcription kit (Takara) according to the manufacturer's instructions. Quantitative real-time PCR (qPCR) was carried out in a 7500 Fast Real-Time PCR System (ABI). Reaction conditions were as follows: predenaturation at 96°C for 5 minutes, denaturation at 96°C for 30 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 30 seconds (30 cycles), and finally, extension at 72°C for 10 minutes and stored at 4°C. The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method. GAPDH and U6 were used as the internal controls for the mRNA and miRNAs, respectively. The sequences of the primers used were as follows: PVT1 F, 5′-TGAGAACTGTCCTTACGTGACC-3′ and R, 5′-AGAGCACCAAGACTGGCTCT-3′; miR-152-3p F, 5′-ACACTCCAGCTGGGTCAGTGCATGACAG-3′ and R, 5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCAAGTT-3′; E2F3 F, 5′-GTATGATACGTCTCTTGGTCTGC-3′ and R, 5′-CAAATCCAATACCCCATCGGG-3′; MAPK8 F, 5′-TGAGAAGGGTGACTGCATCG-3′ and R, 5′-ACCAAACCATTGACACCGAAG-3′; U6 F, 5′-AAAGCAAATCATCGGACGACC-3′ and R, 5′-GTACAACACATTGTTTCCTCGGA-3′; and GAPDH F, 5′-AGCCACATCGCTCAGACAC-3′ and R, 5′-GCCCAATACGACCAAATCC-3′.

2.9 | Western blot analysis

After transfection, cells were washed with cold PBS and collected. We used RIPA cell lysate to lyse the cells and extract the total protein. The Bradford method was applied to evaluate the amount of protein. Then 40 μg protein was separated with 12% SDS-PAGE. After electrophoresis, the protein was transferred to PVDF membranes by electrotransfer. The membranes were then blocked with skim milk at room temperature for 2 hours. Subsequently, the membranes were incubated with primary Abs overnight at 4°C, then the blots were incubated with the goat anti-rabbit secondary Ab at room temperature for 2 hours. Finally, the membranes were visualized using an ECL fluorescence detection kit (Beyotime) and analyzed with ImageJ software (Bio-Rad).

2.10 | Dual luciferase reporter assay

Bioinformatics software was used to predict the target genes of miR-152-3p. The WT and mutant sequence containing the binding sites of miR-152-3p were synthesized and cloned into the pGL3.1 reporter plasmid. The WT and mutant plasmids, along with the miR-152-3p mimic and its negative control, were transfected into CRC cells. After 48 hours, a microplate reader was used to detect firefly and *Renilla* luciferase activity. The ratio of firefly fluorescence was normalized to *Renilla*.

2.11 | RNA pull-down

A biotin-labeled miR-152-3p probe and a negative control were obtained from Sangon Biotech. Colorectal cancer cells were transfected with specific probes; 48 hours later, CRC cells were harvested and lysed. The lysates were then incubated with precoated beads overnight. RNA enrichment was evaluated by qPCR assay.

2.12 | Fluorescence in situ hybridization

Cy3-labeled miR-152-3p and FAM-labeled PVT1 probes were obtained from GenePharma. Signals from the cy3-labeled miR-152-3p and FAM-labeled PVT1 probes were detected using an in situ hybridization kit (RiboBio) according to the manufacturer's instructions. Cell nuclei were then stained with DAPI for 5 minutes at room temperature. Finally, the images were photographed with a confocal microscope (Leica).

2.13 | Xenograft model

Six-week-old male nude mice (Beijing Vital River Laboratory Animal Technology) weighing 20-25 g were used for the in vivo study. Each mouse was injected subcutaneously with 5×10^6 CRC cells. Tumor size was measured every 3 days (volume = length (mm) × width $(mm)^2 \times 0.5$). At the end of the experiment, the mice were killed, and the tumors were removed and weighed.

2.14 | Statistical analysis

The statistical analysis was undertaken using SPSS 21.0 software. Data were expressed as mean ± SD. Student's *t* test was used for comparison between two groups, and ANOVA was used for comparison among multiple groups. $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | High expression of PVT1 in CRC

To study the biological role of lncRNA in CRC, we first screened the dysregulated lncRNAs in CRC. Bioinformatics analysis based on

the GEO database showed that PVT1 was highly expressed in CRC tissues (Figure 1A,B). We used a qPCR assay to verify this result. As shown in Figure 1C, PVT1 was significantly upregulated in CRC tissues. Moreover, PVT1 was significantly upregulated in CRC cells compared to intestinal epithelial cells, which were more potent in LOVO and SW620 (Figure 1D). Therefore, these two types of cells were chosen for the further research.

3.2 | Plasmacytoma variant translocation 1 promotes malignant behavior of CRC cells

To study the impacts of PVT1 on CRC progression, we investigated the effects of PVT1 on the proliferation, invasion, and migration of CRC cells. The expression level of PVT1 was significantly increased after transfection with PVT1 overexpression plasmids and reduced by PVT1 knockdown plasmids, indicating that cells were successfully transfected (Figure 2A). The proliferation of CRC cells was inhibited by knockdown of PVT1 but promoted by PVT1 overexpression (Figure 2B,C). Moreover, overexpression of PVT1 promoted the migration and invasion of CRC cells, whereas knockdown of PVT1 had the opposite effect (Figure 2D,E).

3.3 | Plasmacytoma variant translocation 1 sponges miR-152-3p in CRC cells

A bioinformatics analysis showed the binding site between miR-152-3p and PVT1 (Figure 3A). Luciferase assay revealed that miR-152-3p could bind with PVT1 directly (Figure 3B). RNA pull-down confirmed that PVT1 could bind with miR-152-3p (Figure 3C). Pearson analysis showed that the expression of PVT1 was negatively correlated with that of miR-152-3p (Figure 3D). Fluorescence in situ hybridization showed that PVT1 and miR-152-3p were colocalized in CRC cells (Figure 3E).

