# Downregulation of miR-505-3p predicts poor bone metastasis-free survival in prostate cancer

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**Abstract.** The principal issue deriving from prostate cancer (PCa) is its propensity to metastasize to bone. To date, bone metastasis remains incurable, and therapeutic strategies are limited. Therefore, it is of paramount importance to explore predictive markers for bone metastasis of PCa. In the present study, we reported that miR-505-3p was significantly downregulated in bone metastatic PCa tissues compared with that in non-bone metastatic PCa tissues, but there was no significant difference in miR-505-3p expression between PCa and adjacent normal tissues. miR-505-3p expression was inversely associated with serum PSA levels, Gleason grade, N and M classification, and short bone metastasis-free survival in PCa patients, but had no effect on overall survival in PCa patients. Furthermore, upregulation of miR-505-3p suppressed the activity of TGF-β signaling by directly targeting downstream effectors of TGF-β signaling, SMAD2 and SMAD3, further inhibiting the invasion and migration abilities of PCa cells. Therefore, our findings unraveled a novel mechanism by which miR-505-3p inhibits bone metastasis of PCa, supporting the notion that miR-505-3p may serve as a predictive marker for bone metastasis of PCa.

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## Introduction

Prostate cancer (PCa) is one of the most common cancers and the second leading cause of cancer-related deaths in men worldwide (1). Although there has been substantial progress in the treatment of primary PCa, distant bone metastasis remains incurable, due to the lack of effective therapeutic avenues, contributing to the mortality of PCa (2). Therefore, exploring a novel marker to predict bone metastasis of PCa will facilitate the development of antimetastatic therapeutic strategies against PCa.

miRNAs are a class of small non-coding regulatory RNAs that mechanistically function by binding to the 3' untranslated region (3'UTR) of downstream mRNAs, giving rise to mRNA degradation or repression of translation (3,4), and play crucial roles in many biological processes, including cell apoptosis, proliferation and differentiation (3). Numerous studies have demonstrated that aberrant expression of miRNAs is implicated in the progression and metastasis of several human cancers (5-9). Furthermore, several miRNAs have been identified as critical mediators of bone metastasis in human cancer (10,11), holding promise as a potential predictive factor for bone metastasis. Our previous studies demonstrated that downregulation of miR-145 by loss of wild-type p53 in PC-3 cells promoted bone metastasis of PCa by regulating several positive regulators of EMT, including ZEB2 and HEF1 (12-14). Therefore, it is imperative to discern a novel miRNA to predict bone metastasis of PCa.

In the present study, we found no evident difference in miR-505-3p expression in PCa tissues compared with that in adjacent normal tissues. Notably, miR-505-3p was significantly reduced in bone metastatic PCa tissues, and

miR-505-3p expression was inversely associated with poor clinicopathological characteristics in PCa patients. More importantly, low expression of miR-505-3p was positively associated with shorter bone metastasis-free survival in PCa patients, but was not associated with overall survival of PCa patients. Our results further revealed that upregulation of miR-505-3p inhibited TGF-β signaling activity by targeting SMAD2 and SMAD3, which further inhibited the invasion and migration abilities of PCa cells. Therefore, our results demonstrated that downregulation of miR-505-3p was associated with poor bone metastasis-free survival in PCa, suggesting that miR-505-3p may be used as a novel marker to predict bone metastasis of PCa.

## Materials and methods

*Cell lines and cell culture*. Human RWPE-1, DU145, LNCaP, 22RV1, PC-3 and VCaP cells were obtained from the American Type Culture Collection (ATCC; American Type Culture Collection, Manassas, VA, USA) and cultured according to the manufacturer's instructions. The C4-2B cell line was purchased from MD Anderson Cancer Center (Houston, TX, USA). RWPE-1 cells were grown in defined keratinocyte-SFM (1X) (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). LNCaP, 22RV1, C4-2B and PC-3 cells were grown in RPMI-1640 medium supplemented with 10% FBS, while DU145 and VCaP cells were grown in Dulbecco's modified Eagle's medium (all from Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS. All cells were incubated at 37°C in a humidified atmosphere with 5% $CO_2$.

*Patients and tumor tissues*. The 127 archived PCa tissues, including 81 non-bone metastatic PCa tissues and 46 bone metastatic PCa tissues were obtained during surgery or needle biopsy at Jiangmen Central Hospital (Guangzhou, China). Patients were diagnosed based on clinical and pathological evidence, and the specimens were immediately snap-frozen and stored in liquid nitrogen tanks. For the use of these clinical materials for research purposes, prior informed consents from patients and approval from the Institutional Research Ethics Committee were obtained from Jiangmen Central Hospital (Jiangmen, China). The clinicopathological features of the patients are presented in Table I.

*RNA extraction, reverse transcription, and real-time PCR*. Total RNA from tissues or cells was extracted using TRIzol (Life Technologies; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Messenger RNA (mRNA) and miRNA were reverse-transcribed from total mRNA using the Revert Aid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Real-time PCR was performed according to a standard method, as previously described (15). The list of primers used is presented in Table II. Primers for U6 and miR-505-3p were synthesized and purified by Guangzhou RiboBio Co., Ltd. U6 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control for miRNA or mRNA, respectively. Relative fold expression was calculated using the comparative threshold cycle ($2^{-\Delta\Delta Cq}$) method (16).

