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Pathological significance and prognostic role of LATS2 in prostate cancer

Tsuyoshi Matsuda MD[1] | Yasuyoshi Miyata MD, PhD[1] | Yuichiro Nakamura MD, PhD[1] | Asato Otsubo MD[1] | Yuta Mukae MD[1] | Junki Harada MD[1] | Kensuke Mitsunari MD, PhD[1] | Tomohiro Matsuo MD, PhD[1] | Kojiro Ohba MD, PhD[1] | Bungo Furusato MD, PhD[2] | Hideki Sakai MD, PhD[1]

[1]Department of Urology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[2]Department of pathology, Nagasaki University Graduate School of Biomedical Scieneces, Nagasaki, Japan

Correspondence

Yasuyoshi Miyata, MD, PhD, Department of Urology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
Email: yasu-myt@nagasaki-u.ac.jp

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

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.[1,2] Several investigators have recently focused attention on pathological roles of the Hippo pathway, such as in the growth, progression, and outcome of various cancers.[3,4] Yes-associated protein (YAP) is believed to be a key regulator of the Hippo pathway.[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.[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.[9,10] *In vivo* and *in vitro* studies have shown that, LATS1/2 are negatively associated with carcinogenesis, malignant potential, and cancer cell progression.[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.[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.[16] Neoadjuvant hormonal therapy (NHT) before radical prostatectomy (RP) improves the outcome in some patients; however, there is no useful predictive marker.[17] Therefore, detailed understanding of the pathological characteristics and the molecular regulatory mechanisms of PC cells is essential to devise novel therapeutic strategies for PC.

The Hippo pathway plays important roles in carcinogenesis and malignant aggressiveness of PC cells.[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.[20,21] LATS2 suppresses tumor growth and progression of PC cells[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.

2 | METHODS

2.1 | Cell culture and small-interfering RNA transfection

Two human prostate cancer cell lines, LNCaP and PC-3, were obtained from the American Type Culture Collection (Manassas, VA). LNCaP cells were cultured in ATCC-formulated RPMI-1640 medium supplemented with 10% fetal bovine serum at 37 °C in an atmosphere of 5% CO_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_2 and 95% air. LATS2 was knocked down in both these cell lines using Lipofectamine® 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.[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.[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

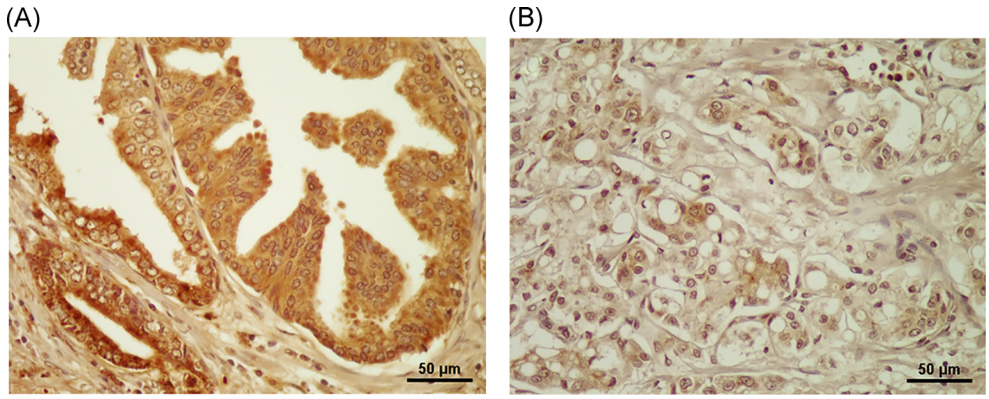

FIGURE 1 Immunoreactivity of LATS2 in non-tumoral glands (A) and prostate cancer tissues (B). Magnification ×400

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 and 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 ($p < .001$).