3.4 | MicroRNA-152-3p reversed effects of PVT1 on malignant behavior of CRC cells

To further study the relationship between PVT1 and miR-152-3p, we undertook rescue experiments. The results showed that overexpression of PVT1 significantly reduced the level of miR-152-3p, whereas the miR-152-3p mimic transfection notably enhanced the level of miR-152-3p (Figure 4A). The PVT1-induced CRC cell proliferation was also reversed by miR-152-3p (Figure 4B,C). These findings were consistent with the results from wound healing and Transwell assays. As shown in Figure 4D,E, miR-152-3p antagonized the effects of PVT1 on the migration and invasion of CRC cells.

3.5 | MicroRNA-152-3p directly targets E2F3 in CRC cells

To elucidate the mechanism underlying miR-152-3p, we predicted the potential targets of miR-152-3p using TargetScan, DIANA, and miRWalk. As illustrated in Figure 5A, all three software programs suggested 440 genes as the potential targets of miR-152-3p. The DAVID software was then used to carry out enrichment analysis (Figure 5B). E2F3 was reported to be involved in both cell proliferation and transcriptional modulation. We therefore investigated the role of E2F3. Figure 5C shows the target region between E2F3 and

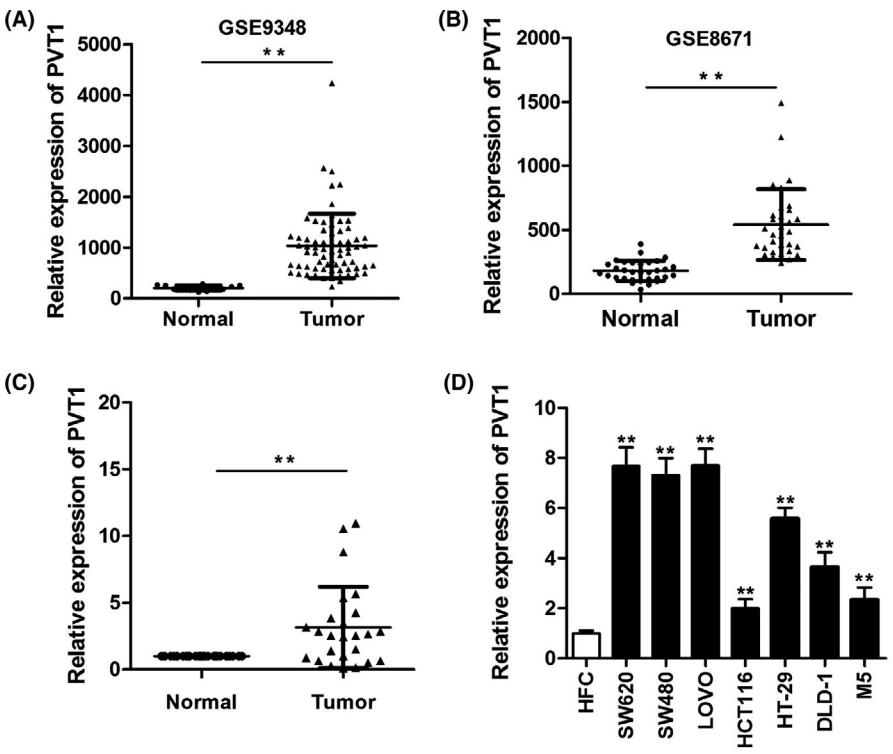

FIGURE 1 Long noncoding RNA plasmacytoma variant translocation 1 (PVT1) is highly expressed in colorectal cancer (CRC). A, B, Bioinformatics analysis based on the GEO database was carried out to evaluate the dysregulated expression of PVT1 in CRC. C, D, Quantitative real-time PCR was used to detect the expression of PVT1 in normal tissues, CRC tissues, intestinal epithelial cells, and CRC cell lines. $^{**}P < .01$