Table I. The relationship between the expression level of miR-505-3p and the clinicopathological characteristics in 127 PCa patients.

| | | miR-505-3p expression | | |
|---|---|---|---|---|
| Parameters | Number of cases | Low | High | P-values |
| Age (years) | | | | |
| ≤72 | 58 | 29 | 29 | 0.935 |
| >72 | 69 | 35 | 34 | |
| T classification | | | | |
| T1-T2 | 50 | 28 | 22 | 0.309 |
| T3-T4 | 77 | 36 | 41 | |
| N classification | | | | |
| N0 | 93 | 38 | 55 | <0.001 |
| N1 | 34 | 26 | 8 | |
| M classification | | | | |
| M0 | 78 | 18 | 60 | <0.001 |
| M1 | 49 | 46 | 3 | |
| Gleason score | | | | |
| ≤7 | 48 | 15 | 33 | <0.01 |
| >7 | 79 | 49 | 30 | |
| Serum PSA at diagnosis, μg/ml | | | | |
| <77.6 | 52 | 20 | 32 | <0.05 |
| >77.6 | 75 | 44 | 31 | |
| BM status | | | | |
| nBM | 81 | 24 | 57 | <0.001 |
| BM | 46 | 40 | 6 | |

PCa, prostate cancer; PSA, prostate-specific antigen; BM, bone metastasis; nBM, non-bone metastasis.

Table II. List of primers used for real-time RT-PCR.

| Primers | |
|---|---|
| CTGF | F: GCTACCACATTTCCTACCTAGAAATCA |
| | R: GACAGTCCGTCAAAACAGATTGTT |
| PTHRP | F: ACTCGCTCTGCCTGGTTAGA |
| | R: GGAGGTGTCAGACAGGTGGT |
| IL11 | F: TGAAGACTCGGCTGTGACC |
| | R: CCTCACGGAAGGACTGTCTC |
| SMAD2 | F: CACGCTAGGAAAACAGCCTC |
| | R: TCGGAAGAGGAAGGAACAAA |
| SMAD3 | F: CGGCAGTAGATGACATGAGG |
| | R: TCAACACCAAGTGCATCACC |
| GAPDH | F: ATTCCACCCATGGCAAATTC |
| | R: TGGGATTTCCATTGATGACAAG |

F, forward; R, reverse.

*Plasmid, small interfering RNA and transfection*. The (CAGAC) 12/pGL3 TGF-β/Smad-responsive luciferase reporter plasmid and control plasmids (cat. no. 32177; Clontech; Takara Bio, Inc., Tokyo, Japan) were used to quantitatively assess the transcriptional activity of TGF-β signaling components. The 3'UTR regions of SMAD2 and SMAD3 were PCR-amplified from genomic DNA and cloned into the pmirGLO luciferase reporter vector (Promega Corporation, Madison, WI, USA). The miR-505-3p mimics were obtained from Guangzhou RiboBio Co., Ltd. Transfection of siRNAs and plasmids was performed using Lipofectamine 3000 (Life Technologies; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions.

*Invasion and migration assays*. Migration and invasion were performed using Transwell chambers consisting of 8-mm membrane filter inserts (Corning Incorporated, Corning, NY, USA) coated with or without Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) as previously described (17).

*Luciferase assay*. Cells ($4x10^4$) were seeded in triplicate in 24-well plates and cultured for 24 h as previously described (18). Cells were transfected with 250 ng pTGF-β/Smad reporter luciferase plasmid, or pmirGLO-SMAD2-3'UTR and -SMAD3-3'UTR luciferase plasmid, plus 5 ng pRL-TK *Renilla* plasmid (Promega Corporation) using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Luciferase and *Renilla* signals were assessed 36 h after transfection using a Dual-Luciferase Reporter Assay kit (Promega Corporation) according to the manufacturer's protocol.

*RNA immunoprecipitation*. Cells were co-transfected with HA-Ago2 (cat. no. 10822; Addgene, Inc., Cambridge, MA, USA), followed by HA-Ago2 immunoprecipitation using an HA-antibody (1:1,000; cat. no. H3663; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), as previously described (19). Real-time PCR analysis of the IP material was used to assess the association of SMAD2 and SMAD3 mRNA with the RISC complex.

*Western blotting*. Western blotting was performed according to a standard method, as previously described (20). Proteins were visualized using ECL reagents (Pierce; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Antibodies against SMAD2 (cat. no. 5339), SMAD3 (cat. no. 9523), pSMAD2/3 (cat. no. 9510) and SMAD2/3 (cat. no. 8685) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA), and p84 (cat. no. PA5-27816) was obtained from Invitrogen; Thermo Fisher Scientific, Inc. All antibodies aforementioned were diluted 1:1,000. The membranes were stripped and reprobed with an anti-α-tubulin antibody (1:5,000; cat. no. ab7291, Abcam, Cambridge, UK) as the loading control.

*Generation and analysis of TCGA datasets and Gene Set Enrichment Analysis (GSEA)*. The miRNA expression levels and clinical profile datasets from PCa patients were downloaded from The Cancer Genome Atlas (TCGA; https://tcga-data.nci.nih.gov/tcga/). The log2 values of miRNAs in each sample were analyzed using Excel 2010 and GraphPad 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). The miRNA expression levels of all PCa tissues were statistically analyzed using paired t-tests or unpaired t-tests. The miRNA expression levels in each sample were analyzed as previously described (21). Briefly the high and low expression levels of miR-505-3p were stratified by the medium expression level of miR-505-3p in PCa tissues. Gene set enrichment analysis was performed using Molecular Signatures Database (MSigDB) v5.2.

*TargetScan and miRanda analysis*. TargetScan (http://www.targetscan.org/vert_71/) and miRanda (http://34.236.212.39/microrna/home.do) datasets were analyzed as previously described respectively (22,23).

*Statistical analysis*. All values are presented as the means ± standard deviation (SD). Significant differences were determined using GraphPad 5.0 software. Student's t-test was used to determine significant differences between two groups. The chi-square test was used to analyze the relationship between miR-505-3p expression and clinicopathological characteristics. Survival curves were plotted using the Kaplan Meier method and compared by log-rank test. $P<0.05$ was considered to indicate a statistically significant difference. All experiments were repeated three times.