TABLE 1 Correlation with clinicopathological features

Variables	N	LATS-2 expression		p value
		Negative	Positive	
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				
pT stage				.017
pT2	45	16/35.6	29/64.4	
pT3	28	18/64.3	10/35.7	

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

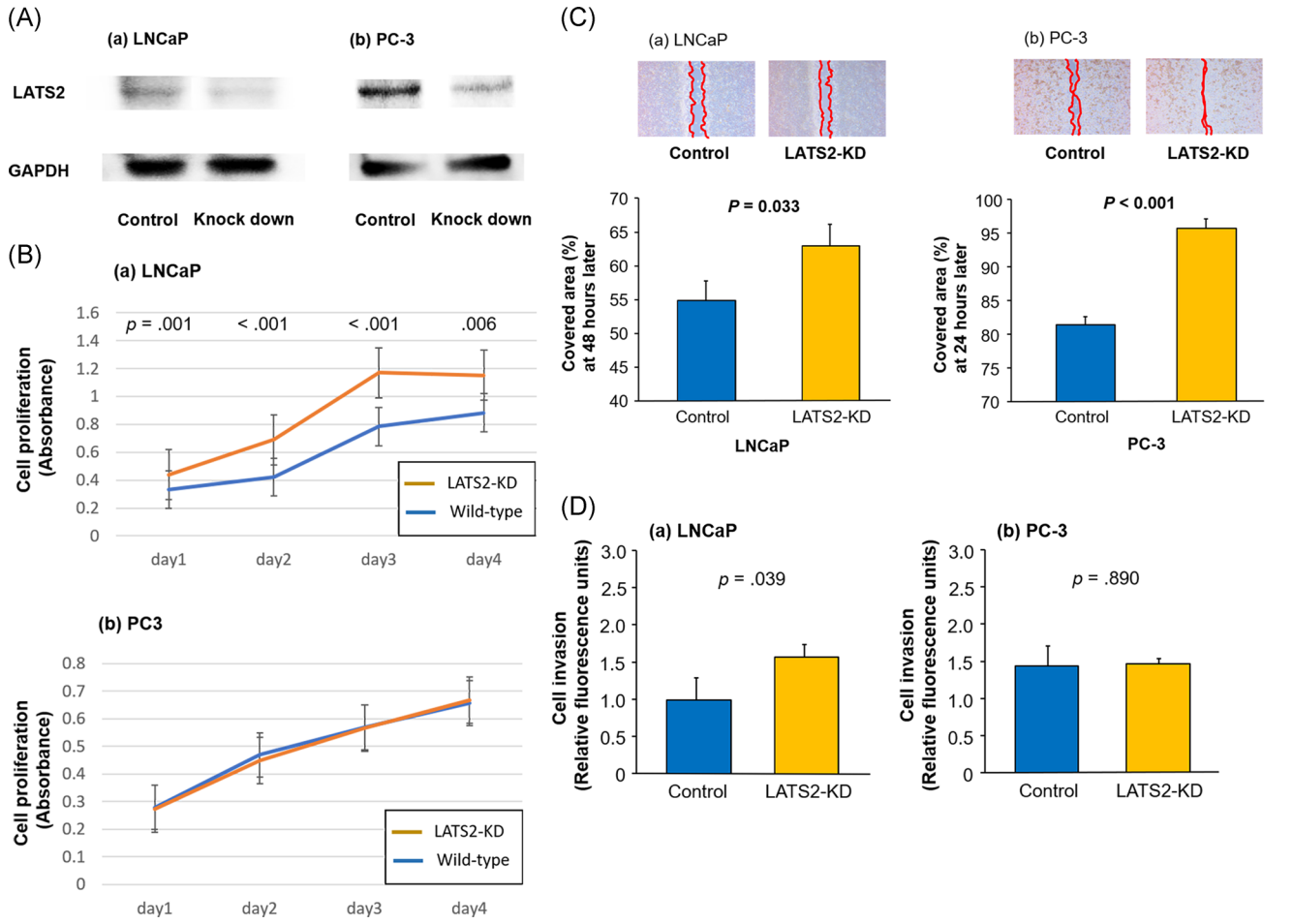

FIGURE 2 (A) Western blot analysis of LATS2 expression, in control and LATS2 knockdown (KD) LNCaP (a) and PC-3 (b) cells. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the control. (B) Growth curves of wild-type (WT) and LATS2 knockdown (KD) LNCaP (a) and PC-3 (b) cells. (C) Migration of wild-type (WT) cells and LATS2 KD LNCaP (a) and PC-3 (b) cells as assessed using wound healing assay. Quantitation of migration at 48 h after siRNA-mediated knockdown of LATS2 in LNCaP cells and at 12 h in PC-3 cells. (D) Evaluation of the invasion of wild-type (WT) and LATS2 knockdown LNCaP (a) and PC-3 (b) cells using the invasion assay