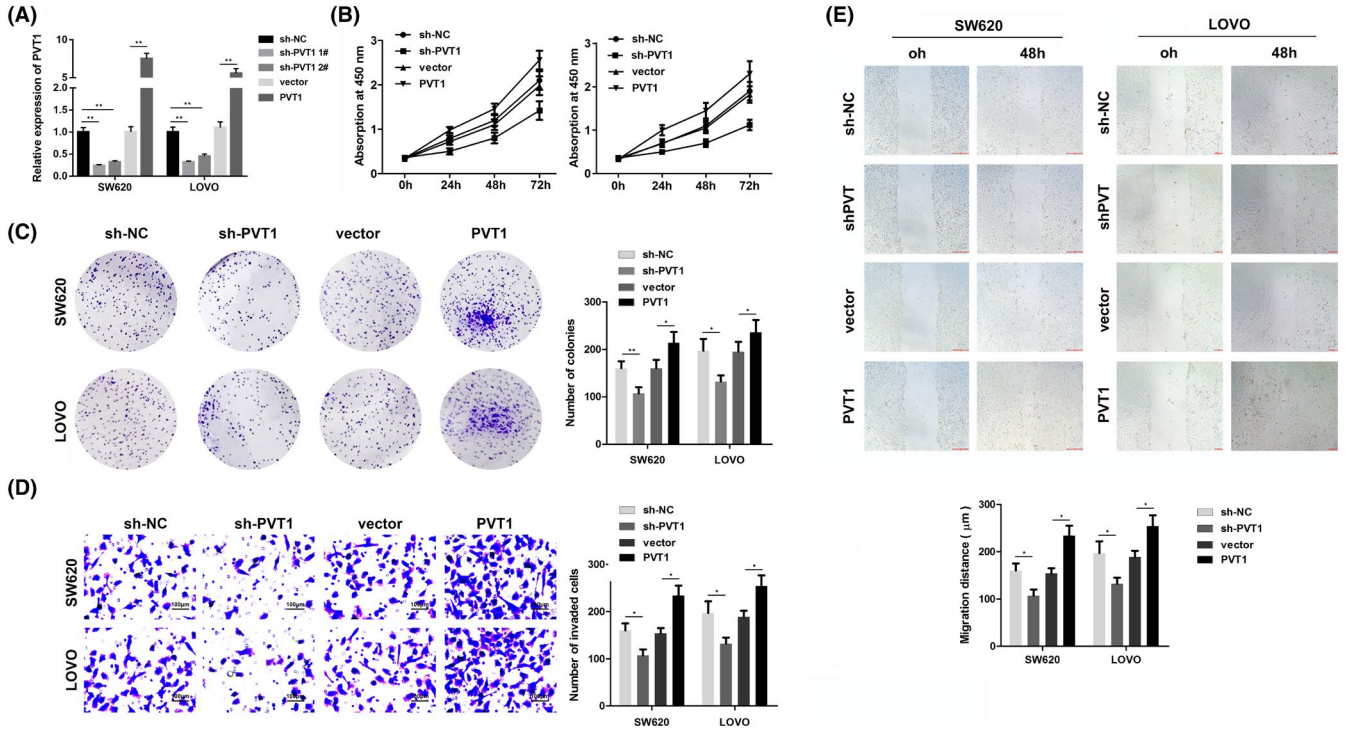

FIGURE 2 Plasmacytoma variant translocation 1 (PVT1) promoted the malignant behavior of colorectal cancer (CRC). pSilencer/sh-PVT1 and pcDNA3.1/PVT1 were transfected to knockdown or overexpressing PVT1, respectively. A, Quantitative real-time PCR was used to assess PVT1 expression. B, C, MTT and colony formation assays were used to detect the proliferation of CRC cells after overexpression or knockdown of PVT1. D, Transwell assay was used to evaluate the invasion of CRC cells. E, Cell migration was investigated using a wound healing assay. $^{*}P < .05$, $^{**}P < .01$. NC, negative control

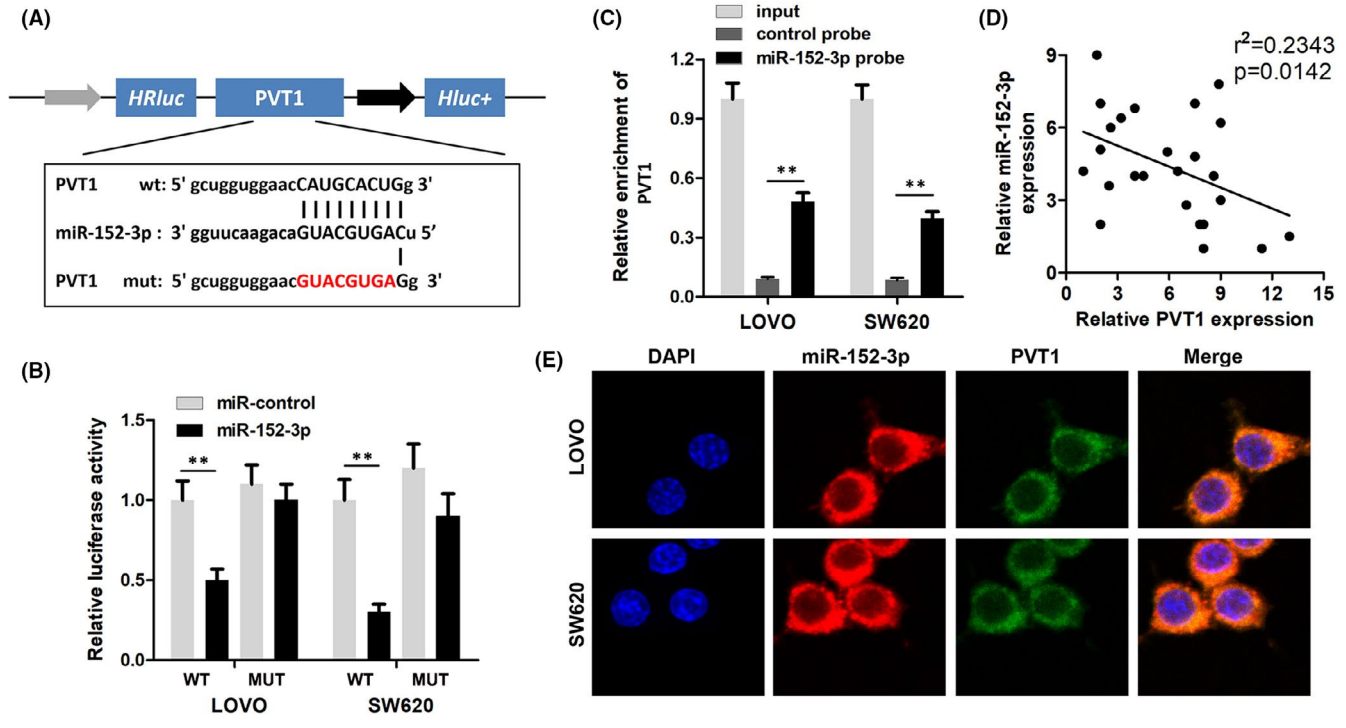

FIGURE 3 Plasmacytoma variant translocation 1 (PVT1) sponges microRNA (miR)-152-3p in colorectal cancer cells. A, Bioinformatics analysis showed the binding site between PVT1 and miR-152-3p. B, Luciferase assay verified the combination of PVT1 and miR-152-3p. C, RNA pull-down confirmed the interaction between PVT1 and miR-152-3p. D, Pearson analysis evaluated the correlation between PVT1 and miR-152-3p. E, FISH detected the colocalization of miR-152-3p and PVT1. $^{**}P < .01$

