

ACTC1 Drives Prostate Cancer Progression through Upregulation of BMP4 Expression

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Abstract

Prostate cancer (PRAD) represents a significant health burden for men globally. By integrating publicly accessible datasets with immunohistochemistry, this investigation found that ACTC1 expression is markedly elevated in PRAD. In vitro experiments demonstrated that forced overexpression of ACTC1 stimulated cellular proliferation and migratory capacity, whereas its silencing inhibited these aggressive traits. These observations were corroborated in vivo, where xenograft models revealed that ACTC1 accelerated tumor expansion. Comprehensive transcriptomic profiling and pathway enrichment analyses indicated that ACTC1 modulates immune and inflammatory cascades, with BMP4 emerging as a critical downstream mediator. Notably, ectopic expression of BMP4 reversed the suppressive effects of ACTC1 depletion on cell growth and movement, establishing that ACTC1 exerts its pro-tumorigenic effects primarily via BMP4 signaling. Collectively, these data position ACTC1 as a novel oncogenic factor in prostate cancer progression and nominate the ACTC1–BMP4 signaling axis as a promising therapeutic target.

Keywords: ACTC1, Prostate cancer, Prognostic marker, Oncogene

Introduction

Prostate cancer continues to rank among the most common cancers diagnosed in men and remains a major contributor to cancer-associated deaths worldwide [1]. Although substantial advances have been made in mapping its genomic alterations and in designing innovative treatments, the precise molecular pathways governing tumor onset and progression remain incompletely elucidated, which limits the development of more effective precision therapies. Consequently, the discovery of previously uncharacterized molecular

drivers is vital to deepen biological understanding and improve patient care.

ACTC1 (actin alpha cardiac muscle 1) codes for the major cardiac isoform of alpha-actin, a protein well known for its essential contributions to cardiac muscle contractility and cytoskeletal architecture [2]. Although ACTC1 has been studied predominantly in cardiovascular biology, growing evidence indicates that various cytoskeletal elements, once regarded merely as structural scaffolds, can actively influence cancer development by modulating processes such as cell division, motility, and invasiveness [3, 4]. Despite this, the function of ACTC1 outside cardiac tissue—especially within prostate cancer—has received limited attention.

Remodeling of the cytoskeleton constitutes a defining feature of prostate cancer progression, facilitating alterations in cellular morphology, movement, and tissue invasion. Multiple cytoskeletal components have been

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linked to disease advancement [5, 6]. While certain actin-related molecules, including ACTN4 [7], Ezrin [8], and FLNA [9], have been linked to metastatic spread and treatment resistance, the full repertoire of cytoskeletal regulators operating in prostate cancer remains incompletely mapped. Our comprehensive review of several publicly available prostate cancer expression datasets identified ACTC1, a cardiac actin variant rarely examined in solid malignancies, as one of the most consistently overexpressed cytoskeletal genes when malignant tissues were compared with normal prostate controls. This consistent upregulation implied an underappreciated pro-oncogenic contribution of ACTC1 to prostate cancer pathobiology.

Additional transcriptomic and experimental data have shown that ACTC1 alterations are associated with cancer progression in a context-specific manner [10]. For example, abnormal ACTC1 levels have been tied to increased cellular migration and metastatic capability in glioblastoma [11] and ovarian carcinoma [12]. At the same time, reduced expression has been reported in bladder cancer, raising the possibility of a tumor-restraining function in that setting [13]. Such conflicting findings emphasize the context-dependent nature of ACTC1 activity and the necessity of clarifying its precise contributions in prostate cancer. Early functional tests in our laboratory further revealed that ACTC1 levels correlated with more aggressive phenotypes in prostate cancer cell models, supporting the hypothesis that ACTC1 could serve as a previously unrecognized driver in this malignancy.

In the present work, we systematically explored the involvement of ACTC1 in prostate cancer progression. Integrating clinical cohort analyses, laboratory-based functional studies (both in vitro and in vivo), and mechanistic investigations, we aimed to determine whether ACTC1 facilitates tumor expansion and metastasis, identify its key downstream targets, and assess its potential as a biomarker or therapeutic target. Our results underscore the cancer-promoting activity of ACTC1 and uncover a novel ACTC1–BMP4 signaling axis implicated in prostate cancer development.

