Pathological significance and prognostic role of LATS2 in prostate cancer

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Abstract

Background: Large tumor suppressor 2 (LATS2) is an important regulator of the Hippo pathway and it plays crucial roles in cell survival and behaviors. Herein, we evaluated the pathological roles of LATS2 in prostate cancer (PC), for which very little information is available.

Methods: Cell proliferation, migration, and invasion in response to the siRNA-mediated knockdown (KD) LATS2 expression were evaluated in two PC cell lines (LNCaP and PC3). The expression of LATS2 in specimens from 204 PC patients was investigated immunohistochemically, and the relationships between its expression and clinicopathological features, proliferation index (PI; measured using an anti-Ki-67 antibody), and biochemical recurrence (BCR) were investigated.

Results: KD of LATS2 increased the growth, migration, and invasion in LNCaP cells and only increased migration in PC3 cells. The expression of LATS2 was negatively associated with the grade group, T, N, M stage, and PI. In addition, the expression of LATS2 was a useful predictor of the histological effects of neoadjuvant hormonal therapy and BCR-free survival periods. A multivariate analysis model including clinicopathological features showed that negative expression of LATS2 had a significantly higher risk of BCR (odds ratio = 2.95, P < 0.001).

Conclusions: LATS2 acts as a tumor suppressor in PC. LATS2 expression is a useful predictor for BCR. LATS2-related activities are possibly dependent on the androgen-dependency of PC cells. Therefore, we suggest that LATS2 could be a potential therapeutic target and a useful predictor for outcome in patients with PC.

Keywords: biochemical recurrence, malignant aggressiveness, prostate cancer, LATS2, tumorigenesis

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1 | INTRODUCTION

The Hippo pathway, an intracellular information transmission pathway, is an important regulator of organ size and tissue homeostasis under physiological and pathological conditions.\textsuperscript{1,2} Several investigators have recently focused attention on pathological roles of the Hippo pathway, such as in the growth, invasion, and outcome of various cancers.\textsuperscript{3,4} Yes-associated protein (YAP) is believed to be a key regulator of the Hippo pathway.\textsuperscript{5} The expression of YAP is positively associated with the proliferation, invasion, and metastasis of malignant cells, and with the prognosis in many types of cancers.\textsuperscript{6-8} The pathological activities of YAP are regulated by other components of the Hippo pathway. Human large tumor suppressor (LATS) proteins, namely, LATS1 and LATS2, are important inhibitors of the Hippo-YAP pathway.\textsuperscript{9,10} In vivo and in vitro studies have shown that, LATS1/2 are negatively associated with carcinogenesis, malignant potential, and cancer cell progression.\textsuperscript{11,12}

Prostate cancer (PC) is the most common cancer in men, and the prognosis has improved with the development of new treatment agents and methods.\textsuperscript{13-15} However, there is several limitations in the treatment. Development of new treatment strategies for patients with castration-resistant PC (CRPC) is essential, because their survival remains unsatisfactory despite the use of new treatment methods.\textsuperscript{16} Neoadjuvant hormonal therapy (NHT) before radical prostatectomy (RP) improves the outcome in some patients; however, there is no useful predictive marker.\textsuperscript{17} Therefore, detailed understanding of the pathological characteristics and the molecular regulatory mechanisms of PC is essential to devise novel therapeutic strategies for PC.

The Hippo pathway plays important roles in carcinogenesis and malignant aggressiveness of PC cells.\textsuperscript{18,19} Most studies on the pathological significance and prognostic roles of the Hippo pathway in PC have focused on the YAP-axis of the Hippo pathway. In recent years, the pathological roles of LATS1 in PC have become clear.\textsuperscript{20,21} LATS2 suppresses tumor growth and progression of PC cells\textsuperscript{12,22,23}, however, detailed pathological roles, such as the relationship between LATS2 expression and pathological features including grade group (GG) and metastasis, tumor growth, and prognosis in patients with PC, are not fully understood. Therefore, we aimed to unravel the relationships between the expression of LATS2 and tumorigenesis, malignant aggressiveness including tumor growth, invasion, and metastasis, and outcome in PC through in vivo and in vitro studies. In addition, the prognostic role of LATS2 expression on the histological effects of NHT and biochemical recurrence (BCR) was analyzed using univariate and multivariate analyses, in PC patients subjected to RP.