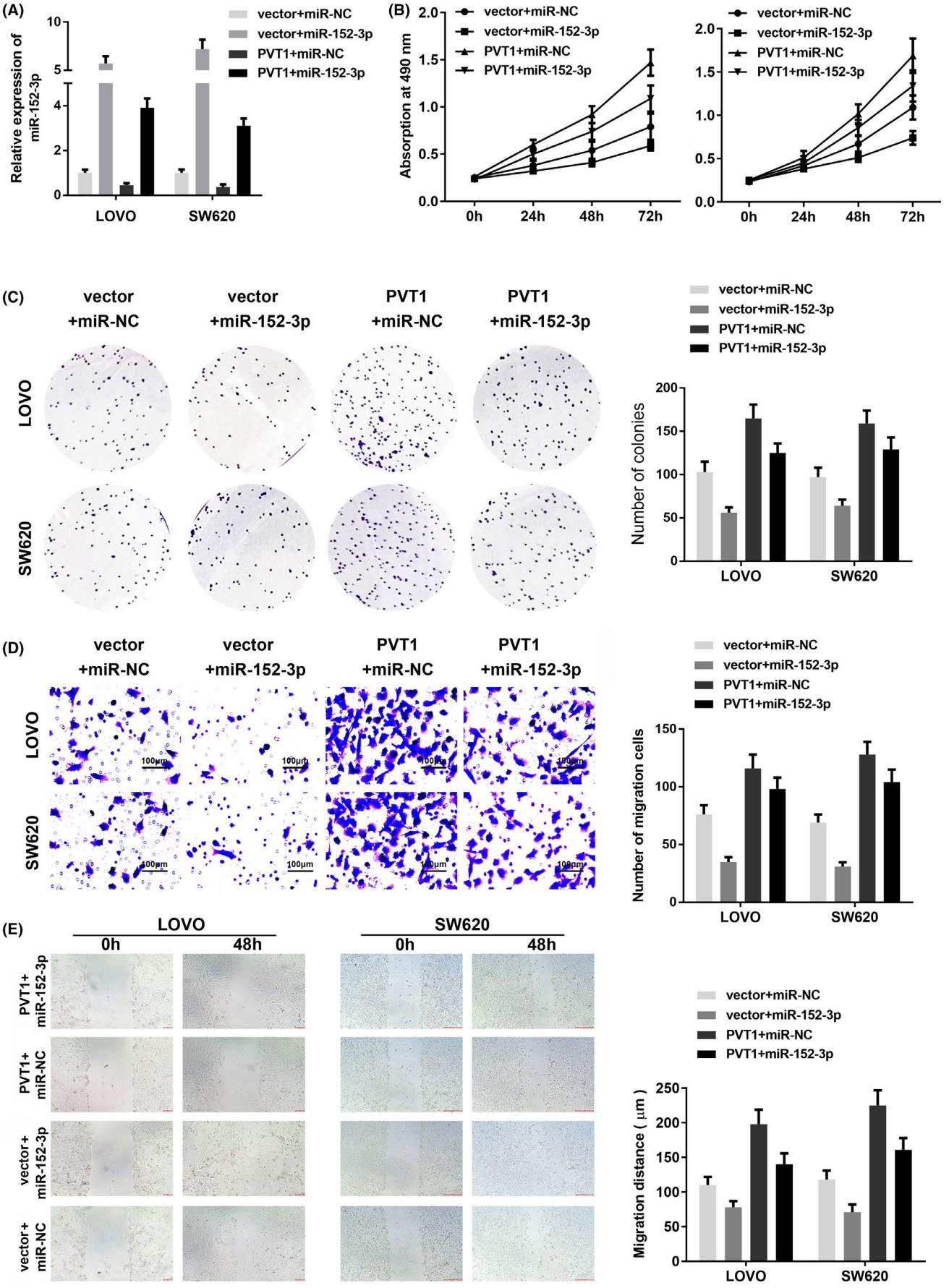
(A)
Relative expression of miR-152-3p
vector+miR-NC
vector+miR-152-3p
PVT1+miR-NC
PVT1+miR-152-3p
LOVO
SW620
(B)
Absorption at 490 nm
0h
24h
48h
72h
(C)
vector +miR-NC
vector +miR-152-3p
PVT1 +miR-NC
PVT1 +miR-152-3p
Number of colonies
(D)
Number of migration cells
(E)
0h
48h
Migration distance (μm)

FIGURE 4 MicroRNA (miR)-152-3p reversed the effect of plasmacytoma variant translocation 1 (PVT1) in colorectal cancer (CRC) cells. CRC cells were transfected with pcDNA3.1/ PVT1, miR-152-3p mimics, or their combination. A, miR-152-3p expression was detected by quantitative real-time PCR. B, C, MTT and colony formation assays were used to detect the proliferation of CRC cells. D, Transwell assay was used to evaluate the invasion of CRC cells. E, Cell migration was investigated using a wound healing assay. $^{**}P < .01$. NC, negative control