## Results

*miR-505-3p is downregulated in bone metastatic PCa tissues*. To investigate the aberrant miRNA expression between bone metastatic PCa tissues and non-bone metastatic PCa tissues, we analyzed the miRNA sequencing datasets of PCa tissues from TCGA. We found that that there was no significant difference in miR-505-3p expression between 498 PCa tissues and 52 adjacent normal tissues (ANT) (Fig. 1A), or between 52 paired PCa tissues compared with the expression in the matched ANT (Fig. 1B). Notably, miR-505-3p expression was significantly downregulated in bone metastatic PCa tissues compared with the expression in non-bone metastatic PCa tissues (Fig. 1C). The miR-505-3p expression in our 81 non-bone metastatic PCa tissues and 46 bone metastatic PCa tissues was further validated via real-time PCR and the results revealed that miR-505-3p expression was significantly downregulated in bone metastatic PCa tissues compared with the expression in non-bone metastatic PCa tissues (Fig. 1D). The percentage of low miR-505-3p expression was much higher in bone metastatic PCa tissues compared to that in non-bone metastatic PCa tissues (Fig. 1E), which was consistent with the results of TCGA (Fig. 1F). Therefore, these results indicated that downregulation of miR-505-3p was involved in bone metastasis of PCa.

*Low expression of miR-505-3p is ssociated with advanced clinicopathological features and poor bone metastasis-free survival in PCa patients*. We further investigated the clinical association of miR-505-3p expression with clinicopathological characteristics in PCa patients and found that miR-505-3p expression was downregulated in N1, M1 or Gleason grade (>7) PCa tissues, but not in T3-T4 PCa tissues (Fig. 2A-D). Statistical

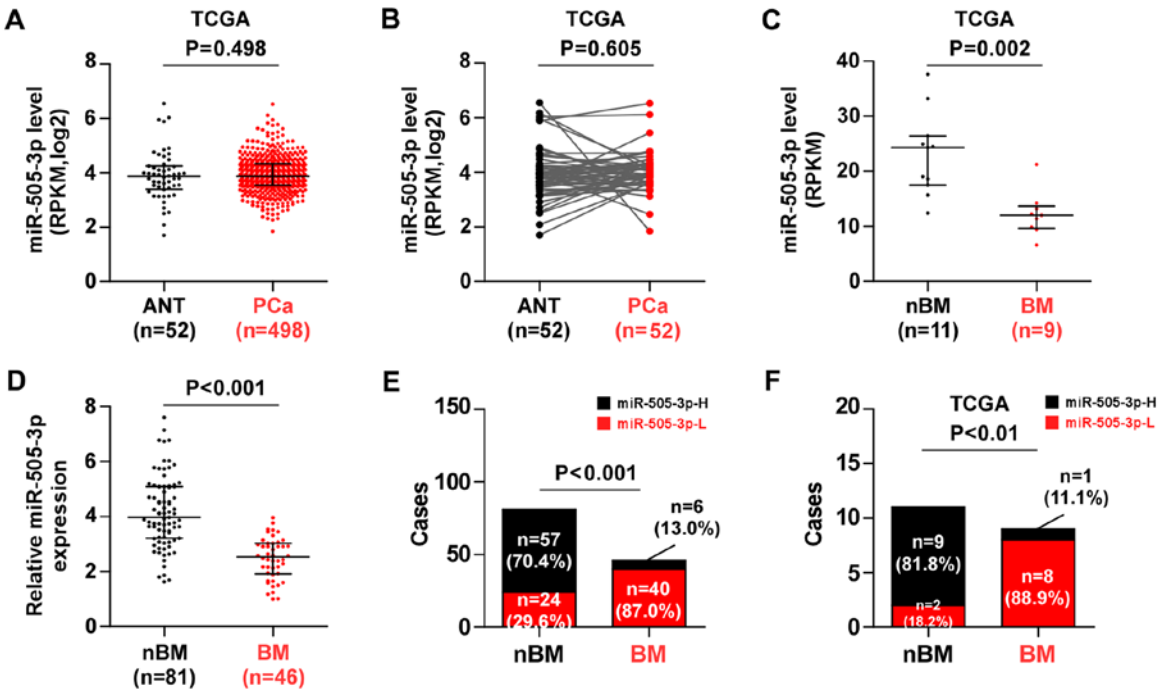


Figure 1. miR-505-3p is downregulated in bone metastatic prostate cancer (PCa) tissues. (A) The expression level of miR-505-3p in 498 PCa tissues and 52 ANT in the PCa datasets from TCGA. (B) The expression level of miR-505-3p in 52 paired PCa tissues and the matched ANT in the PCa datasets from TCGA. (C) The expression level of miR-505-3p in BM PCa tissues was downregulated compared with that in the nBM PCa tissues in the PCa datasets from TCGA. (D) miR-505-3p was downregulated in 46 BM PCa tissues compared with the expression in 81 nBM PCa tissues. (E) The percentages and number of samples exhibiting high or low miR-505-3p expression in our PCa patients with different bone metastasis status. (F) The percentages and number of samples exhibiting high or low miR-505-3p expression in PCa patients with different bone metastasis status from TCGA. PCa, prostate cancer; ANT, adjacent normal tissues; TCGA, The Cancer Genome Atlas; BM, bone metastatic; nBM, non-bone metastatic.