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 ($p = 0.013$). 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

	Univariate analysis			Multivariate analysis		
Biopsy at diagnosis						
For high PI	OR	95% CI	*p* value	OR[c]	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[d]	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[e]	95% CI	*p* value
LATS2; negative	3.46	1.61–7.41	.001	1.48	0.59–3.73	.407
For BCR[a]	HR	95% CI	*p* value	HR[f]	95% CI	*p* value
LATS2; negative	2.63	1.50–4.60	.001	2.95	1.60–5.41	.001
RP specimens						
For BCR[b]	HR	95% CI	*p* value	HR[g]	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

[a]In patients treated with radical prostatectomy.

[b]In patients without neoadjuvant hormonal therapy

[c]Adjusted by high GG, high T stage, and presence of metastasis

[d]Adjusted by high GG and presence of metastasis

[e]Adjusted by high GG and high T stage

[f]Adjusted by high GG, high T stage, and neoadjuvant hormonal therapy

[g]Adjusted by high GG and high pT stage

TABLE 3 Correlation with histological effects of neoadjuvant hormonal therapy

LUTS2 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	

diagnosis and in RP specimens without NHT (Table 2). Univariate analyses showed that the expression of LATS2 in both biopsy and RP specimens was a worse predictive factor ($p = .001$ and .004, respectively), and similar results were detected in the multivariate analysis model using biopsy ($p = .001$) and RP specimens ($p = .018$; Table 2).

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.[28,29] However, in a previous *in vivo* study, the expression of LATS2 was investigated only in 10 human PC tissues.[28] Therefore, this study is the first report that a substantially clarifies the anti-cariogenic activity of LATS2 expression in patients with PC. *In vitro* studies indicated that LATS2 suppressed proliferation, invasion, and migration of LNCaP cells, which is pathologically significant. LATS2 inhibits the miR-372-induced stimulation of proliferation and migration of androgen-dependent PC (DU145) cells.[30] In addition, LATS2 regulates FOXP3-mediated tumor growth as a tumor suppressor in LNCaP cells.[29] These reports support our results; however, there is a contrasting opinion that LATS2 promotes miR-93-induced proliferation and invasion in both LNCaP and DU145 cells.[23] 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.[31] 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.[12,32,33] The results from this study

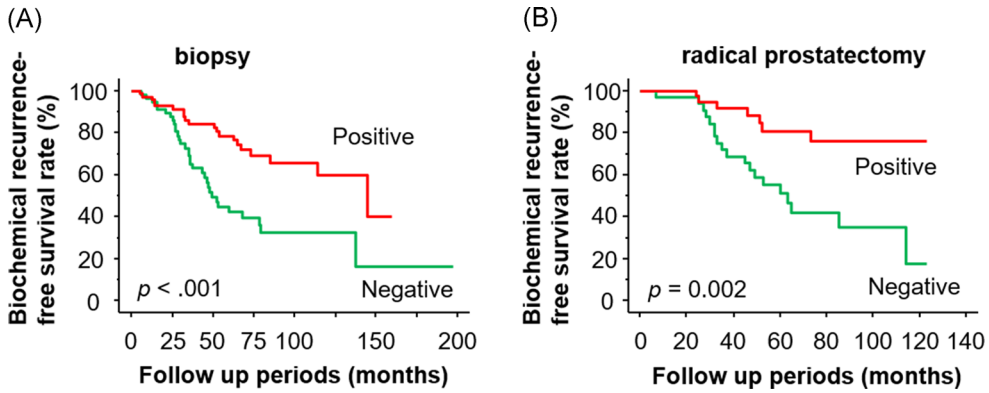

FIGURE 3 Kaplan–Meier survival curves for biochemical recurrence-free survival following radical prostatectomy according to the expression of LATS2. The analysis was performed in 133 biopsy specimens at diagnosis (A) and in 60 radical prostatectomy specimens (B)

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.