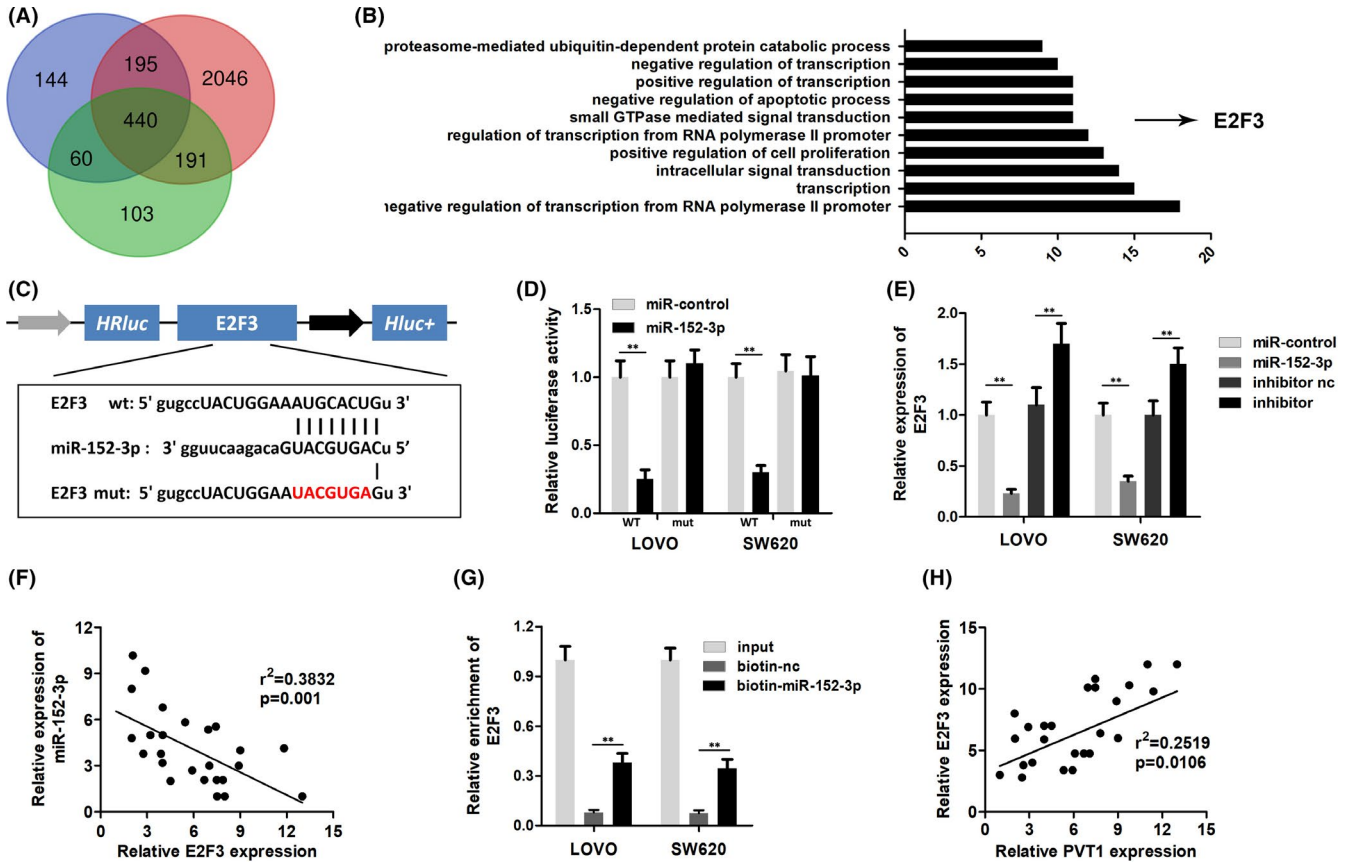

FIGURE 5 MicroRNA (miR)-152-3p targets E2F3 in colorectal cancer cells. A, Bioinformatics prediction based on the TargetScan, DIANA, and miRWalk databases screened the targets of miR-152-3p. B, Enrichment analysis of the 440 potential target genes was carried out using DAVID software. C, Binding site between miR-152-3p and E2F3. D, Luciferase assay verified the combination of miR-152-3p and E2F3. E, Pearson analysis was conducted to detect the correlation between PVT1 and miR-52-3p. F, Pearson analysis evaluated the correlation between miR-152-3p and E2F3. G, RNA pull-down confirmed the interaction between miR-152-3p and E2F3. H, Quantitative real-time PCR assessed the E2F3 expression in each group. $^{**}P < .01$. NC, negative control

miR-152-3p. As the luciferase assay indicated, overexpression of miR-152-3p reduced the luciferase activity of the reporter carrying WT E2F3 3′-UTR ($P < .05$) (Figure 5D). In addition, overexpression of miR-152-3p significantly decreased the level of E2F3, whereas knockdown of miR-152-3p exerted the opposite effect (Figure 5E). Pearson analysis showed that miR-152-3p was negatively correlated with E2F3 expression (Figure 5F). RNA pull-down of LOVO and SW620 cells showed that there was a positive interaction between miR-152-3p and E2F3 (Figure 5G). In addition, the expression of PVT1 was positively correlated with E2F3 (Figure 5H).

3.6 | Knockdown of E2F3 reversed the effect of PVT1

The expression of E2F3 was significantly increased by PVT1 overexpression but decreased by E2F3 knockdown (Figure 6A). Plasmacytoma variant translocation 1 promoted the proliferation of CRC cells, whereas knockdown of E2F3 could reverse the effect of PVT1 (Figure 6B,C). The results of cell invasion and migration experiments showed that PVT1 promoted CRC cell invasion and migration, whereas knockdown of E2F3 could reverse the effect of PVT1 on CRC cell invasion and migration (Figure 6D,E).