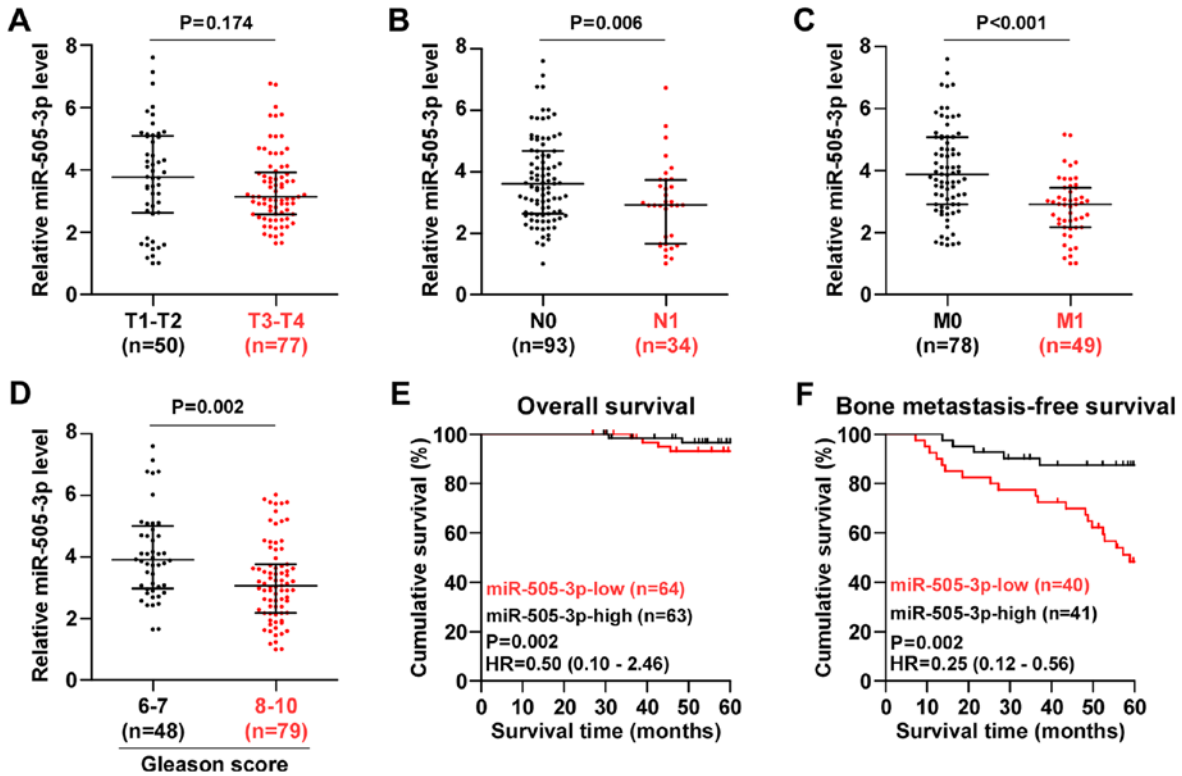


Figure 2. miR-505-3p expression is inversely associated with clinicopathological characteristics and bone metastasis-free survival. (A) The expression levels of miR-505-3p in PCa tissues with different tumor stages. (B) The expression levels of miR-505-3p in PCa tissues with different lymph node metastasis status. (C) The expression levels of miR-505-3p in PCa tissues with different distant metastasis status. (D) The expression levels of miR-505-3p in PCa tissues with different Gleason scores. (E) Kaplan-Meier analysis of the overall survival curves of PCa patients with high miR-505-3p expression (n=63) vs. low miR-505-3p expression (n=64). (F) Kaplan-Meier analysis of bone metastasis-free survival curves of PCa patients with high miR-505-3p expression (n=41) vs. low miR-505-3p expression (n=40). PCa, prostate cancer.

