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# β-arrestin1-medieated inhibition of FOXO3a contributes to prostate cancer cell growth in vitro and in vivo

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Recently, β-arrestin1 has been indicated as a prostate cancer promoter through promoting cell proliferation and epithelial to mesenchymal transition, but its underlying mechanism remains unclear. Here, our data revealed that β-arrestin1 could promote cell growth through inhibiting the transcriptional activity and expression of FOXO3a in prostate cancer cells in vitro and in vivo. We found that β-arrestin1 could promote the cell and tumor growth of prostate cancer, and β-arrestin1 expression represented a negative correlation with FOXO3a expression but not FOXO1 expression in prostate cancer cell lines and tissues. In addition, forced expression of β-arrestin1 induced a significant decrease of FOXO3a expression but had no clear effect on FOXO1 expression. Mechanistically, β-arrestin1 could interact with FOXO3a and MDM2, respectively, and promote the interaction between FOXO3a and MDM2, whereas it had no obvious interaction with FOXO1. Furthermore, β-arrestin1 could inhibit the transcriptional activity of FOXO3a via Akt and ERK1/2 pathways. Together, our results revealed a novel mechanism for β-arrestin1 in the regulation of the prostate cancer procession through inhibiting FOXO3a.

**KEYWORDS**
β-arrestin1, cell growth, FOXO3a, prostate cancer, ubiquitylation

## 1 | INTRODUCTION

Prostate cancer (PCa) is the most commonly diagnosed tumor and a major cause of cancer-related deaths among men worldwide.[1] Although androgen-ablation therapies initially lead to disease regression, advanced PCa ultimately progresses to castration-resistant prostate cancer (CRPC), which is refractory to androgen-ablation therapies and remains the major challenge for the treatment of PCa. To overcome the resistance of PCa to androgen-ablation therapies, it is essential to investigate the underlying mechanisms of CRPC cell proliferation and apoptosis.

β-arrestin1 is one of the well-known negative regulators of G-protein-coupled receptor (GPCR) signaling. Accumulating evidence reveals that β-arrestin1 can also serve as an adaptor to guide signals from distinct pathways that play important roles in cell proliferation, apoptosis and cancer progression through interacting with different signaling molecules.[2,3] Recently, β-arrestin1 has been identified as a potential tumor promoter by promoting cell proliferation and epithelial to mesenchymal transition in PCa, but its underlying mechanism is still not well clarified[4,5]

The Forkhead box-O (FOXO) class of transcription factors in mammalian consists of 4 members (FOXO1, FOXO3a, FOXO4 and FOXO6)

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that play a pivotal role in tumor suppression through regulating multiple genes that are involved in cell cycle arrest, DNA damage repair, apoptosis and energy metabolism.[6-8] In PCa, FOXO1 and FOXO3a are the predominantly expressed members of the FOXO subfamily.[9] Previous studies have revealed that decreased transcriptional expressions and activities of FOXO1 and FOXO3a were correlated with increasing tumor grade and associated with cancer progression.[10-13] In addition, both FOXO1 and FOXO3a are involved in the regulation of cell apoptosis, proliferation and invasion in PCa.[9,14-20] However, the precise molecular mechanism of the abnormal expression of FOXO remains unclear.

In the present study, we investigated the role of FOXO in β-arrestin1-medieated cell growth of CRPC cell lines and the results demonstrated for the first time that β-arrestin1 could promote cell growth through inhibiting the activity and expression of FOXO3a, rather than FOXO1, in CRPC cells, thus representing a novel mechanism of β-arrestin1 in the regulation of cell growth via FOXO3a in the prostate cancer process.

## 2 | METHODS

### 2.1 | Cell culture and drugs

All prostate cell lines, including RWPE-1, LNCaP, C4-2, PC3 and DU145, were purchased from ATCC (USA) and cultured, as recommended, in a humidified incubator containing 5% $CO_2$ at 37°C. HEK293 cells were maintained in DMEM) supplemented with 10% FBS, 1% penicillin/streptomycin and 10 mmol/L HEPES buffer. PC3 monoclonal cells expressing pEGFP-N1-β-arrestin1 (GFP-β-arr1) or empty vector pEGFP-N1 (GFP-N1) were generated and cultured in the presence of G418 (0.5 mg/mL). Insulin-like growth factor 1 (IGF-1) and epidermal growth factor (EGF) were purchased from R&D Systems. The proteasome inhibitor MG132, PI3-K inhibitor LY294002 and ERK1/2 inhibitor U0126 were purchased from Selleck Chemicals.

### 2.2 | Cell transfection and plasmids

Short hairpin RNA (shRNA) was synthesized by RiBoBio (China) as previously described.[5] The shRNA sequence targeting human β-arrestin1 was 5′-GGCCTGACCTTTCGCAAGGACTT-3′. The sequence of unrelated shRNA was 5′-TTCTCCGAACGTGTCACGT-3′ (shRNA-NC). The full lengths of β-arrestin1, FOXO3a and MDM2 were cloned into a modified pEGFP-N1 vector or pcDNA3.1 vector in-frame with HA, respectively. FHRE-Luc was a gift from Michael Greenberg (Addgene plasmid #1789). The luciferase reporter 3× IRS-luc was constructed as previously described.[19] For transient transfections, cells were seeded in 60-mm dishes and transfected at 70% confluence. The transfections were conducted with shRNA or plasmids using Lipofectamine LTX according to the manufacturer's instructions (Invitrogen).