3.7 | Knockdown of PVT1 inhibits growth of CRC cells

To study the biological effects of PVT1, we carried out an in vivo study. The findings of animal experiments showed that silencing of PVT1 reduced tumor volume and weight. Silencing of miR-152-3p significantly reversed the antitumor effect of PVT1 knockdown (Figure 7A-C). qPCR was used to detect the expression of PVT1 and

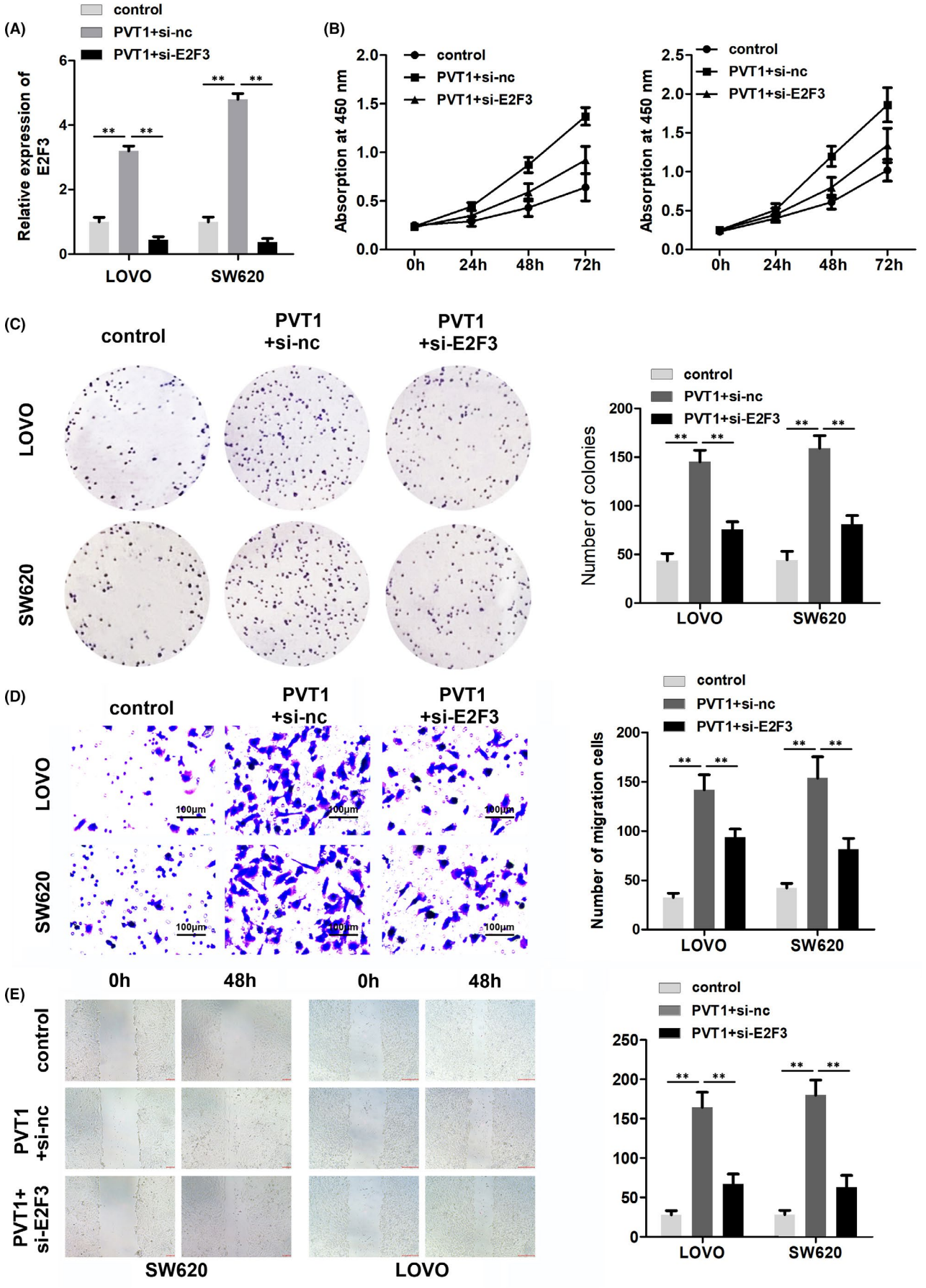
(A)
control
PVT1+si-nc
PVT1+si-E2F3
Relative expression of E2F3
LOVO
SW620
(B)
Absorption at 450 nm
0h
24h
48h
72h
(C)
control
PVT1 +si-nc
PVT1 +si-E2F3
LOVO
SW620
Number of colonies
(D)
Number of migration cells
100μm
(E)
0h
48h
PVT1+ si-E2F3

FIGURE 6 Knockdown of E2F3 reversed the effects of plasmacytoma variant translocation 1 (PVT1). Colorectal cancer (CRC) cells were transfected with pcDNA3.1/PVT1, si-E2F3, or their combination. A, Quantitative real-time PCR was used to detect E2F3 expression. B, C, MTT and colony formation assays were used to detect the proliferation of CRC cells. D, Transwell assay was carried out to evaluate the invasion of CRC cells. E, Cell migration was investigated using a wound healing assay. $^{**}P < .01$. NC, negative control