supplemented with 10% fetal bovine serum at 37 °C in an atmosphere of 5% CO\textsubscript{2} and 95% air. PC-3 cells were cultured in ATCC-formulated F-12K medium supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO\textsubscript{2} and 95% air. LATS2 was knocked down in both these cell lines using Lipofectamine\textsuperscript{®} RNAiMAX reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA), according to the manufacturer’s protocol. The expression of LATS2 was confirmed using western blot analysis. Clones of cells exhibiting knockdown (KD) of LATS2 were selected for use in further experiments. To rule out nonspecific effects, a group of cells was simultaneously transfected with a negative control siRNA (QIAGEN, Maryland, USA). Detailed methods about cell culture and siRNA are provided in our pervious report.\textsuperscript{24}

2.2 | Evaluation of proliferation, migration, and invasion of prostate cancer cells in vitro

To evaluate the proliferation and growth of cancer cells, we performed the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described in our previous report.\textsuperscript{24} Briefly, the relative numbers of viable cells were determined using the MTT assay kit (Roche Molecular Systems, Inc., CA). LNCaP and PC3 cells were grown in 96-well plates, and MTT labeling reagent was added to each well of the plate. The cultures were incubated at 37 °C for 4 h. The solubilization solution was added to each well and the cells the plates were incubated overnight in a humidified atmosphere. The number of cells was determined by measuring the absorbance at 550 nm.

Cell migration was evaluated using the CytoSelect™ 24-well wound healing assay kit (CELL BIOLABS, INC. San Diego, CA); inserts were used to create a wound area with a defined 0.9 mm gap, and cell migration rates were measured. LNCaP cells were incubated for 48 h and PC3 cells were incubated for 12 h.

The invasive potential of LNCaP and PC-3 cells, in response to knockdown (KD) of LATS2 expression, was assessed using CytoSelect™ 24-Well Cell Invasion Assay, according to the manufacturer’s protocol. The respective wild type (WT) cells were used as the control. A suspension of these cells was added to the membrane insert and incubated for 24 h. The cells that passed through the polycarbonate membrane were transferred to a new well containing a cell staining solution, and the absorbance was measured using a plate reader (at 560 nm).

2.3 | Western blot analysis

Cultured cells were harvested and lysed. Equal amounts of proteins were electrophoresed on Criterion™ TGX™ precast gels (Bio-Rad Laboratories, Inc. Hercules, CA, USA) and transferred onto a nitrocellulose membrane. After blocking with 5% skimmed milk prepared in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) for 1 h at room temperature, the membranes were incubated
overnight with anti-LATS2 antibody (Abcam PLC, Cambridge, UK) at 4 °C. After three washes with TBS-T, the membrane was incubated with the appropriate secondary antibody for 1 h at room temperature. Specific protein bands were detected using ECL Prime (Cytiva Inc., Marlborough, MA, USA).

2.4 | Patients and human prostatic tissues

We determined the expression of LATS2 in 204 PC patients, who were histologically diagnosed with adenocarcinoma at Nagasaki University Hospital. The staging of the cancer was done using magnetic resonance imaging of the prostate, computed tomography of the abdomen and pelvis, bone scanning, and lung X-ray photography. Among the 204 patients, 133 patients with organ-confined PC were treated with RP with (n = 60) or without (n = 73) NHT. Detailed information on NHT is provided in our previous report.25 Sixty non-tumoral specimens obtained through transurethral resection (TUR) from patients with benign prostatic hyperplasia were also stained for LATS2. When serum concentrations of prostate-specific antigen were ≥ 20 ng/mL, the patients were considered to be exhibiting BCR. The histopathological effects of NHT were evaluated based on the Japanese Urological Association guidelines in the “General Rule for Clinical and Pathological Study on Prostate Cancer.” In short, the histological response to NHT was classified according to cancer cell viability as follows: grade 0, ≥ 50% of the cancer cells were viable; grade 1, non-viable cells were < 25% of the total number of cancer cells; grade 2, non-viable cells were ≥ 50% of the total number of cancer cells; and grade 3, almost all cancer cells were non-viable or were not detected. This criterion was used to evaluate the anticancer effects of NHT in organ-confined PC patients subjected to RP.26

This study protocol was approved by the Ethics Committee of the Nagasaki University Hospital (No. 12052899). All procedures involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments. Written informed consent was obtained from all participants.