### 2.3 | Cell growth assay

The cell growth was detected using trypan blue staining, MTS and colony formation methods. The cell number was counted in triplicates of samples at indicated time points after transfection. The cells were trypzinized and resuspended in a 1:1 mixture of PBS and 0.5% trypan blue, and the number of viable cells was counted using a hemocytometer. The MTS assay was carried out using the Cell Titer 96 AQueous One Solution Cell Proliferation Assay kit (Promega). In brief, equal amounts of cells were seeded in a 96-well plate after transfection and cultured in medium supplemented with serum for 48 hour, at the end of the experiment, 10 μL of MTS (5 mg/mL in PBS) were added and the cells were incubated for 2 hour in the humidified incubator that contained 5% $CO_2$ at 37°C. The relative cell proliferation was obtained by scanning with an ELISA reader with a 490-nm filter. For the colony formation assay, cells transfected with indicated shRNA or plasmids were seeded in 6-well plates at a density of $2 \times 10^3$ per well and grown in a medium containing 10% FBS for 7 days; then the cells were fixed with 4% paraformaldehyde and stained with crystal violet, and the clone number was counted.

### 2.4 | Western blot analysis

Western blot analysis was conducted as previous described.[19] In brief, cells were lysed in RIPA buffer and equal amounts of protein were separated on a 10% SDS polyacrylamide gel, transferred to a nitrocellulose membrane and immunoblotted with antibodies. The primary antibodies used included antibodies against β-arrestin1, phospho-Akt (Ser473), Akt, phospho-ERK1/2, ERK1/2, phospho-FOXO3a (Ser253), phospho-FOXO3a (Ser294), FOXO1 and FOXO3a (Cell Signaling Technology), HA-Tag, GFP-Tag (Abmart), phospho-FOXO1 (Ser256, SAB), MDM2 (Abcam), Ubiquitin and GAPDH (Santa Cruz). The secondary antibodies were anti-mouse and anti-rabbit IgG conjugated with HRP (Santa Cruz). The band intensities were quantified with respect to GAPDH using ImageJ software and presented as bar graphs after testing statistical validity.

### 2.5 | Correlation test

Public data of β-arrestin1, FOXO1 and FOXO3a expressions in PCa patients were obtained from The Cancer Genome Atlas Project (TCGA). After excluding patients with incomplete information or normal types, 498 PCa patients' paired expression data were analyzed in total.

### 2.6 | Luciferase assay

Cells were plated in 24-well plates and the plasmids were transfected using Lipofectamine LTX according to the manufacturer's instructions. After transfections, cell lysates were prepared, and luciferase activity was determined using the Dual-Luciferase Assay (Promega) according to the manufacturer's instructions. The relative luciferase activity was measured as the ratio of firefly luciferase activity to Renilla luciferase activity.

### 2.7 | Immunoprecipitation

Immunoprecipitation was conducted as we previously described.[5,19] In brief, cells were mechanistically broken using a 29-gauge needle in ice-cold RIPA buffer with protease inhibitors and incubated with indicated antibody or control IgG antibody (Santa Cruz) at 4°C for overnight. The lysate antibody mixture was centrifuged at 2500 *g*/min for 5 minute and washed 3 times with lysis buffer. The precipitated proteins were eluted with SDS sample buffer for western blot analyses.

### 2.8 | Nude mice xenograft experiment

Twenty 6-week-old male nude mice were purchased from the Experimental Animal Center of Guangdong Province (Guangzhou, China) and divided into 2 groups of 6 animals each. A total of 100 μL cells ($2 \times 10^6$ cells/mL) were subcutaneously injected into the right side of axillary region of each mouse. Tumor size was measured twice a week with a vernier caliper. Tumor volume was calculated by the formula $0.524 \times (\text{length}) \times (\text{width})^2$. After 4 weeks of observation, the mice were killed and the tumors were dissected, weighted and immunohistochemical stained. All procedures were performed in accordance with the Animal Management Rules of the Ministry of Health of the People's Republic of China, and were approved by the Animal Care Commission of the First Affiliated Hospital of Guangzhou Medical University.

### 2.9 | Immunohistochemical staining

Immunohistochemistry was performed on paraffin-embedded tissue sections from xenograft tumors. The tissue samples were fixed, paraffin-embedded, sectioned at 4-μm thickness and then stained according to standard immunohistochemistry protocol. Images were obtained with a PathScope 4S scanner (DigiPath, USA) and quantified using Image Pro Plus software.

### 2.10 | Statistical analysis

The data are reported as the means ± SD of at least 3 independent experiments. The mean differences were compared using ANOVA and the Student's *t* test. A *P*-value of <.05 was considered to be statistically significant.

## 3 | RESULTS

### 3.1 | β-arrestin1 promotes the cell growth of castration-resistant prostate cancer cells

At first, we investigated the role of β-arrestin1 in the regulation of cell growth in different CRPC cell lines, including PC3 and DU145 cells. As shown in Figure 1, compared with the negative control group, shRNA-mediated knockdown of β-arrestin1 expression significantly decreased the cell growth and colony formation in PC3 and DU145 cells, whereas the forced overexpression of β-arrestin1 had opposite effects, suggesting that β-arrestin1 is required for the cell growth in CRPC cells. These results were consistent with the results by Zecchini et al. (2014) that revealed that β-arrestin1 promotes the cell proliferation of PCa C4-2 cells.[4]