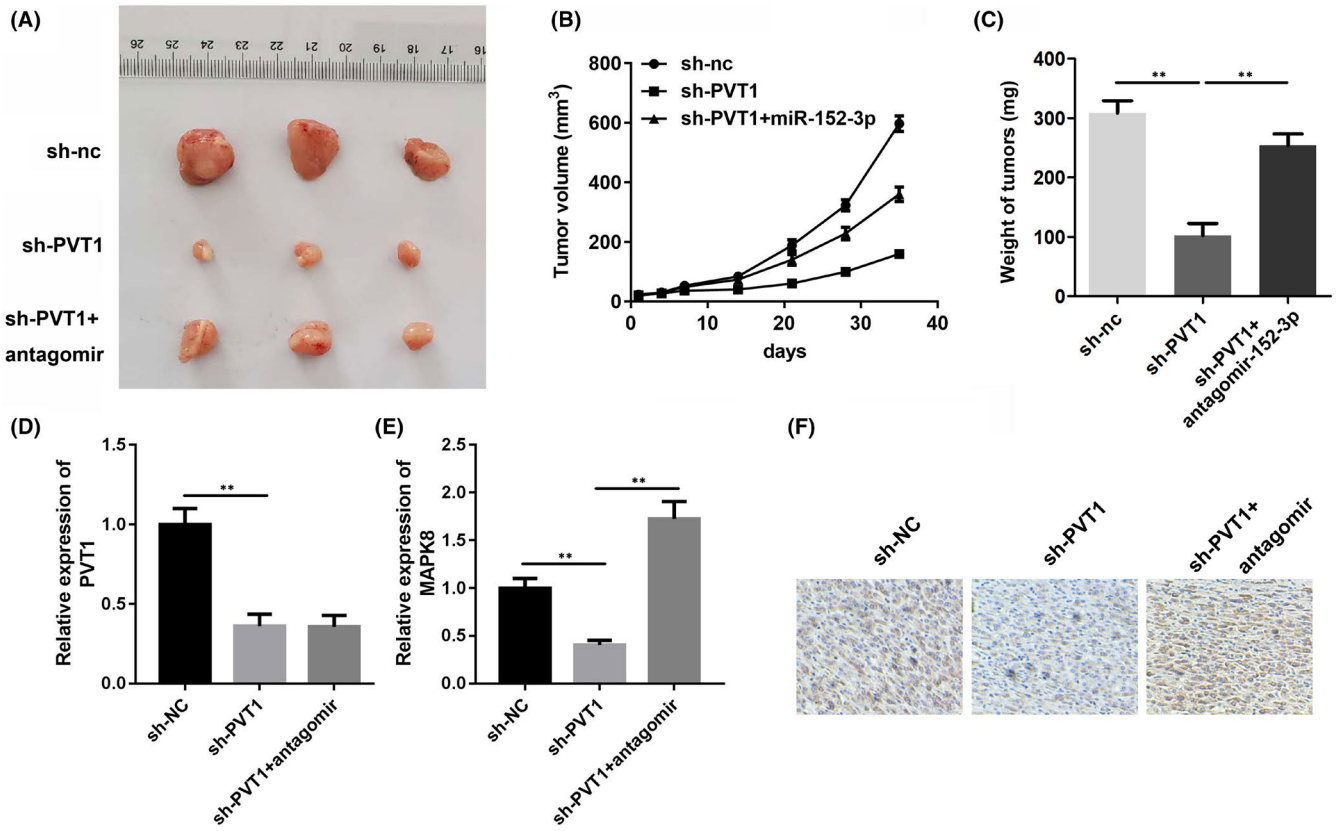

FIGURE 7 Long noncoding RNA plasmacytoma variant translocation 1 (PVT1) promoted tumor growth. SW620 cell line stably expressing pSilencer/sh-PVT1 was used to establish the xenograft mouse model. Antagomir-152-3p (80 mg/kg) was injected into the tail vein once a week. A, Image of tumors in each group. B, Growth curves of tumors were constructed. C, Weight of tumors in each group was detected. D, E, qPCR was performed to detect the expression of PVT1 and MAPK8 in the tumors. F, IHC was used to evaluate the expression of MAPK8 in the tumors. $^{**}P < .01$. NC, negative control

MAPK8 in the tumors. sh-PVT1 sigfinicantly inhibited the expression of PVT1 and MAPK8. Antagomir has no effect of the expression of PVT1 but notably elevated the expression level of MAPK8 (Figure 7D, E). Moreover, IHC was performed to evaluate the expression of MAPK8, sh-PVT1 attenuated the expression of PVT1 and antagomir elevated the expression level of MAPK8 (Figure 7F).

3.8 | Long noncoding RNA PVT1/miR-152-3p/E2F3 axis promotes CRC progression by regulating MAPK8

To study the role of the PVT1/miR-152-3p/E2F3 axis in CRC, we tested the expression of the downstream gene *MAPK8*. The experimental results showed that PVT1 and E2F3 promoted the transcriptional activation of MAPK8 (Figure 8A,B). Chromatin immunoprecipitation showed that E2F3 could bind with the promoter of the MAPK8 (Figure 8C). In addition, overexpression of PVT1 and E2F3 promoted the expression of MAPK8, while knockdown of PVT1 and E2F3 inhibited that (Figure 8D-F).

4 | DISCUSSION

Colorectal cancer is a common gastrointestinal tumor with high morbidity and mortality.[6,21] There has been limited development in lncRNA-targeted therapies. Some lncRNAs regulate the biological process of CRC, which in turn induces the pathogenesis of tumors. For instance, lncRNA MIR100HG, miR-100, and miR-125b mediate the resistance of CRC cells to cetuximab by inhibiting the activation of the Wnt/β-catenin pathway.[22] Long noncoding RNA CCAT1 functions as competing endogenous RNA (ceRNA) to promote the activation of ITPKB by sponging miR-410, inhibiting the proliferation of CRC cells.[23]

The purpose of this study was to explore the role of the PVT1/miR-152-3p axis in the malignant progression of CRC and the underlying mechanism. Takahashi et al[10] reported that PVT1 is highly expressed in CRC tissues and cell lines. Overexpression of PVT1 is closely related to CRC lymph node metastasis, vascular invasion, and tumor stages. Thus, PVT1 can be an independent influencing factor for CRC patients with a poor prognosis. In addition, downregulation of