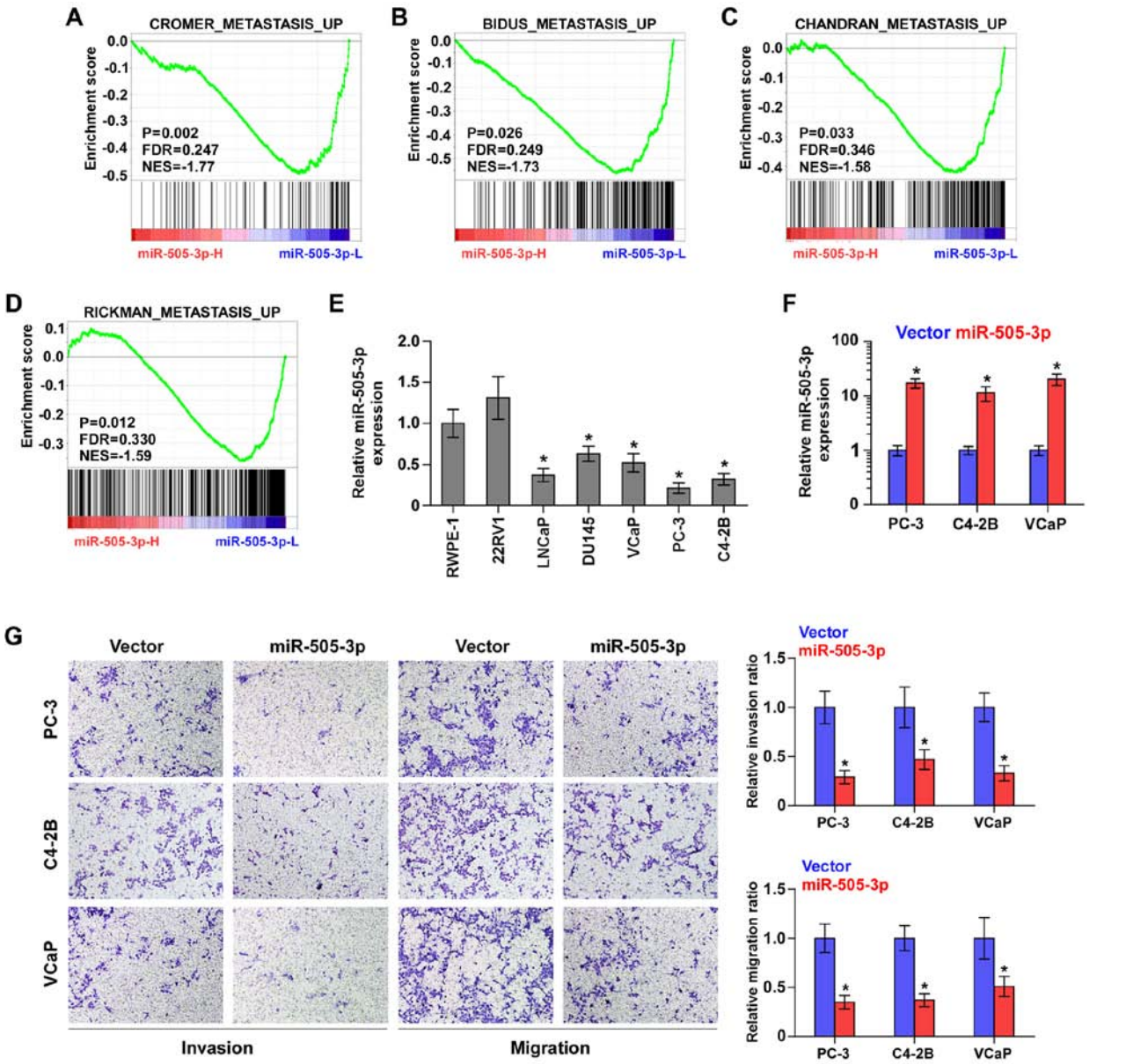


Figure 3. Upregulation of miR-505-3p inhibits the invasion and migration in PCa cells. (A-D) GSEA revealed that miR-505-3p downregulation was significantly and positively associated with metastatic propensity. (E) miR-505-3p expression in normal prostate epithelial cells RWPE-1 and 6 PCa cell lines. *P<0.05. (F) Real-time PCR analysis of miR-505-3p expression after transfection with miR-505-3p mimics in PCa cells. Transcript levels were normalized by *U6* expression. *P<0.05. (G) Overexpression of miR-505-3p suppressed the invasive and migration abilities of PCa cells. *P<0.05. PCa, prostate cancer; GSEA, gene set enrichment analysis.

analysis of PCa tissue samples revealed that miR-505-3p expression was inversely associated with serum PSA levels, Gleason grade, N classification, M classification and bone metastasis status in PCa patients (Table I). Kaplan Meier analysis revealed that miR-505-3p expression levels had no effect on the overall 5-year survival in PCa patients (Fig. 2E); whereas low expression of miR-505-3p was strongly and positively associated with shorter bone metastasis-free survival in PCa patients (Fig. 2F). Thus, our results demonstrated that downregulation of miR-505-3p predicted poor bone metastasis-free survival in PCa patients.

*Upregulation of miR-505-3p inhibits invasion and migration in PCa cells*. To determine the biological role of miR-505-3p in bone metastasis of PCa, we performed gene set enrichment (GSEA) analysis of miR-505-3p expression against the oncogenic signatures collection of MSigDB, and the results revealed that downregulation of miR-505-3p was significantly and positively associated with metastatic propensity (Fig. 3A-D). Therefore, we further examined whether miR-505-3p has an effect on the invasion and migration of PCa cells. We first examined miR-505-3p expression in normal prostate cells (RWPE-1) and 6 PCa cell lines and found that miR-505-3p expression was differentially downregulated in metastatic cell lines, including lymph node metastatic cell line LNCaP, brain metastatic PCa cells DU145 and bone metastatic PCa cell lines (PC-3, C4-2B and VCaP), particularly in PC-3 and C4-2B, but slightly elevated in primary PCa 22RV1 cells (Fig. 3E). This finding further elucidated the pro-metastatic roles of miR-505-3p downregulation in PCa.