### 3.2 | Correlation between the expressions of β-arrestin1 and Forkhead box-O

As previous studies have revealed that the activities of FOXO, including FOXO1 and FOXO3a, are inhibited by activated Akt, whose activation is mediated by β-arrestin1 in response to growth factor stimulation,[17,21,22] we established their expression levels in distinct types of prostate cell lines, including benign prostate RWPE-1 cells, LNCaP cells and CRPC cells (PC3, DU145 and C4-2). As shown in Figure 2A,B, compared with the benign prostate RWPE-1 cells, the expression of β-arrestin1 in PCa cells was significantly increased, whereas FOXO3a expression was decreased and seemed to be negatively correlated with β-arrestin1 expression, especially in the CRPC cells. In contrast, FOXO1 expression was decreased in PC3 and DU145 cells but increased in LNCaP and C4-2 cells, and had no obvious correlation with β-arrestin1 expression. In addition, the correlation between the β-arrestin1 and FOXO1 (or FOXO3a) expressions in human PCa tissues, which were obtained from 498 PCa patients' expression data from TGCA database, was calculated using both Pearson and Spearman correlation coefficients. By using both statistical analyses, we found that β-arrestin1 and FOXO1 expression were weekly positively correlated (Pearson's coefficient = .127; *P* value < .05, and Spearman's coefficient = .18; *P* value < .001), whereas the correlation between the β-arrestin1 and FOXO3a expression was negatively correlated (Pearson's coefficient = −.27; *P* value < .0001, and Spearman's coefficient = −.31; *P* value < .0001), indicating an inverse relationship between the β-arrestin1 and FOXO3a expression (Figure 2C,D). Because FOXO plays a pivotal role in the regulation of cell proliferation and apoptosis, these results suggested that β-arrestin1 may promote cell growth through inhibiting FOXO3a but not FOXO1 in PCa.

### 3.3 | β-arrestin1 decreases FOXO3a expression via the ubiquitylation pathway

To assess the correlation between β-arrestin1 and FOXO expression, PC3 monoclonal cells with stable overexpression of β-arrestin1 (PC3-β-arr1) were generated through G418 selection. As shown in Figure 3A, there was no obvious difference between the expressions of FOXO1 in PC3-N1 cells and PC3-β-arr1 cells, whereas the FOXO3a expression was significantly decreased in the PC3-β-arr1 cells compared to the PC3-N1 cells. Because ubiquitylation is a key mechanism to FOXO for degradation,[23] we then investigated the effect of β-arrestin1 on FOXO ubiquitylation as previously described[24,25] and found that the ectopic expression of β-arrestin1 markedly increased the ubiquitylation of FOXO3a but had no significant effect on FOXO1 ubiquitylation (Figure 3B

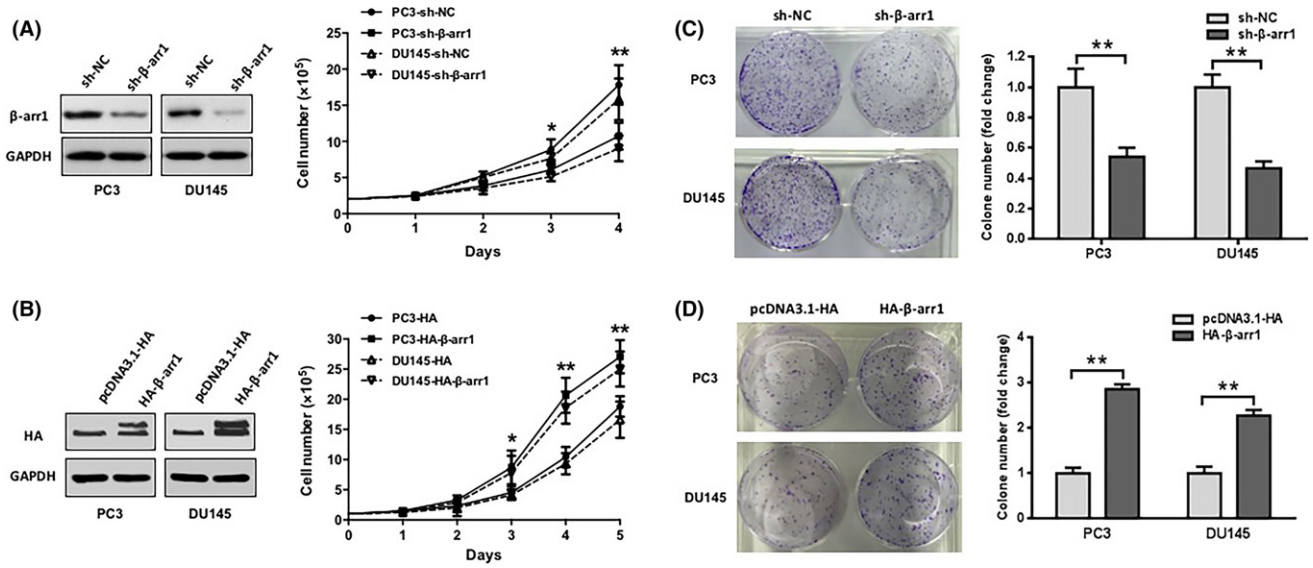


**FIGURE 1** β-arrestin1 promotes the cell growth of castration-resistant prostate cancer (CRPC) cells. A, B, The expressions of the indicated proteins in the cells transfected with indicated shRNA or plasmids were detected using western blot. The cell growth was detected using trypan blue staining and a hemocytometer at indicated days after transfection. C, D, The cells transfected with indicated shRNA or plasmids were seeded in 6-well plates and cultured for 7 d, then cells were fixed and stained. The clone number was counted and expressed as a fold change of the control group. * denotes $P \leq .05$; ** denotes $P \leq .01$. β-arr1, β-arrestin1; NC, negative control