Materials and Methods

Public dataset analysis

Gene expression data were retrieved from open-access GEO repositories, specifically GSE55945, GSE3325, and GSE69223. Differential expression analyses were

conducted independently for each dataset, contrasting prostate tumor samples with matched benign tissues, using the edgeR package as previously outlined [14, 15]. Candidate hub genes were identified by intersecting lists of differentially expressed genes (DEGs) across multiple datasets and further validating them against findings from earlier publications. In parallel, survival analyses were executed using TCGA-PRAD clinical and expression data to gauge the prognostic significance of selected genes. DEGs were defined as follows: fold change (FC) ≥ 2.0 or ≤ 0.5 , with both P-value and false discovery rate (FDR) < 0.05 .

Patient information

Tumor specimens along with corresponding adjacent non-malignant prostate tissues were obtained from 75 individuals diagnosed with prostate cancer. Immediately following surgical excision, all samples were preserved in formalin. The research protocol received ethical clearance from the Ethics Committee of Nanjing Medical University, and each participant provided written informed consent before enrollment.

Immunohistochemistry (IHC)

Both cancerous prostate tissues and paired non-tumorous prostate samples were prepared following standard procedures [16]. IHC staining was executed as reported in prior publications [17, 18]. Briefly, sections were deparaffinized, subjected to antigen retrieval in citrate buffer (pH 6), and incubated with anti-ACTC1 antibody (YM6671, Immunoway, 1:2000). The streptavidin–biotin–peroxidase detection system was used, with diaminobenzidine as the chromogenic substrate. Negative controls were generated by excluding the primary antibody or substituting it with non-relevant immunoglobulins. Two independent evaluators, one of whom was a pathologist, scored the staining. ACTC1 immunoreactivity was assessed by multiplying staining intensity (scale 0–3) by the proportion of positively stained cells (scale 0–3). Composite scores were subsequently classified as negative (0), weak (1–2), moderate (3–4), or strong (5–6) [19].

Cell culture

The human prostate cancer cell lines LNCaP, PC-3, and DU145 were acquired from the American Type Culture Collection (ATCC, Bethesda, USA). Cells were routinely cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 0.1 mg/ml

streptomycin. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂, as per established protocols [20]. All lines underwent short tandem repeat (STR) authentication and were regularly screened for mycoplasma contamination.

Construction of stable cell lines

Stable cell line generation was performed following previously documented methodologies [20, 21]. Lentiviral vectors were employed to achieve ACTC1 overexpression (OE) and knockdown (KD). For rescue studies, BMP4 overexpression constructs were additionally introduced. Transduced cells were selected and maintained under puromycin pressure.

Cell proliferation assays

Cells were seeded at 4000 cells per well into 96-well plates and incubated at 37 °C for up to 6 days. Proliferative activity was quantified using CCK-8 reagent at designated intervals following genetic manipulation. Growth curves were plotted to evaluate the consequences of ACTC1 OE or KD, with or without concurrent BMP4 overexpression.

Colony formation assay

Prostate cancer cells were plated at sparse densities and allowed to grow for 10–14 days. The resulting colonies were fixed, stained with crystal violet solution, and manually quantified.

Migration, invasion, and wound-healing assays

Cell migratory and invasive abilities were evaluated using Transwell inserts, with or without Matrigel coating. Wound-healing experiments involved creating a linear scratch in confluent cell monolayers and monitoring wound closure at 24 hours.

RNA-seq and analysis

Cellular RNA was harvested with TRIzol reagent (Invitrogen), followed by DNase I digestion (TaKaRa) to completely remove contaminating genomic DNA. Sample quality was verified using a NanoDrop ND-2000 instrument (Thermo). Only RNA preparations fulfilling rigorous standards—OD_{260/280} values of 1.8–2.2, OD_{260/230} ≥ 2.0, RIN score ≥ 6.5, 28S/18S ratio ≥ 1.0, and total amount > 10 µg—were selected for downstream library construction. Sequencing libraries were generated and subjected to high-throughput sequencing on the Illumina HiSeq 4000 system at Shanghai Majorbio Bio-

pharm Technology Co., Ltd (China). All sequencing experiments were performed with three independent biological replicates for each condition.

Quantitative RT-PCR

RNA was extracted and converted into complementary DNA. Target gene transcript levels were quantified by real-time PCR, normalized to the housekeeping gene GAPDH. Fold changes in expression were determined via the $2^{-\Delta\Delta C_t}$ calculation method.