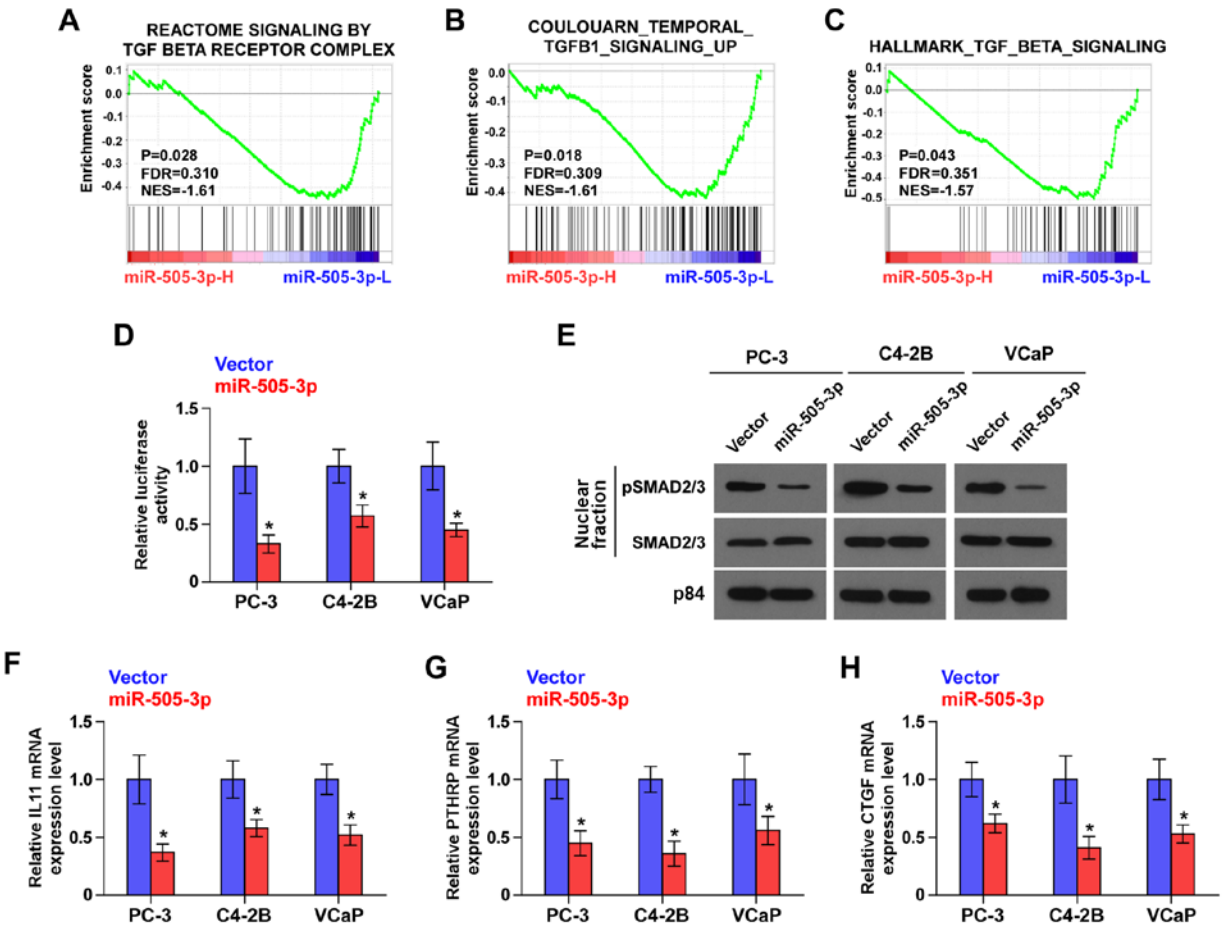


Figure 4. miR-505-3p inhibits the activation of TGF-β signaling by targeting SMAD2 and SMAD3 in PCa cells. (A-C) GSEA revealed that miR-505-3p downregulation was positively associated with TGF-β signaling activity. (D) Transcriptional activity based on a TGF-β/Smad-responsive luciferase reporter as assessed in the indicated cells. *P<0.05. (E) Western blotting of nuclear pSMAD2/3 expression. The nuclear protein p84 was used as a nuclear protein marker. (F-H) Real-time PCR analysis of downstream target gene expression of TGF-β signaling, including IL11, PTHRP and CTGF in the indicated cells. *P<0.05. PCa, prostate cancer; GSEA, gene set enrichment analysis.

According to the expression levels of miR-505-3p shown in Fig. 3E, we upregulated miR-505-3p in bone metastatic PC-3, C4-2B and VCaP cells via transfection with miR-505-3p mimics to further evaluate the effects of miR-505-3p on the invasion and migration of PCa cells (Fig. 3F). As shown in Fig. 3G, miR-505-3p overexpression significantly decreased the invasive and migratory abilities of PCa cells. Therefore, these results indicated that low expression of miR-505-3p was implicated in bone metastasis of PCa cells via promotion of the invasion and migration abilities of PCa cells.

*miR-505-3p inhibits TGF-β signaling activity by targeting SMAD2 and SMAD3*. GSEA analysis of miR-505-3p expression revealed that downregulation of miR-505-3p was positively associated with TGF-β signaling activity (Fig. 4A-C). These results indicated that miR-505-3p may negatively regulate the TGF-β signaling pathway, which has been demonstrated to play an important role in bone metastasis of PCa (24). As shown in Fig. 4D, miR-505-3p overexpression reduced the transcriptional activity of the TGF-β/Smad-responsive luciferase reporter in PCa cells. Cellular fractionation and western blot analysis revealed that overexpression of miR-505-3p decreased nuclear accumulation of pSMAD2/3 (Fig. 4E). Real-time PCR analysis revealed that upregulation of miR-505-3p differentially reduced downstream target genes of TGF-β signaling in PCa cells, including IL11, PTHRP and CTGF (Fig. 4F-H). Thus, these results indicated that miR-505-3p inhibited the activity of TGF-β signaling in PCa cells.

*miR-505-3p directly targets SMAD2 and SMAD3 in PCa cells*. Using the publicly available algorithms TargetScan and miRanda, we found that the critical downstream effectors of TGF-β signaling SMAD2 and SMAD3 were potential targets of miR-505-3p (Fig. 5A). Real-time-PCR and western blot analysis revealed that miR-505-3p overexpression reduced the mRNA and protein expression levels of SMAD2 and SMAD3 (Fig. 5B and C). Luciferase assays revealed that miR-505-3p overexpression suppressed the luciferase reporter activity of SMAD2 and SMAD3, but not the expression of SMAD2 and SMAD3 with the 3'UTR mutation within the miR-505-3p-binding seed regions in PCa cells (Fig. 5D-F). Microribonucleoprotein (miRNP) immunoprecipitation (IP) assays revealed a direct association of miR-505-3p with SMAD2 and SMAD3 transcripts (Fig. 5G-I), further elucidating the direct suppressive effects of miR-505-3p on SMAD2 and SMAD3. Collectively, these results demonstrated that miR-505-3p inhibited the activity of TGF-β signaling by targeting SMAD2 and SMAD3in PCa cells.