2.5 | Immunohistochemical staining

Immunohistochemical staining was performed using formalin-fixed and paraffin-embedded samples. Five-micrometer thick sections were deparaffinized and rehydrated; antigen retrieval was performed at 96 °C for 40 min and at 121 °C for 15 min in 0.01 M sodium citrate buffer (pH 6.0) for anti-LATS2 and anti-Ki-67 antibodies, respectively. To block endogenous peroxidase, all the slides were treated with 3% hydrogen peroxide for 30 min, and then incubated overnight at 4 °C with the anti-LUTS2 (Abcam, MA) or anti-Ki-67 (Dako Corp., Glostrup, Denmark) antibody. All the slides were then treated with peroxidase using the labeled polymer method using EnVision+ Peroxidase (Dako Corp., Glostrup, Denmark) for 60 min. Peroxidase was visualized using a liquid 3,3-diaminobenzidine tetrahydrochloride (DAB) substrate kit (Thermo Fisher Scientific, Rockford, IL).

The expression of LATS2 was evaluated using a semi-quantitative method to assess both the intensity and extent of staining, as described in a previous report.27 In short, the intensity was scored as 0 (no staining), 1 (weak), 2 (moderate), or 3 (strong), and the extent of staining was scored on a scale of 0 to 3, according to the percentage of cells (0%, 0.1%–10%, 10.1%–50%, or 50.1%–100% for scores 0, 1, 2, and 3, respectively). Scores of 0–3 were considered as low expression, and scores 4–9 were considered as high expression of LATS2.27 For determining the proliferation of cancer cells, the proliferation index (PI) was calculated as follows: PI (%) = number of Ki-67-positive cancer cells/total number of cancer cells × 100. When PI was over the median (> 7.4%), the tissues were considered as having a high PI. Skeletal muscle (stained with the anti-LATS2 antibody) and tonsil (stained with the anti-Ki-67 antibody) tissues were used as positive controls.

2.6 | Statistical analyses

Data are expressed as mean ± standard deviation (SD). The Student’s t-test or Mann–Whitney U-test was performed for continuous

**FIGURE 1** Immunoreactivity of LATS2 in non-tumoral glands (A) and prostate cancer tissues (B). Magnification ×400
variables. The chi-square test was used for categorical comparisons. To determine the predictive value for BCR, Kaplan–Meier survival curves and log-rank tests were used, and multivariate Cox proportional hazards analysis was also performed (described as hazard ratios [HRs] with 95% confidence intervals [CIs], together with p values). The crude and adjusted effects were estimated using the logistic regression analysis (described as odds ratios [ORs] with 95% CIs, together with p values). In some statistical analyses, GG1–2 and GG3–5 were classified as low and high GG, respectively. For T staging, T3 or 4 disease was classified as having a high T stage. Significance was defined as p < .050. Statistical analyses were performed on a personal computer using the StatView for Windows (version 5.0; Abacus Concepts, Berkeley, CA).

3 | RESULTS

3.1 | Expression of LATS2 in human prostate tissues

LATS2 immunostaining was detected mainly in the cytoplasm in both the non-tumoral gland (A) and PC tissues (B); the ratio of positive staining for LATS2 in PC tissues (104 of 204 specimens, 52.0%) was significantly lower (p < .001) than that in non-tumoral glands (48 of 60 specimens, 80.0%) (Figure 1). The relationships between the expression of LATS2 and clinicopathological features are shown in Table 1. In GG1 specimens, the ratio of positive staining for LATS2 was 75.6%; while in GG5 specimens, it was only 21.1%. Therefore, there is a significant negative correlation between GG (p < .001). In addition, the expression of LATS2 has a negative correlation with each TNM classification (Table 1). The ratio of positive staining for LATS2 in T4 tumors (21.7%) was significantly lower (p < .001) than that in T1 (67.6%), and a negative relationship was observed with the metastasis into lymph node (p = .038) and distant organs (p < .001).