Primer sequences utilized in this work are provided here:

ACTC1-F: GGTGATGAAGCCCAGAGCAA;
 ACTC1-R: GTGGTGACAAAGGAGTAGCC;
 BMP4-F: CTGGTCTTGAGTATCCTGAGCG;
 BMP4-R: TCACCTCGTTCTCAGGGATGCT;
 GAPDH-F: GCACCACCAACTGCTTA;
 GAPDH-R: AGTAGAGGCAGGGATGAT

In vivo tumor growth assay

The DU145 cell line was selected for subcutaneous xenograft studies owing to its androgen-independent properties and reliable tumor-forming ability in vivo, which enabled robust evaluation of growth kinetics. Male nude mice were sourced from Obio Technology (Shanghai, China). Mice were randomly assigned to control and ACTC1-overexpressing (OE) cohorts, with 7 animals per group. Each mouse received a subcutaneous injection of 1×10^6 cells into the flank at six weeks of age. Tumor dimensions were monitored with calipers at three-day intervals, and volumes were computed using the standard formula: length $\times$ width² $\times$ 0.5 (mm³) [22]. Growth curves were statistically compared using a two-way repeated-measures ANOVA, accounting for both time-dependent progression and group differences while adjusting for individual animal variability. All tumor assessments and data processing were carried out by personnel unaware of group assignments to avoid observer bias. Euthanasia was performed by CO₂ asphyxiation at a flow rate of 35% vol/min inside a specialized chamber (dimensions 630 $\times$ 480 $\times$ 500 mm). CO₂ delivery continued for a minimum of 1 minute after breathing stopped. Death verification included confirmation of absent heartbeat and respiration, development of rigor mortis, and fixed, dilated pupils. All animal experiments were approved by the Ethics Committee of Nanjing Medical University and conducted in accordance with the ethical principles of the Declaration of Helsinki. Reporting followed the

ARRIVE guidelines (Ethics approval reference: 20230531).

Functional annotation and pathway enrichment analysis
Functional interpretation of gene lists was performed with the Database for Annotation, Visualization, and Integrated Discovery (DAVID) platform, applying Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses [23-26]. Only terms and pathways reaching a significance threshold of $P < 0.05$ were retained.

Statistical analysis

Quantitative data are reported as mean $\pm$ SEM. Two-group comparisons were evaluated with unpaired Student's t-test. For analyses involving three or more groups, a one-way ANOVA was conducted, with multiple testing corrected using the Benjamini–Hochberg false discovery rate (FDR) procedure. A P value below 0.05 was regarded as statistically significant. Computations were executed in GraphPad Prism and R software (version 3.3.0).

Results and Discussion

Identification of ACTC1 as the critical gene in prostate cancer

To identify pivotal genes driving prostate cancer, we performed separate bioinformatic analyses of three GEO datasets: GSE55945, GSE3325, and GSE69223 (**Figures 1a and 1b**). In each dataset, genes showing differential expression were defined by stringent criteria (P and FDR < 0.05 , fold change ≥ 2.0 or ≤ 0.5). The intersection of these DEG lists across all datasets was generated to isolate genes that displayed consistent dysregulation between prostate tumors and adjacent normal tissues. This analysis highlighted ACTC1, SRP9, and DAB2IP as repeatedly altered candidates (**Figure 1c**). Detailed expression profiles for these genes are presented in **Figure 2a**. Prognostic evaluation using survival data demonstrated that, of the three candidates, elevated ACTC1 expression alone was significantly linked to unfavorable patient outcomes (**Figure 2b**). Consistent with the transcriptomic results, immunohistochemical analysis of patient-derived tissues confirmed substantially increased ACTC1 protein expression in cancerous prostate samples compared with matched benign controls (**Figure 2c**). These convergent lines of evidence establish that ACTC1 is strongly

upregulated in prostate cancer and likely contributes to disease advancement.