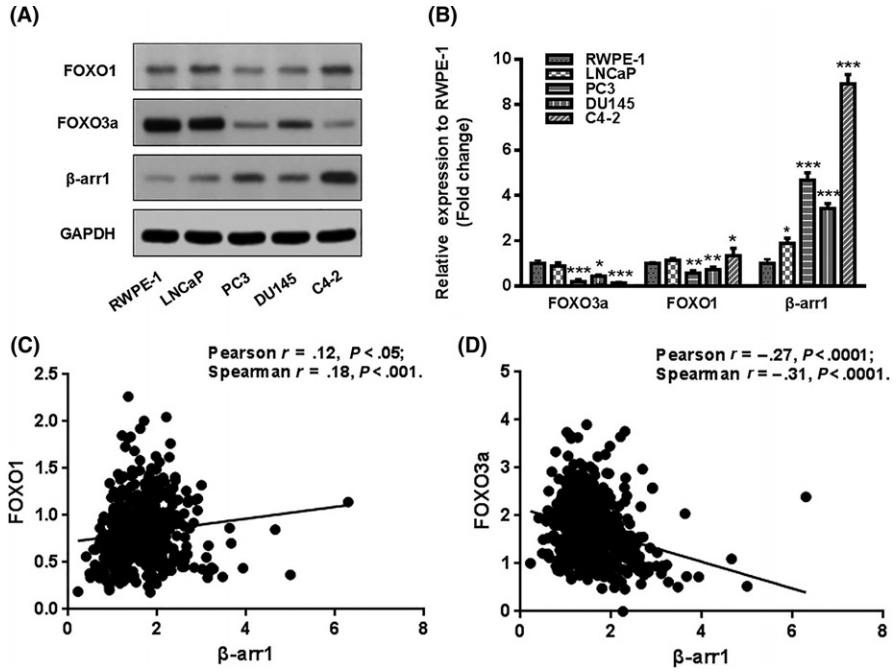


**FIGURE 2** β-arrestin1 and Forkhead box-O (FOXO) expressions in human prostate cells and tissues. A, B, The expressions of indicated proteins in distinct prostate cell lines were detected using western blot and quantified with respect to GAPDH using ImageJ software. C, D, Correlation between β-arrestin1 and FOXO expression in human prostate cancer tissues. * denotes $P \leq .05$, **denotes $P \leq .01$ and *** denotes $P \leq .001$ vs RWPE-1

and Figure S1). Moreover, previous studies have revealed that β-arrestins could scaffold the MDM2 E3 ubiquitin ligase to target protein and facilitate the proteasomal degradation of target protein, and the ubiquitylation modification of FOXO could be mediated by MDM2;[24-26] we then examined whether β-arrestin1 serves as an adapter to promote the interaction between MDM2 and FOXO, and the results revealed that β-arrestin1 could form a complex with FOXO3a and MDM2, respectively, but not FOXO1