3.2 | Correlation with proliferation, migration, and invasion of prostate cancer cells

The downregulation of LATS2 in LNCaP and PC3 cells, following the transfection of siRNA, was confirmed (Figure 2A). The growth curves showed that the proliferation of LATS2-KD LNCaP cells was significantly higher, when compared to that of the control cells; however, there was no significant difference in proliferation between the control and LATS2-KD PC-3 cells (Figure 2B). The KD of LATS2 expression resulted in a significant increase in the migration of both LNCaP and PC-3 cells (p = .033 and p < .001, respectively; Figure 2C). The KD of LATS2 increased the invasion of LNCaP (p = 0.039); however, no significant change was found in PC-3 cells (Figure 2D).

We investigated the relationship between malignant behavior and the expression of LATS2 in human PC tissues. With regard to cancer cell proliferation, the PI in LATS2-positive tissues (6.2/4.1%) was significantly lower (p < .001) than that in LATS2-negative tissues (10.6/4.7%). In addition, univariate logistic regression analyses showed that negative staining for LATS2 was associated with a 6.34-times higher risk for high PI; a similar finding was obtained using multivariate analysis model, including high GG, high T stage, and presence of metastasis (Table 2). To investigate the relationship between the expression of LATS2 in human PC tissues and the invasive potential, the relationship between LATS2 and T stage was analyzed. LATS2-negative PC tissues had a 3.38-times higher risk of high T stage, compared to that of LATS2-positive tissues (Table 2), and a

| TABLE 1 Correlation with clinicopathological features |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Variables                   | N                          | LATS-2 expression           | p value                     |
| At diagnosis                |                             |                             |                             |
| Grade group (GG)            |                             |                             | .001                        |
| GG1: N/%                    | 45                         | 11/24.4                     | 34/75.6                     |
| GG2                         | 44                         | 13/29.2                     | 31/30.8                     |
| GG3                         | 35                         | 21/60.0                     | 14/40.0                     |
| GG4                         | 42                         | 23/54.8                     | 19/45.2                     |
| GG5                         | 38                         | 30/78.9                     | 8/21.1                      |
| Low GG (1–2)                | 89                         | 24/27.0                     | 65/73.0 < .001             |
| High GG (3–5)               | 105                        | 74/64.3                     | 41/35.7                     |
| T stage                     |                             |                             | .001                        |
| T1                          | 37                         | 12/32.4                     | 25/67.6                     |
| T2                          | 92                         | 36/39.2                     | 56/60.9                     |
| T3                          | 52                         | 32/61.5                     | 20/38.5                     |
| T4                          | 23                         | 18/78.3                     | 5/21.7                      |
| Low T stage (1–2)           | 129                        | 48/37.2                     | 81/62.8 < .001             |
| High T stage (3–4)          | 75                         | 50/66.7                     | 25/33.3                     |
| N stage                     |                             |                             | .038                        |
| N0                          | 177                        | 80/45.2                     | 97/54.8                     |
| N1                          | 27                         | 18/66.7                     | 9/33.3                      |
| M stage                     |                             |                             | < .001                      |
| M0                          | 168                        | 71/42.3                     | 97/57.7                     |
| M1                          | 36                         | 27/75.0                     | 9/25.0                      |
| Metastasis                  |                             |                             | .001                        |
| None (N0M0)                 | 165                        | 70/42.5                     | 95/57.6                     |
| Presence                    | 39                         | 28/71.8                     | 11/28.2                     |
| Radical prostatectomy       |                             |                             | .017                        |
| pT2                         | 45                         | 16/35.6                     | 29/64.4                     |
| pT3                         | 28                         | 18/64.3                     | 10/35.7                     |
similar trend was detected between the expression of LATS2 and the pT stage in RP specimens without NHT (OR = 3.26, 95% CI = 1.22–8.74, \( p = .019 \)). A multivariate analysis indicated a similar trend; however, the relationship between the expression of LATS2 and high T stage was not statistically significant (\( p = .052 \); Table 2). A similar logistic regression analysis was performed for the presence of metastasis, and a significant negative correlation was found in univariate analysis (\( p = .001 \)); however, the independent role of LATS2 expression was not detected in the multivariate analysis (\( p = .407 \), Table 2).