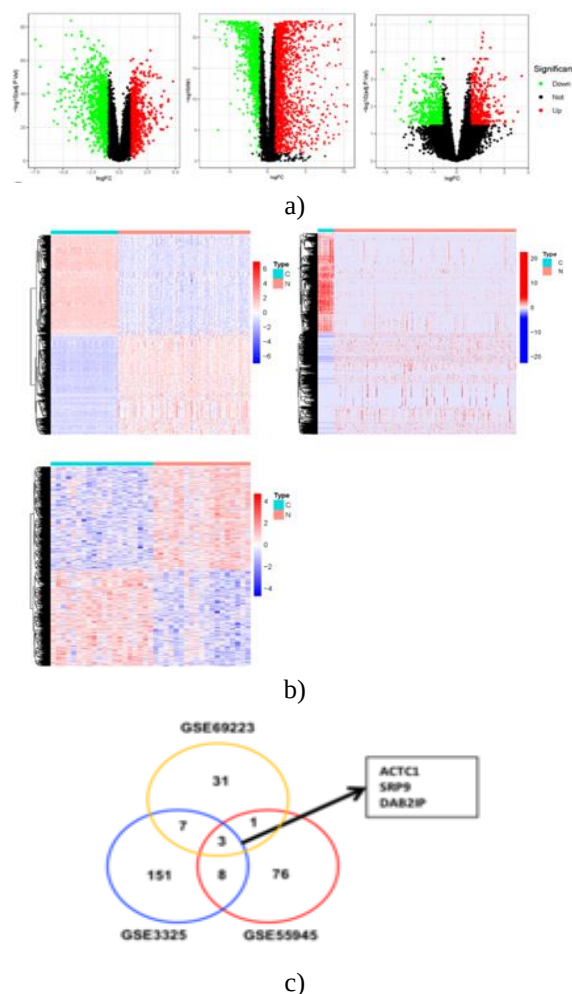


Figure 1. Detection of genes showing altered expression levels in prostate cancer cohorts

Volcano plots depicting the distribution of differentially expressed genes obtained from comparisons between malignant prostate tissues and nearby non-cancerous prostate tissues in the GEO series GSE55945, GSE3325, and GSE69223. Significantly upregulated transcripts are marked in red, downregulated transcripts in green, and genes with no notable changes in black; (b) Heatmaps visualizing the unique expression signatures of these DEGs when contrasting tumor (c) samples against normal (N) controls, along with differences observed between microsatellite unstable (MSI) and microsatellite stable (MSS) categories; and (c) Venn diagram showing the common DEGs across the three GEO datasets, with ACTC1, SRP9, and DAB2IP as the central overlapping genes.

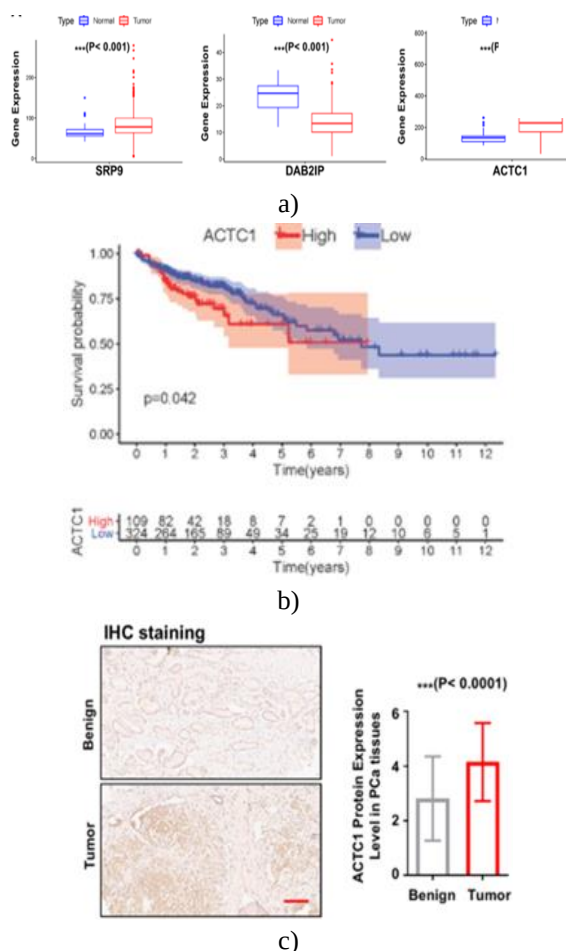


Figure 2. Upregulation of ACTC1 in prostate cancer tissues correlates with adverse patient prognosis

(a) Box plots illustrating mRNA expression levels of SRP9, DAB2IP, and ACTC1 in malignant prostate samples compared to normal controls. ACTC1 displayed prominent overexpression in tumor tissues ($***P < 0.001$). Error bars: mean $\pm$ SEM; (b) Kaplan–Meier curves showing that increased ACTC1 expression is associated with shorter overall survival among prostate cancer cases ($P = 0.042$); and (c) Representative immunohistochemistry images depicting ACTC1 protein distribution in non-cancerous prostate glands and prostate cancer lesions. Scoring quantification confirmed markedly elevated ACTC1 levels in tumors ($N = 75$, original magnification $\times 150$, $***P < 0.0001$). Scale bar = 100 μm . Error bars: mean $\pm$ SEM.