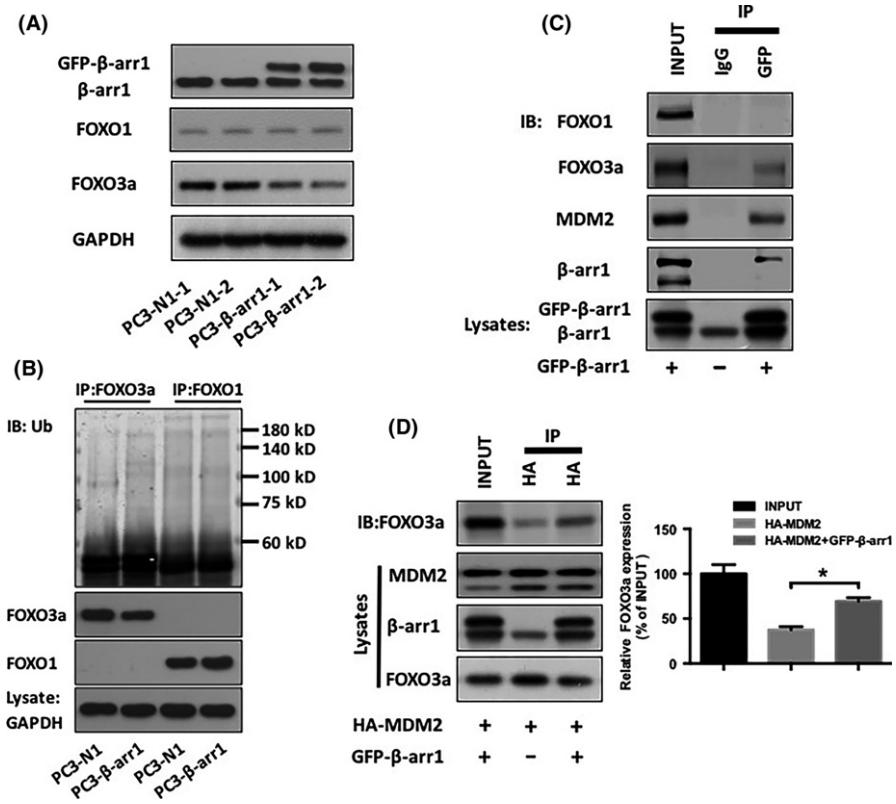


**FIGURE 3** β-arrestin1-mediated Forkhead box-O (FOXO) expression and ubiquitylation. A, PC3 monoclonal cells that stably expressed GFP-N1 or GFP-β-arrestin1 were cultured in a growth medium for 24 h, and the expressions of the indicated proteins were detected using western blot. B, PC3 monoclonal cells were cultured in a growth medium for 24 h and treated with or without MG132 (10 μmol/L) for 6 h; then the cell lysates of PC3 monoclonal cells were subjected to immunoprecipitation with FOXO1 and FOXO3a antibody, respectively, and ubiquitination was detected using western blot with anti-ubiquitin antibody. The cell lysates were probed with anti-GAPDH antibody to demonstrate the total protein expression. C, D, Forty-eight hours after transfection with the indicated plasmids, the cell lysates and immunoprecipitated proteins were analyzed using western blotting with the indicated antibodies. The FOXO3a expression (D, top line) was quantified using ImageJ software. * denotes $P \leq .05$

(Figure 3C). In addition, β-arrestin1 significantly accelerated the interaction between FOXO3a and MDM2 (Figure 3D). Taken together, these data suggested that β-arrestin1 could downregulate FOXO3a expression, at least partially, through promoting the MDM2-mediated ubiquitylation pathway, whereas it has no obvious effect on FOXO1 expression.

## 3.4 | β-arrestin1 inhibits the transcriptional activity of FOXO3a

As FOXO is functionally inhibited by phosphorylation in response to IGF-1 and EGF stimulation through Akt and ERK1/2 kinase, whose activations were mediated by β-arrestin1 in response to growth factor stimulation, respectively,[17,22,24,27] we then investigated the role of β-arrestin1 in IGF-1-induced and EGF-induced phosphorylation of FOXO. Compared with the negative control group, the knockdown of the endogenous expression of β-arrestin1 significantly decreased IGF-1-induced or EGF-induced phosphorylation of FOXO3a, as well as the expression of total FOXO3a (Figure 4A,B). In addition, both LY294002 (an Akt inhibitor) and U0126 (an ERK1/2 inhibitor) could significantly attenuate β-arrestin1-promoted phosphorylation of FOXO3a in response to IGF-1 or EGF stimulation, respectively, suggesting that β-arrestin1 could decrease the transcriptional activity of FOXO3a via Akt and ERK1/2 pathways (Figure 4C,D). By contrast, although the effect of β-arrestin1 on IGF-1-induced phosphorylation of FOXO1 was similar to its effect on FOXO3a, β-arrestin1 had no obvious effect on FOXO1 expression, and EGF stimulation had no significant effect on the FOXO1 phosphorylation (Figure 4A-D). Furthermore, the ectopic expression of β-arrestin1 resulted in a significant decrease in the transcriptional activity of endogenous FOXO3a and obviously decreased the reporter gene activity activated by exogenous FOXO3a, suggesting that β-arrestin1 inhibits the transcriptional activities of both endogenous and transfected FOXO3a (Figure 4E). In contrast, β-arrestin1 had no obvious effects on the transcriptional activities of both endogenous and exogenous FOXO1 (Figure 4F).

## 3.5 | FOXO3a attenuates β-arrestin1-induced cell growth in castration-resistant prostate cancer cells

To assess the role of FOXO3a in the regulation of β-arrestin1-mediated cell growth. HA-β-arrestin1 and GFP-FOXO3a were