### 3.3 Relationships between the expression of LATS2 and outcome of NHT

In this study population, 60 patients received NHT for clinical organ-confined PC. We investigated the prognostic roles of the expression of LATS2 for anticancer effects, by evaluating the histological changes in response to NHT. The percentage of specimens with histological grade 3 (almost all cancer cells were non-viable or cancer cells were not detected) among LATS2-positive specimens was only 6.9%; in contrast, the ratio among the LATS2-negative specimens was 32.3% (Table 3). LATS2 expression was positively associated with the histological effects of NHT (\( p = .013 \)). Multivariate analysis was used to analyze the poor pathological effects; negative expression of LATS2 was independently associated with poor histological responses following NHT (OR = 8.85, 95% CI = 1.19–66.01, \( p = .034 \)).

Kaplan–Meier survival curves were used to assess the relationship between the expression of LATS2 and BCR. BCR-free survival periods following RP in 133 patients with LATS2-negative tumors were significantly shorter than that in LATS2-positive patients (\( p < .001 \), Figure 3A). A similar analysis was performed to assess the expression of LATS2 in samples from patients subjected to RP without NHT; negative expression of LATS2 was identified as a worse predictor of BCR (\( p = .002 \), Figure 3B). Cox proportional hazard analyses were performed for BCR-free survival periods in patients with LATS2 expression, in biopsy specimens collected at...
TABLE 2 Correlation with malignant behaviors by uni and multivariate analyses

| Biopsy at diagnosis | Univariate analysis | Multivariate analysis |
|---------------------|---------------------|----------------------|
|                      | OR 95% CI p value    | OR 95% CI p value    |
| LATS2; positive      | 1.00 - -             | 1.00 - -             |
| negative             | 6.46 3.50–11.92 .001 | 6.34 3.24–12.39 .001 |
| For high T stage     | OR 95% CI p value    | OR 95% CI p value    |
| LATS2; negative      | 3.38 1.86–6.13 .001  | 2.06 0.99–4.28 .052  |
| For metastasis       | OR 95% CI p value    | OR 95% CI p value    |
| LATS2; negative      | 3.46 1.61–7.41 .001  | 1.48 0.59–3.73 .407  |
| For BCR³             | HR 95% CI p value    | HR 95% CI p value    |
| LATS2; negative      | 2.63 1.50–4.60 .001  | 2.95 1.60–5.41 .001  |
| RP specimens         |                      |                      |
| For BCR³             | HR 95% CI p value    | HR 95% CI p value    |
| LATS2; negative      | 3.62 1.52–8.63 .004  | 3.00 1.24–7.28 .012  |

PI, proliferation index; OR, odds ratio; CI, confidential interval, BCR, biochemical recurrence, HR, hazard ratio

³In patients treated with radical prostatectomy.
²In patients without neoadjuvant hormonal therapy
³Adjusted by high GG, high T stage, and presence of metastasis
³Adjusted by high GG and presence of metastasis
³Adjusted by high GG and high T stage
³Adjusted by high GG, high T stage, and neoadjuvant hormonal therapy
³Adjusted by high GG and high pT stage

TABLE 3 Correlation with histological effects of neoadjuvant hormonal therapy

| LATS2 expression | Histological effect; grade, N/% | p value |
|------------------|---------------------------------|--------|
|                  | 0/1 2 3                          |        |
| Negative         | 10/34.5 7/24.1 10/34.4 2/6.9     | 0.013  |
| Positive         | 5/16.1 12/38.7 4/12.9 10/32.3    |        |