ACTC1 stimulates cell growth, motility, and xenograft tumor development in prostate cancer models

To investigate the oncogenic potential of ACTC1, we engineered prostate cancer cell lines (LNCaP, DU145,

and PC-3) with stable ACTC1 overexpression. Results from CCK-8 viability assays indicated that elevated ACTC1 significantly enhanced proliferative activity, yielding 1.5- to 3-fold increases in cell numbers across all three lines relative to empty vector controls (**Figure 3a**). Parallel Transwell chamber experiments further showed that ACTC1 overexpression augmented migratory ability by 1.5- to 1.75-fold in these cell models (**Figure 3b**). In vivo studies using DU145 xenografts in immunodeficient mice revealed that ACTC1-overexpressing tumors exhibited approximately 1.5-fold greater volume and substantially higher final weights than control tumors (**Figure 3c**). Overall, these data support a tumor-promoting role for ACTC1 in prostate cancer.

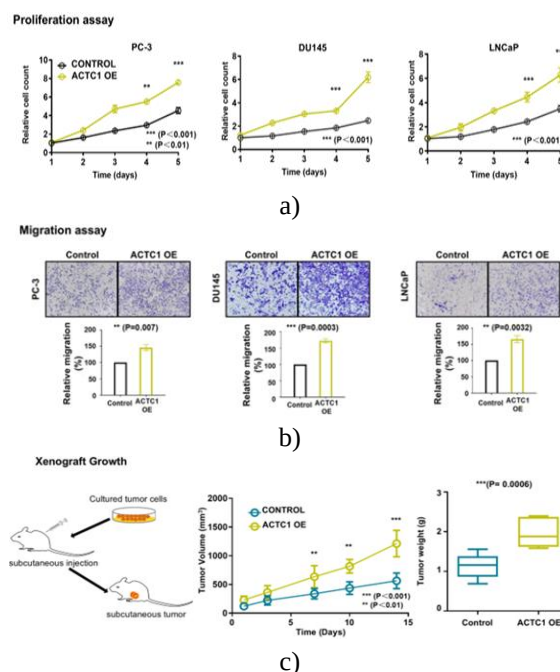


Figure 3. ACTC1 stimulates proliferative activity and cell migration in prostate cancer models

(a) Growth curves measured at multiple time points in LNCaP, PC-3, and DU145 cell lines ($**P < 0.01$, $***P < 0.001$, $N = 3$). Error bars represent mean $\pm$ SEM; (b) Quantification of migrated cells in Transwell assays performed on the specified prostate cancer lines ($N = 3$). Error bars represent mean $\pm$ SEM, and (c) Analysis of subcutaneous tumor development in DU145 xenografts expressing either control vector or ACTC1 ($**P < 0.01$, $***P < 0.001$). Error bars represent mean $\pm$ SEM.

RNA sequencing uncovers immune-related and inflammatory signaling cascades controlled by ACTC1

To delineate the downstream mechanisms by which ACTC1 contributes to tumor development, a comprehensive RNA-seq analysis was conducted in prostate cancer cells engineered to overexpress ACTC1 compared with control cells. This profiling identified 334 genes exhibiting clear differential expression between the groups. Volcano plot visualization revealed broad changes in the transcriptional landscape of DU145 cells after ACTC1 upregulation, particularly the activation of numerous immunity-linked genes (**Figure 4a**). Specifically, 96 transcripts were increased. At the same time, 237 were suppressed in the ACTC1-overexpressing condition. To interpret the biological implications of these transcriptional shifts, enrichment analyses were executed using the DAVID online platform. Gene Ontology (GO) evaluation pointed to significant involvement in processes including immune response, chemotaxis, and type I interferon signaling (**Figure 4b**). In parallel, KEGG pathway mapping demonstrated enrichment in several immune and inflammatory routes, such as cytokine–cytokine receptor interaction, Toll-like receptor signaling, and pathways associated with viral responses (**Figure 4c**).