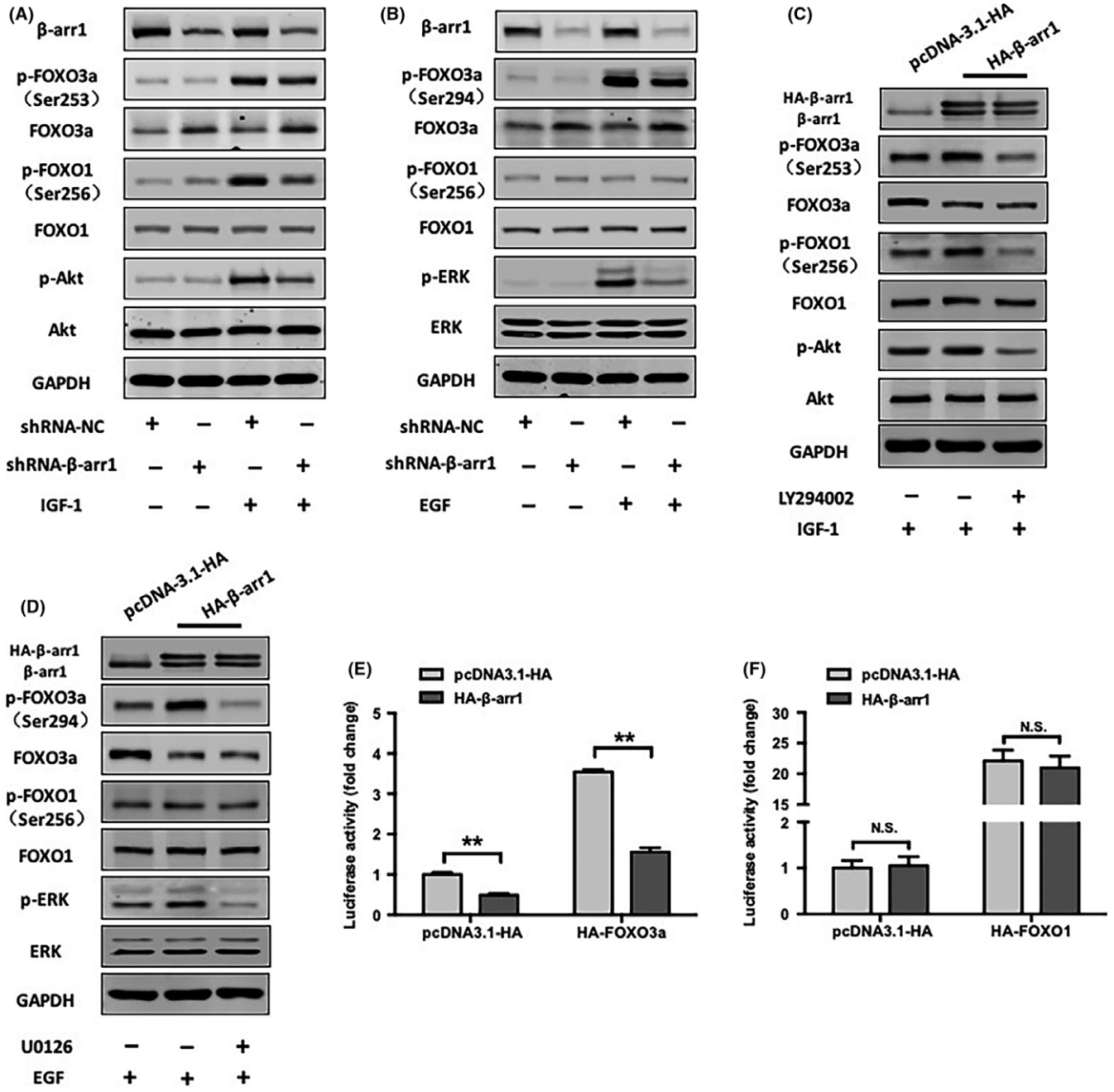


**FIGURE 4** β-arrestin1-mediated transcriptional activity of FOXO3a. A, B, Forty-eight hours after transfection with the indicated shRNA, C4-2 cells were incubated in serum-free medium for 1 h and followed by IGF-1 (100 ng/mL) or epidermal growth factor (EGF; 100 ng/mL) stimulation for 10 min. The expressions of the indicated proteins were subsequently detected using western blot. C, D, Forty-eight hours after transfection with the indicated plasmids, RWPE-1 cells were incubated in serum-free medium for 1 h followed by LY294002 (50 μmol/L) or U0126 (10 μmol/L) treatment for 1 h prior to IGF-1 or EGF stimulation (100 ng/mL, 10 min). The expressions of the indicated proteins were subsequently detected using western blot. E, F, RWPE-1 cells were transiently cotransfected with FHRE-Luc or 3× IRS-luc plasmid along with the indicated plasmids. Luciferase measurement and data analysis were performed 24 h after transfection. ** denotes $P \leq .01$. N.S., not significant

cotransfected into CRPC cells (Figure 5A); then the cell growth was detected using MTS and colony formation methods. As shown in Figure 5B, compared with the control group, the cell proliferation was significantly accelerated in the cells transfected with HA-β-arr1, whereas the cell proliferation was markedly inhibited in the cells transfected with GFP-FOXO3a. In addition, the ectopic expression of FOXO3a 'clearly attenuated HA-β-arr1-induced cell proliferation in PC3 and DU145 cells. Meanwhile, the colony formation assay presented similar results to the MTS detection (Figure 5C). These findings, together with the above results, suggested that β-arrestin1-induced cell growth of CRPC cells, at least in part, is mediated by FOXO3a.

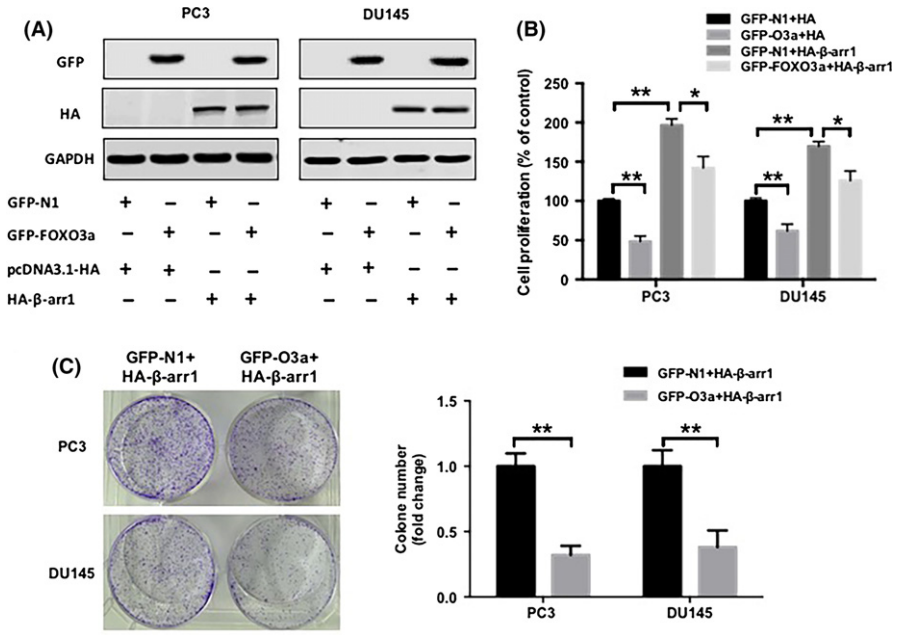


**FIGURE 5** FOXO3a attenuates β-arrestin1-induced cell growth of castration-resistant prostate cancer (CRPC) cells. A, Forty-eight hours after transfection, the expressions of the indicated proteins in the cells transfected with indicated plasmids were detected using western blot. B, Twenty-four hours after transfection, equal amounts of cells were seeded in a 96-well plate and cultured for 2 d; then the cell proliferation was detected using MTS array and expressed as a percent of the control group which were transfected with GFP-N1 + HA. C, Colony formation assay was carried out as described in Figure 1. * denotes $P \leq .05$ and ** denotes $P \leq .01$