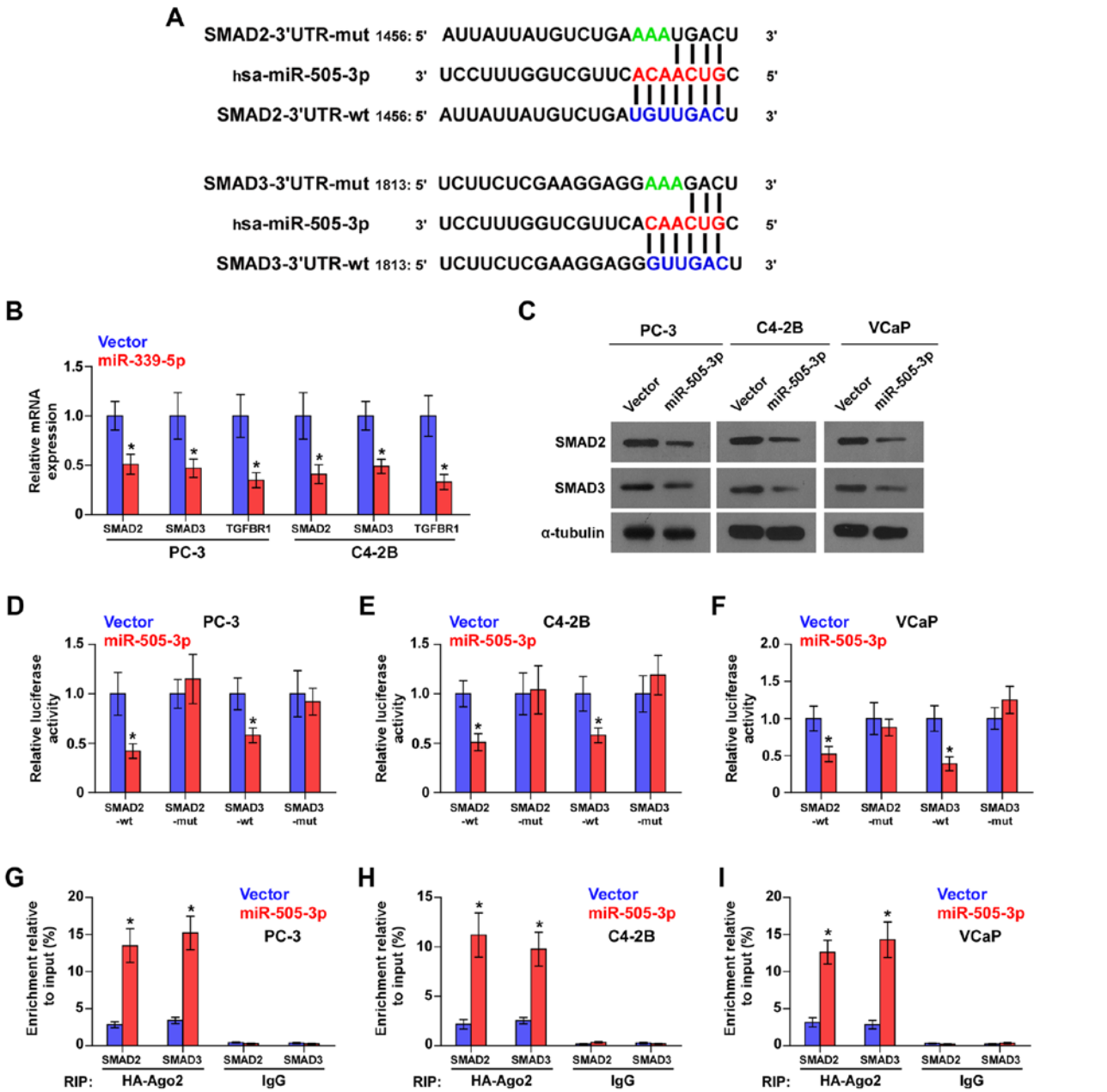


Figure 5. miR-505-3p targets SMAD2 and SMAD3 in PCa cells. (A) Predicted miR-505-3p targeting sequence and mutant sequences in the 3'UTRs of SMAD2 and SMAD3. (B) Real-time PCR analysis of SMAD2, SMAD3 and TGFBR1 expression in the indicated cells. *P<0.05. (C) Western blot analysis of SMAD2 and SMAD3 expression in the indicated cells. α-tubulin served as the loading control. (D-F) Luciferase assay of the 3'UTR reporter of SMAD2 and SMAD3 in the indicated cells. *P<0.05. (G-I) MiRNP IP assay revealing the association between miR-505-3p and SMAD2 and SMAD3 transcripts in PCa cells cells. Pulldown of the IgG antibody served as the negative control. *P<0.05. PCa, prostate cancer; 3'UTRs, 3'untranslated regions.

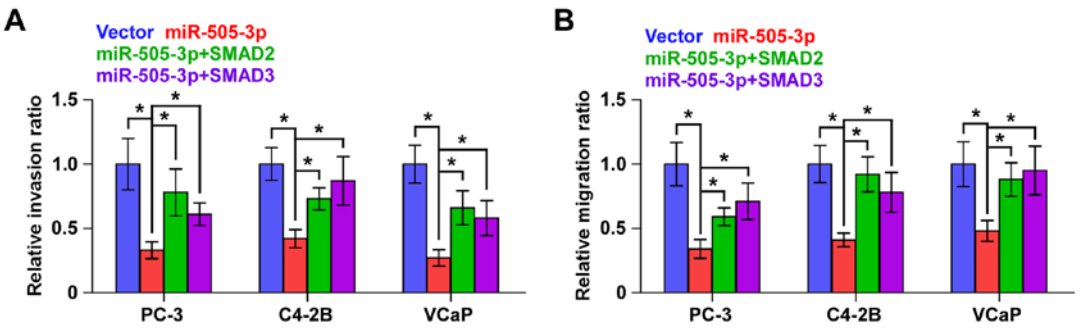


Figure 6. SMAD2 and SMAD3 reverse the effects of miR-505-3p on the invasion and migration in PCa cells. (A) The inhibitory effects of miR-505-3p on the invasion ability of PCa cells were reversed by individual upregulation of SMAD2 and SMAD3. *P<0.05. (B) The inhibitory effects of miR-505-3p on the migration ability of PCa cells were reversed by individual upregulation of SMAD2 and SMAD3. *P<0.05. PCa, prostate cancer.

*SMAD2 and SMAD3 reverse the effects of miR-505-3p on the invasion and migration in PCa cells*. We further investigated whether SMAD2 and SMAD3 had an effect on the invasion and migration abilities in miR-505-3p-overexpressing PCa cells and found that individual upregulation of SMDA2 and SMAD3 markedly reversed the inhibitory effects of miR-505-3p on the invasion and migration abilities in PCa cells (Fig. 6). These results indicated that miR-505-3p suppresses the invasion and migration abilities by inhibiting SMAD2 and SMAD3 activity in PCa cells.