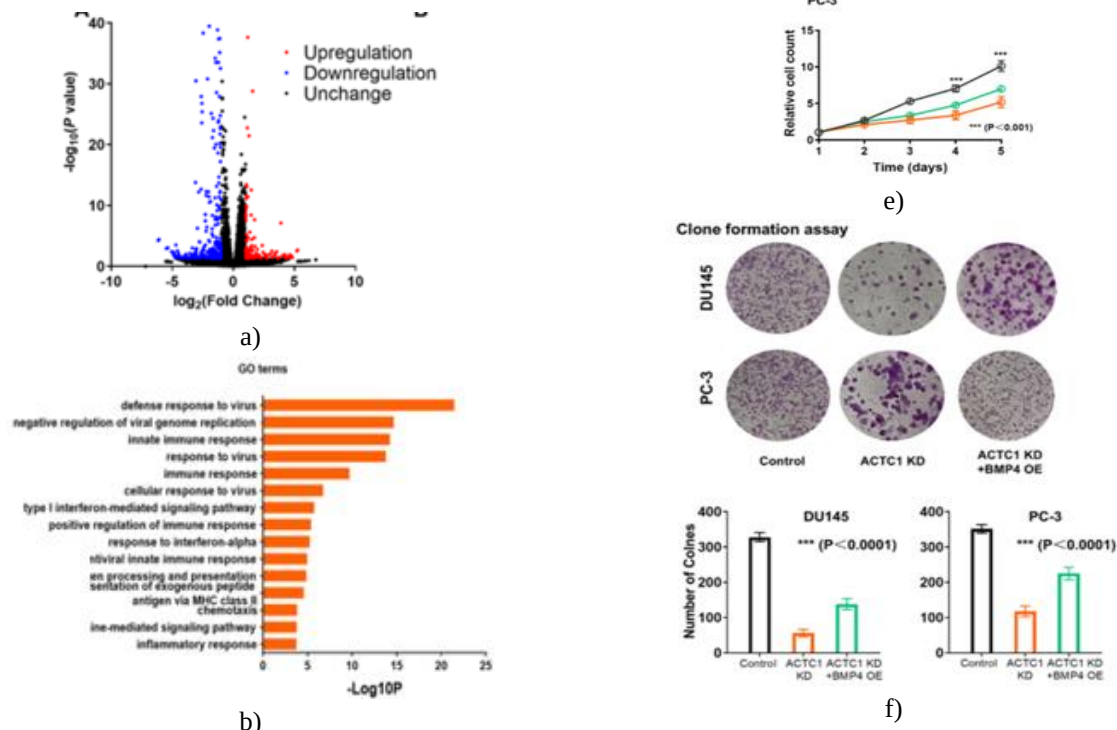


Figure 4. ACTC1 accelerates prostate cancer cell growth by upregulating BMP4

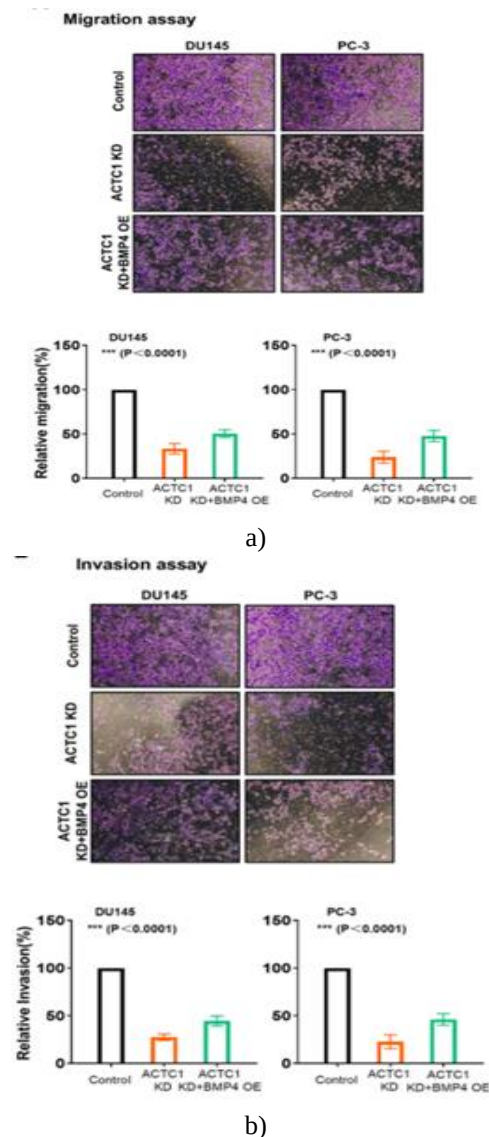
(a) Volcano plot displaying differentially expressed genes from RNA-seq performed on DU145 cells with ACTC1 overexpression; (b) Top GO terms enriched among the DEGs; (c) Significantly enriched KEGG pathways among the DEGs; (d) qRT-PCR measurement of BMP4 transcript abundance in prostate cancer cells expressing control vector versus ACTC1-overexpressing constructs (* $P < 0.05$, *** $P < 0.001$, $N = 3$). Error bars: mean $\pm$ SEM; (e) Forced BMP4 expression counteracts the proliferative deficit induced by ACTC1 silencing in DU145 cells, as determined by one-way ANOVA (** $P < 0.01$, *** $P < 0.001$, $N = 3$). Error bars: mean $\pm$ SEM; (f) BMP4 overexpression reverses the reduction in colony formation caused by ACTC1 knockdown (*** $P < 0.001$, $N = 3$). Error bars: mean $\pm$ SEM.