### 3.6 | Effects of β-arrestin1 on castration-resistant prostate cancer cell growth and FOXO3a expression in vivo

Finally, nude mice xenograft tumor model assays were conducted to further confirm the effects of β-arrestin1 on CRPC cell growth and FOXO3a expression in vitro. As shown in Figure 6A, the tumor volumes were significantly larger in the mice of the PC3-β-arr1 group than those of the PC3-N1 group at the indicated time point of the experiment. The representative images of the tumors excised from each group are shown in Figure 6B. In addition, the average weight of tumors was significantly higher in the PC3-β-arr1 group compared with the PC3-N1 group (Figure 6C). Furthermore, immunohistochemical staining also revealed that the FOXO3a expression in the tumors of the PC3-β-arr1 group was significantly lower than that in the PC3-N1 group (Figure 6D). Together, these results suggested that β-arrestin1 could accelerate PCa growth through, at least in part, decreasing FOXO3a in vivo.

## 4 | DISCUSSION

In the present study, our data indicated that β-arrestin1 could promote the cell growth of CRPC cell lines, which is associated with its inhibitory effects on the activity and expression of FOXO3a but not FOXO1, thereby representing a novel potential mechanism of CRPC progress mediated by β-arrestin1.

As negative regulators of GPCR-mediated signaling, β-arrestins, including β-arrestin1 and β-arrestin2, can also function as scaffold proteins and interact with various signaling molecules to regulate different signaling pathways.[3] Previous studies have indicated that β-arrestins play an impressive role in tumor progression through regulating signal transductions which are responsible for tumor viability and metastasis.[2,28] Although the role of β-arrestin1 in cell survival and proliferation has been well established in ovarian, gastric, lung and breast cancers,[28] and more recently, β-arrestin1 was identified to act as a potential tumor promoter in PCa, its underlying mechanism has still not been well clarified.[4,5]

In the present study, our results revealed that similar to its roles in lung, breast and liver cancer, β-arrestin1 promoted the cell growth of CRPC cells in vitro and in vivo.[29-32] We investigated the basal expression of β-arrestin1 in distinct prostate cell lines and found that compared with benign prostate RWPE-1 cells, β-arrestin1 expression was significantly increased in PCa cells. In addition, our results revealed that the faster growing, highly tumourigenic and more aggressive PCa cells, including PC3, DU145 and C4-2, display higher β-arrestin1 expression compared to LNCaP cells, suggesting that β-arrestin1 may exert a regulatory function in CRPC progress. This result is consistent with a previous report that had not compared the β-arrestin1 expression between RWPE-1 and PCa cells.[4] Interesting, there was an inverse relationship between the β-arrestin1 and FOXO3a expression, whereas the β-arrestin1 and FOXO1 expression were positively correlated in human PCa tissues. However, in general, all FOXO could regulate the same set of genes through binding to their shared DNA-binding sequence, and, indeed, specificity of the individual FOXO is likely to be obtained through interactions with coregulators.[6] Here, our data suggested that β-arrestin1-induced cell growth of CRPC cells may be mediated by FOXO3a rather than FOXO1.