4 | DISCUSSION

LATS2 functions as a tumor suppressor in androgen-dependent PC (LNCaP) cells and in patients with hormone-naïve PC. The ratio of positive staining of LATS2 in PC tissues was significantly lower than that in non-tumor tissues. Similar results have been reported in PC tissues of humans and mouse models. However, in a previous in vivo study, the expression of LATS2 was investigated only in 10 human PC tissues. Therefore, this study is the first report that substantially clarifies the anticariogenic activity of LATS2 expression in patients with PC. In vitro studies indicated that LATS2 suppressed proliferation, invasion, and migration of androgen-dependent PC (DU145) cells. In addition, LATS2 regulates FOXP3-mediated tumor growth as a tumor suppressor in LNCaP cells. These reports support our results; however, there is a contrasting opinion that LATS2 promotes miR-93-induced proliferation and migration of androgen-dependent PC cells. Further studies are necessary to confirm the oncogenic activities of LATS2 in androgen-dependent PC cells. The relationship between LATS2 expression and pathological features in patients with PC were examined only in a few studies. The expression of LATS2 is downregulated in PC tissues with metastasis, compared to that in organ-confined PC. This study clearly demonstrated that the expression of LATS2 is negatively associated with tumor grade and each classification of TNM stage in patients with PC. In other types of malignancies, such as in ovarian cancer, hepatocellular carcinoma, and glioma, LATS2 suppresses tumorigenesis, cell growth, invasion, and metastasis. The results from this study
support the notion that LATS2 plays a role as a tumor suppressor against carcinogenesis, tumor growth, and progression in androgen-dependent/hormone sensitive PC cells.

The expression of LATS2 is a useful predictor for the anticancer effects of NHT in patients with organ-confined PC. LATS2 plays crucial roles in therapeutic resistance/sensitivity against molecular-targeted therapy and chemotherapeutic agents in various types of malignancies.34,35 There is no report on its role in the resistance to hormonal therapy in patients with PC. However, another member of LATS, LATS1, is associated with increased resistance to hormonal therapy in breast cancer.36 In PC, the LATS2-related pathway has important roles in promoting castration resistance.37 We speculated that LATS2 could regulate the anticancer effects of hormonal therapy in PC. The clinical benefits of NHT before RP needs clarification.38 NHT before RP may lead to good control over cancer in patients with high-risk PC.39 New NHT strategies before RP, using new hormonal agents are being developed.40 Therefore, information about predictive markers for the anticancer effects of NHT before RP is important for assessing the treatment strategies; new predictive markers for the anticancer effects of NHT are reported.17 We believe that our results on the relationship between the expression of LATS2 in biopsy specimens and the histological effects of NHT is significant to this discussion.

In addition to the histological effects, the expression of LATS2 is a useful predictor for BCR in organ-confined PC patients subjected to RP. Unfortunately, there is little information regarding the prognostic value of LATS2 expression in patients with PC. Several in vitro studies, using human cancer tissues, show that high expression of LATS2 is a significant predictor for better prognosis and longer survival periods in a variety of cancers, such as gastric cancer, lung cancer, and breast cancer.27,36,41 We have no data on the relationship between the expression of LATS2 and survival periods, because the prognosis of patients with PC is generally good. However, we believe that LATS2 might be a useful predictive marker in patients with PC.

One of the most interesting results in this study was that the pathological roles of LATS2 were different between androgen-dependent and androgen-independent PC cell lines. LATS2 expression was significantly correlated with the proliferation, migration, and invasion of androgen-dependent LNCaP cells; however, it was correlated only with migration in androgen-independent PC-3 cells. We speculate that the pathological roles of LATS2 in PC cells are dependent on the androgen-dependence of PC cells. LATS2 is a tumor suppressor in PC and its expression is significantly associated with AR-mediated activities and AR-dependent gene expression.28 However, we cannot conclude the pathological roles of androgen-independent PC/castration-resistant PC. This is one of the major limitations of this study, and elucidation through future studies is essential. Whether AR affects the degradation of LATS2 through the ubiquitin-mediated pathway is one of the important questions that remains to be answered. We focused on the pathological significance and prognostic role of LATS2; however, the main biological activity of LATS2 is the modulation of Hippo-YAP functioning under various physiological and pathological conditions.9,10 Therefore, detailed analyses of the co-activities of LATS2 and the other members of the Hippo pathway, including YAP/TAZ in PC are critical for discussing the clinical usefulness and limitations of these results.

5 | CONCLUSIONS

LATS2 played important roles in tumor growth, invasion, and metastasis, especially in the proliferation of androgen-dependent and hormone-naïve PC cells. LATS2 expression was a useful predictive factor for BCR following RP and for the histological effects following NHT in patients with organ-confined PC. We speculate that some pathological roles of LATS2 are dependent on the androgen dependence of PC. We conclude that LATS2 is a potential therapeutic target and a useful predictor of outcome in patients with PC.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.
DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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