BMP4 functions as an essential intermediary in ACTC1-mediated enhancement of proliferation, colony-forming capacity, migration, and invasiveness in prostate cancer cells

Among the genes with altered expression, BMP4 was highlighted as a likely direct or indirect target of ACTC1. Although BMP4 displays varying activities depending on tumor context, evidence supports a predominantly oncogenic role in prostate cancer [27]. As part of the bone morphogenetic protein subgroup within the broader TGF- β superfamily, BMP4 functions as a secreted factor that modulates multiple oncogenic processes across different malignancies [28–30], including promoting tumor progression in the prostate [31]. Our qRT-PCR results demonstrated that ACTC1 overexpression robustly elevated BMP4 mRNA levels in DU145 and PC-3 prostate cancer cells relative to control conditions (**Figure 4d**), suggesting that BMP4 is a key downstream component of the ACTC1 signaling network.

Functional validation through rescue experiments confirmed the importance of this relationship. Knockdown of ACTC1 led to clear suppression of cell proliferation in DU145 and PC-3 lines, but BMP4 overexpression restored proliferative rates to levels comparable with those of controls (**Figure 4e**). Analogous findings were observed in colony formation experiments, where ACTC1 depletion markedly reduced the ability of cells to form colonies, and this phenotype was largely rescued by concurrent BMP4 overexpression (**Figure 4f**). These data establish BMP4 as a necessary mediator for the growth-promoting actions of ACTC1. ACTC1 further influenced cell movement capabilities. In Transwell migration and invasion models, silencing

ACTC1 substantially weakened both the migratory and invasive behaviors of DU145 and PC-3 cells; however, BMP4 overexpression effectively reversed these deficiencies (**Figures 5a and 5b**). Supporting scratch-wound assays similarly showed delayed wound closure following ACTC1 knockdown, which was restored by BMP4 re-expression (**Figure 5c**). Collectively, these results demonstrate that BMP4 serves as a critical functional effector through which ACTC1 exerts its tumor-promoting effects on multiple malignant traits in prostate cancer.



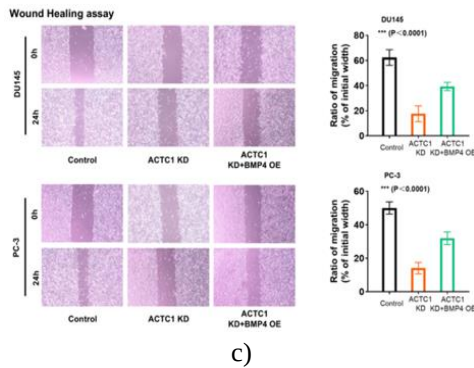


Figure 5. ACTC1 enhances prostate cancer cell migration and invasion by upregulating BMP4

(a) BMP4 overexpression reverses the migration impairment caused by ACTC1 knockdown ($***P < 0.001$, $N = 3$). Error bars: mean $\pm$ SEM; (b) BMP4 overexpression restores invasive capacity reduced by ACTC1 knockdown ($***P < 0.001$, $N = 3$). Error bars: mean $\pm$ SEM; and (c) Wound healing assays demonstrating that BMP4 overexpression corrects the migration defect induced by ACTC1 knockdown ($***P < 0.001$, $N = 3$). Error bars: mean $\pm$ SEM.