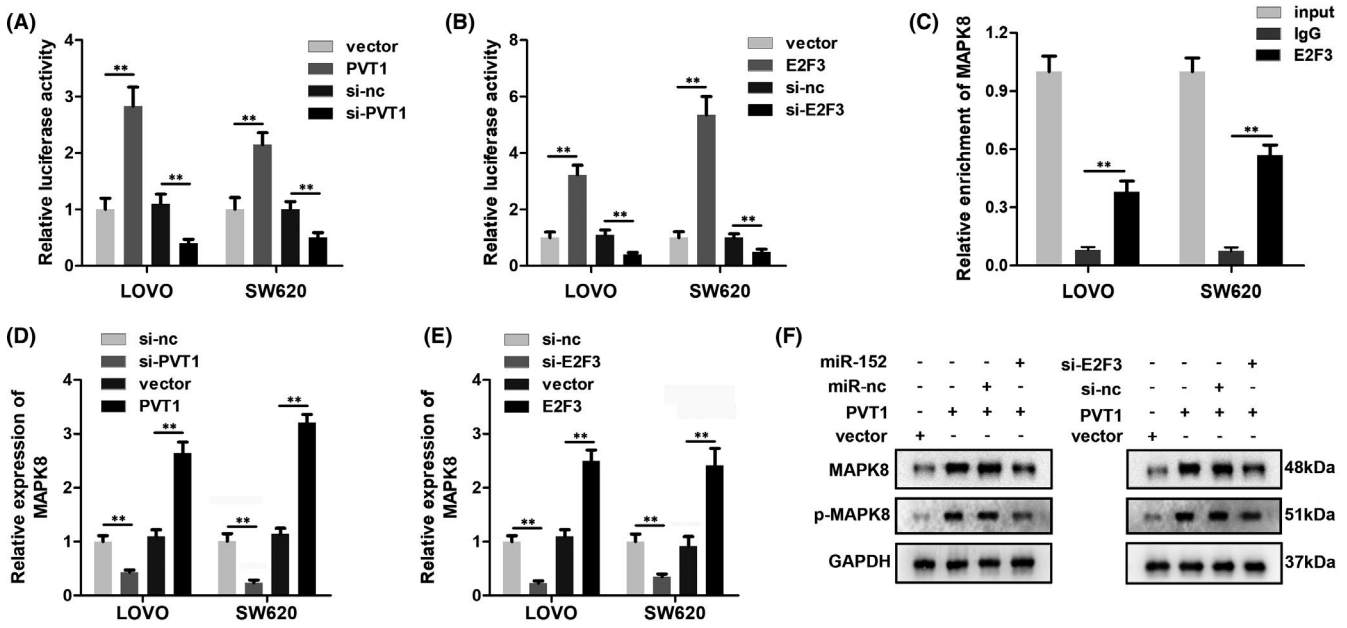

FIGURE 8 Plasmacytoma variant translocation 1 (circPVT1)-microRNA (miR)-152-3p-E2F3 axis promotes the mechanism of MAPK8. A, Luciferase reporter carrier experiment suggests that PVT1 has an effect on the transcriptional activation of MAPK8. B, Luciferase reporter carrier experiment suggests that E2F3 affects the transcriptional activation of MAPK8. C, ChIP confirmed that E2F3 can enrich the *MAPK8* gene. D, Quantitative real-time PCR suggests that both PVT1 and E2F3 promote *MAPK8* gene expression. E, F, Western blot analysis suggests that both PVT1 and E2F3 can regulate MAPK8 protein expression. $^{**}P < .01$. NC, negative control

PVT1 inhibits the proliferation, invasion, and metastasis of CRC cells and promotes the apoptosis of CRC cells. However, the mechanisms underlying these effects of PVT1 are complicated. As reported, PVT1 can increase the antiapoptotic effect of CRC cells and promotes tumor growth by inhibiting the transforming growth factor-β signaling pathway. Meanwhile, it participates in the progression of CRC by modulating the expression of c-Myc and its three target genes, *FUBP1*, *EZH2*, and *NPM1*. A recent study reported that PVT1 stabilizes miR-16-5p and interacts with the vascular endothelial growth factor (VEGF) A/VEGF receptor 1/AKT axis to promote the progression of CRC. These studies indicate that PVT1 plays a critical role in the development of CRC. Continuing to study the effects and mechanism of PVT1 could uncover novel strategies for the treatment of CRC.

In this study, we found that the expression of PVT1 was negatively correlated with that of miR-152-3p. Overexpression of PVT1 significantly promoted tumor proliferation, migration, and invasion.

MicroRNAs play important regulatory roles in the development of various tumors.[24,25] Recent studies have found that some miRNAs promote and inhibit the invasion or metastasis of CRC tumors by adjusting related genes, thus providing potential therapeutic targets for antimetastatic strategies. MicroRNA-152-3p has been confirmed as an anticancer gene in CRC. It inhibits the development of CRC by modulating the PIK3R3 and AKT-ERK pathways. In this study, overexpression of miR-152-3p antagonized the effects of PVT1 on the proliferation and metastasis of CRC cells. This result confirmed the antitumor function of miR-152-3p in CRC. A recent study reported that PVT1 sponges miR-152-3p and regulates the expression of Hepatoma derived growth factor (HDGF) in gastric cancer. We think that this finding supported our conclusion in colon cancer.[26]

To elucidate the mechanism of CRC, we investigated the downstream gene *miR-152-3p* in CRC. MicroRNA-152-3p directly targeted E2F3 in CRC cells. Transcriptional factor E2F is important in cell cycle regulation from the G_1 phase to the S phase. E2F transcription is closely correlated with the occurrence and apoptosis of tumors.[27] In addition, *E2F* acts as an oncogene in the development of many human cancers. Abnormal expression of E2F was found in various cancers, such as prostate cancer, skin cancers, and CRC. In this study, we found that as an oncogene in CRC, *E2F3* might mediate the oncogenic effect of the PVT1/miR-152 axis. As a transcription factor, E2F3 was confirmed to modulate the transcriptional activation of MAPK8.

5 | CONCLUSION

In the present study, PVT1 targeted miR-152-3p and inhibited the anticancer effect of miR-152-3p in CRC. Plasmacytoma variant translocation 1 acts as a ceRNA of E2F3 to modulate its expression and the transcriptional activation of MAPK8. Therefore, our findings revealed new aspects of the cellular functional and pathophysiological role of PVT1 and miR-152-5p, both of which might be considered potential molecular targets for the treatment of CRC.

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