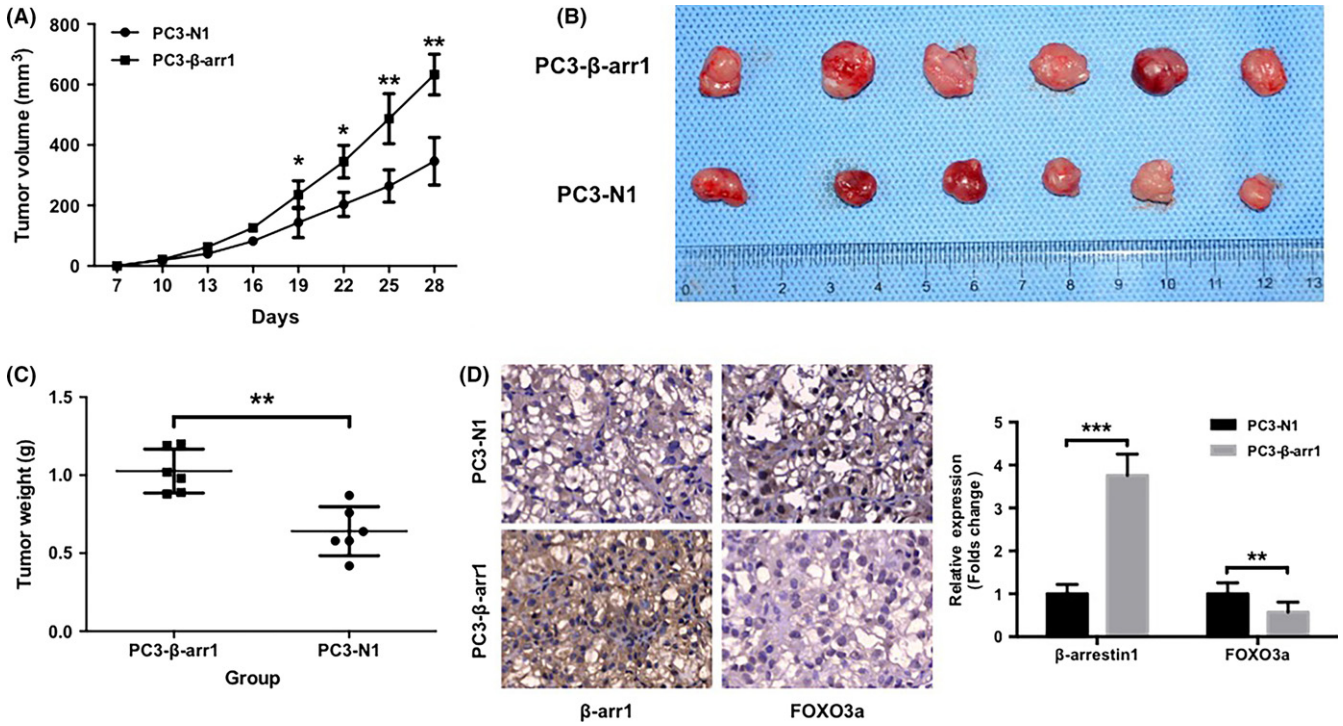


**FIGURE 6** Effects of β-arrestin1 on tumor growth in vivo. A, The xenograft tumor volumes of nude mice injected with PC3-N1 cells or PC3-β-arr1 cells (n = 6). B, The representative photograph of tumors derived from nude mice. C, The tumor weight of nude mice in PC3-N1 and PC3-β-arr1 groups (n = 6). D, Immunohistochemical staining and quantification for β-arrestin1 and FOXO3a in tumor samples. Magnification: ×400. * denotes $P \leq .05$ and ** denotes $P \leq .01$

To assess the correlation between the expressions of β-arrestin1 and FOXO, PC3 monoclonal cells with stable overexpression of β-arrestin1 were generated and the results showed that the forced expression of β-arrestin1 clearly decreased FOXO3a expression but had no obvious effect on the expression of FOXO1. In addition, the results of nude mice xenograft tumor model assays revealed that forced expression of β-arrestin1 could induce the decrease of FOXO3a expression. In general, FOXO1 and FOXO3a are dysregulated in a series of cancers, including PCa, but its underlying mechanism is remains unclear.[7] Because the ubiquitin-mediated degradation of FOXO plays a pivotal role in tumorigenesis,[23] we examined the effect of β-arrestin1 on FOXO ubiquitylation and found that the ectopic expression of β-arrestin1 markedly increased the ubiquitylation of FOXO3a but had no significant effect on FOXO1. Furthermore, as one of the well-characterized binding partners of MDM2, β-arrestin1 acts as an essential adaptor for MDM2 to mediate the ubiquitylation and degradation of isulin-like growth factor 1 receptor (IGF-1R).[26,33] Similar to its effect on IGF-1R, our results revealed that β-arrestin1 could form a complex with FOXO3a and MDM2, whereas it could not interact with FOXO1, demonstrating that β-arrestin1 could decrease FOXO3a expression through the MDM2-mediated ubiquitylation pathway, thus representing a novel mechanism of FOXO3a degradation during CRPC procession. In contrast, our previous study revealed that β-arrestin2, another key member of β-arrestins, could interact with FOXO1 and promote the ubiquitylation and degradation of FOXO1.[19] Thus, the different effects on the regulation of FOXO between β-arrestin1 and β-arrestin2 suggested that β-arrestin1 and β-arrestin2 may promote the prostate cancer process through a distinct mechanism.

The function of FOXO3a is regulated by posttranslational modifications, such as phosphorylation, ubiquitination and acetylation.[8,34] As a key downstream target of Akt and ERK1/2, FOXO3a is functionally inhibited by phosphorylation in response to IGF-1 or EGF stimulation. Phosphorylated Akt or ERK1/2 phosphorylates FOXO3a and results in the inactivation of FOXO3a, as well as its translocation from the nucleus to the cytoplasm, which leads to the transcription suppression of pro-apoptotic genes. Because β-arrestin1 is required for growth factor-induced activation of Akt and ERK1/2,[22,27] there is no surprise that β-arrestin1 is required for IGF-1 or EGF-induced phosphorylation of FOXO3a. Interestingly, our results revealed that β-arrestin1 could inhibit the transcriptional activities of both endogenous and transfected FOXO3a without growth factor stimulation, suggesting that some other unclear mechanisms were involved in the β-arrestin1-mediated regulation of FOXO3a transcriptional activity in CRPC cells. In addition, β-arrestin1 had similar effects on IGF-1-induced phosphorylation of FOXO1 and FOXO3a, but EGF stimulation had no significant effect on FOXO1 phosphorylation, which was different from its effect on FOXO3a.

In conclusion, our data revealed that β-arrestin1 could promote the cell growth of CRPC cells through, at least in part, inhibiting FOXO3a in vitro and vivo, thus representing a novel mechanism of β-arrestin1-mediated cell growth via FOXO3a in PCa cells, as well as the degradation mechanism of FOXO3a mediated by β-arrestin1 via the MDM2-mediated ubiquitylation pathway. We expect that our findings on β-arrestin1-mediated regulation of FOXO3a's activity

and expression will provide useful information for the development of effective therapies against PCa.

## CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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