## Discussion

The primary findings of the present study provide novel insights into the important predictive role of miR-505-3p in bone metastasis of PCa. In the present study, we reported that miR-505-3p was significantly downregulated in bone metastatic PCa tissues, and that miR-505-3p expression was inversely associated with poor clinicopathological characteristics and bone metastasis-free survival in PCa patients. Our results further revealed that upregulation of miR-505-3p inhibited TGF-β signaling activity by targeting SMAD2 and SMAD3, which further inhibited the invasion and migration abilities of PCa cells. Therefore, our results provide evidence that miR-505-3p may serve as a novel marker to predict bone metastasis of PCa.

As one of the originally identified miRNAs, miR-505-3p has been reported to be downregulated in several types of cancers, which predicted poor prognosis in cancer patients (25,26). However, miR-505-3p was found to be upregulated in hepatocellular carcinoma, which facilitated the identification of small-size, early-stage, α-fetoprotein-negative hepatocellular carcinoma in patients at risk (27). These findings indicated that the anticancer or pro-cancer role of miR-505-3p in cancer is tumor type-dependent. However, the expression level and biological role of miR-505-3p in bone metastasis of PCa remains largely unknown. In the present study, we found that miR-505-3p expression was downregulated in bone metastatic PCa tissues, which was positively associated with advanced clinicopathological characteristics and poor bone metastasis-free survival in PCa patients. Upregulation of miR-505-3p inhibited the invasion and migration abilities of PCa cells by targeting SMAD2 and SMAD3, leading to the inactivation of TGF-β signaling activity. Therefore, our findings demonstrated that miR-505-3p plays a tumor-suppressive role in bone metastasis of PCa by suppressing the activity of the TGF-β signaling pathway.

Constitutive activation of TGF-β signaling has been reported in bone metastasis of PCa. Fournier *et al* have reported that TGF-β upregulated the negative regulator of the TGF-β pathway PMEPA1, which inhibited bone metastasis of PCa. Notably, disruption of this negative feedback loop using PMEPA1 siRNA promoted bone metastases *in vivo* (28). Furthermore, downregulation of the negative regulator of the TGF-β signaling PICK1 caused by oncogenic miR-210-3p contributed to bone metastasis in PCa (24). In the present study, we found that the critical downstream effectors of TGF-β signaling SMAD2 and SMAD3 were potential targets of miR-505-3p. Through a series of mechanistic experiments, including real-time-PCR, western blotting, luciferase assay and microribonucleoprotein immunoprecipitation, our results demonstrated that miR-505-3p directly targeted SMAD2 and SMAD3 in PCa cells, resulting in the suppression of TGF-β signaling activity. Since the crucial roles of TGF-β signaling in bone metastasis in a variety of cancers (29-33), including PCa (24,28), have been well established, our results uncovered a novel mechanism responsible for the constitutive activation of TGF-β signaling in PCa, providing theoretical evidence that miR-505-3p plays a tumor suppressive role in bone metastasis of PCa.

miRNAs alone or in clusters have been widely documented to serve as potential markers for the diagnosis and prognosis of cancer. Several studies have shown that high levels of miR-96 were significantly correlated with poor overall and recurrence-free survival in PCa patients (34,35). Moreover, a miRNA classifier, including miR-505-3p, served as a potential biomarker for hepatocellular carcinoma, which was valuable in the detection of preclinical hepatocellular carcinoma (27). However, the clinical significance of miR-505-3p in PCa remains largely unknown. In this study, our results demonstratd that low expression of miR-505-3p was positively associated with poor clinicopathological characteristics and shorter bone metastasis-free survival in PCa patients, but had no significant effect on the overall survival in PCa patients. These findings indicated that miR-505-3p holds promise as a novel prognostic marker for bone metastasis of PCa.

miRNAs can not only serve as potential markers for the diagnosis and prognosis of cancer, but also provide data for the optimization and personalization of therapy in the treatment of cancer. miR-505-3p has been reported as a tumor inhibitor in several human cancer types, suggesting the therapeutic application of miR-505-3p in cancer treatment. For example, lentivirus-mediated miR-505-3p upregulation suppressed cervical cancer cell proliferation and invasion *in vitro*. Notably, upregulation of miR-505-3p markedly reduced tumorigenicity of cervical cancer cells *in vivo* (26). In endometrial carcinoma, overexpression of miR-505 reduced tumorigenicity and inhibited the growth of xenograft tumors in a mouse model of endometrial carcinoma (36). Furthermore, several lines of evidence have reported that miR-505-3p expression levels can predict complete chemotherapeutic response in cancer (37-39). Therefore, it is tempting to investigate, although it remains to be studied, the therapeutic application of miR-505-3p in bone metastasis of PCa via an intra-prostatic injection mouse model or clinical trial.

In summary, our results demonstrated that miR-505-3p negatively regulated TGF-β signaling and was inversely associated with bone metastasis-free survival in PCa. An improved understanding of the underlying molecular mechanisms by which miR-505-3p inhibits bone metastasis of PCa and the prognostic values of miR-505-3p expression for bone metastasis of PCa will increase our knowledge of the biological basis of the development of PCa bone metastasis and facilitate the development of antimetastatic therapeutic strategies against PCa.

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## Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

## Authors' contributions

PX and PH conceived, designed, and wrote the study. YT, BW and XL performed the experiments. XH and WZ analyzed the miR-505-3p expression and clinical correlation with clinicopathological characteristics. SH and XP performed the clinical analysis and drafted the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Ethics approval and consent to participate

For the use of the clinical samples for research purposes, prior informed consents from patients and approval from the Institutional Research Ethics Committee were obtained from Jiangmen Central Hospital (Jiangmen, China).

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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