Prostate cancer ranks as the most prevalent malignancy among men globally, particularly in Western populations [32]. The present investigation establishes ACTC1 as a previously unrecognized oncogenic factor in this disease. Through comprehensive analysis of several GEO datasets combined with immunohistochemical confirmation in patient samples, we showed that ACTC1 expression is consistently higher in prostate tumors than in benign prostate tissue. Subsequent in vitro and in vivo experiments demonstrated that ACTC1 augments cell proliferation, motility, and invasiveness in culture and accelerates tumor growth in xenograft models. RNA sequencing further revealed extensive remodeling of immune and inflammatory gene networks, and follow-up mechanistic work identified BMP4 as a major downstream effector responsible for mediating many of ACTC1's tumor-promoting activities.

These results provide valuable insights into the contributions of cytoskeletal components to prostate cancer pathobiology. Although ACTC1 has historically been examined primarily for its role in cardiac contractility and cytoskeletal integrity, our data indicate that it also plays an active role in cancer development. The identification of BMP4 as an ACTC1 transcriptional target reveals a novel ACTC1–BMP4 signaling module that fuels malignant behavior in prostate tumors. As a member of the TGF- β superfamily, BMP4 influences

key cellular processes, including proliferation, migration, epithelial–mesenchymal transition, and cancer stemness. It can display either tumor-enhancing or tumor-suppressing properties depending on the specific tissue and molecular environment [27, 29, 33]. By connecting ACTC1 to BMP4, this study uncovers a new regulatory pathway that may underlie aggressive prostate cancer phenotypes.

Clinically, both ACTC1 and the BMP4 pathway it controls represent attractive candidates for biomarker development and targeted therapy, especially in advanced and castration-resistant forms of prostate cancer (CRPC). Despite treatment progress, the molecular drivers of disease progression and therapy resistance are still only partially understood, leaving limited options for patients with metastatic or resistant disease. The ACTC1–BMP4 axis therefore offers fresh mechanistic insight and potential new intervention points. While BMP pathway inhibitors such as LDN-193189 and DMH1 have displayed encouraging activity in preclinical models [34, 35], their utility in prostate cancer is complicated by the pleiotropic roles of BMP signaling in normal physiology. In this context, inhibiting upstream modulators such as ACTC1 could provide a more selective means of controlling BMP4 activity. As BMP inhibitors continue to advance in clinical trials, insights from the ACTC1–BMP4 relationship may guide the design of more precise therapeutic strategies for aggressive prostate cancer.

Several limitations should be noted. Although our findings strongly implicate ACTC1 in the progression of prostate cancer, the exact molecular mechanisms by which ACTC1 controls BMP4 expression remain to be elucidated. Experiments examining direct promoter binding or transcriptional regulation were beyond the scope of this work and will be pursued in subsequent studies. While ACTC1 overexpression was reproducibly observed across GEO datasets, TCGA-PRAD data, and our own clinical cohort, validation in additional independent patient series and patient-derived models would reinforce its relevance. Additionally, although TCGA-based survival analysis linked high ACTC1 to poorer outcomes, multivariate analyses were not conducted; larger, comprehensively annotated cohorts will be necessary to establish ACTC1 as an independent prognostic indicator. Finally, our study centered on BMP4, but other downstream targets of ACTC1 may also contribute to its oncogenic effects and merit future investigation.

In conclusion, this work positions ACTC1 as an important oncogenic driver in prostate cancer and underscores the ACTC1–BMP4 signaling axis as a central contributor to tumor progression. These observations broaden our perspective on cytoskeletal proteins, moving beyond their conventional structural roles to reveal regulatory functions in cancer. Future research should delineate the detailed mechanisms connecting ACTC1 to BMP4 and assess the clinical value of targeting this pathway.

Conclusion

This study establishes ACTC1 as a novel oncogenic factor in prostate cancer. ACTC1 is overexpressed in tumor tissues and drives cell proliferation, migration, and in vivo tumor growth by activating the BMP4 signaling pathway. These discoveries uncover a previously unappreciated ACTC1–BMP4 axis with potential utility as both a prognostic biomarker and a therapeutic target in advanced prostate cancer.

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Conflict of Interest: None

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Ethics Statement: None

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