ORCID

Yasuyoshi Miyata http://orcid.org/0000-0001-6272-6657
Tomohiro Matsuo http://orcid.org/0000-0002-9508-0319

REFERENCES

1. Ishihara E, Nishina H. The Hippo-YAP Pathway Regulates 3D Organ Formation and Homeostasis. *Cancers (Basel)*. 2018;10(4):122.
2. Moya IM, Halder G. Hippo–YAP/TAZ signaling in organ regeneration and regenerative medicine. *Nat Rev Mol Cell Biol*. 2019;20:211-226.
3. Ansari D, Ohlsson H, Althini C, et al. The Hippo Signaling Pathway in Pancreatic Cancer. *Anticancer Res*. 2019;39(7):3317-3321.
4. Wang K, Hu YB, Zhao Y, Ye C. Long non-coding RNA ASAP1-IT1 suppresses ovarian cancer progression by regulating Hippo/YAP signaling. *Int J Mol Med*. 2021;47(4):1.
5. Misra JR, Irvine KD. The hippo signaling network and its biological functions. *Annu Rev Genet*. 2018;52:65-87.
6. Tang Y, Fang G, Guo F, et al. Selective Inhibition of STRN3-Containing PP2A Phosphatase Restores Hippo Tumor-Suppressor Activity in Gastric Cancer. *Cancer Cell*. 2020;38(1):115-128.
7. Yamaguchi H, Taouk GM. A Potential Role of YAP/TAZ in the Interplay Between Metastasis and Metabolic Alterations. *Front Oncol*. 2020;10:9282020.
8. Zhang X, Yang L, Szeto P, et al. The Hippo pathway oncoprotein YAP promotes melanoma cell invasion and spontaneous metastasis. *Oncogene*. 2020;39(30):5267-5281.
9. Dasgupta I, McCollum D. Control of cellular responses to mechanical cues through YAP/TAZ regulation. *J Biol Chem*. 2019;294(46):17693-17706.
10. Ma S, Meng Z, Chen R, Guan KL. The Hippo Pathway: Biology and Pathophysiology. *Annu Rev Biochem*. 2019;88:577-604.
11. Malik SA, Khan MS, Dar M, et al. Molecular Alterations and Expression Dynamics of LATS1 and LATS2 Genes in Non-Small-Cell Lung Carcinoma. *Pathol Oncol Res*. 2018;24(2):207-214.
12. Guo C, Liang C, Yang J, Hu H, Fan B, Liu X. LATS2 inhibits cell proliferation and metastasis through the Hippo signaling pathway in glioma. *Oncol Rep*. 2019;41(5):2753-2761.
13. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):7-30.
14. Parker C, Castro E, Fizazi K, et al. Prostate Cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2020;31(9):1119-1134.
15. Rajaram P, Rivera A, Muthima K, Olveda N, Muchalski H, Chen QH. Second-Generation Androgen Receptor Antagonists as Hormonal Therapeutics for Three Forms of Prostate Cancer. *Molecules*. 2020;25(10):2448.
16. Cornford P, Bellmunt J, Bolla M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part II: Treatment of Relapsing, Metastatic, and Castration-Resistant Prostate Cancer. *Eur Urol*. 2017;71(4):630-642.
17. Wang X, Qi M, Zhang J, et al. Differential response to neoadjuvant hormonal therapy in prostate cancer: Predictive morphological parameters and molecular markers. *Prostate*. 2019;79(7):709-719.
18. Collak FK, Demir U, Sagir F. YAP1 Is Involved in Tumorigenic Properties of Prostate Cancer Cells. *Pathol Oncol Res*. 2020;26(2):867-876.
19. Lin M, Bu C, He Q, et al. TAZ is overexpressed in prostate cancers and regulates the proliferation, migration and apoptosis of prostate cancer PC3 cells. *Oncol Rep*. 2020;44(2):747-756.
20. Wang R, Du Y, Shang J, Dang X, Niu G. PTPN14 acts as a candidate tumor suppressor in prostate cancer and inhibits cell proliferation and invasion through modulating LATS1/YAP signaling. *Mol Cell Probes*. 2020;53:101642.
21. Xu N, Wu YP, Yin HB, et al. SHCBP1 promotes tumor cell proliferation, migration, and invasion, and is associated with poor prostate cancer prognosis. *J Cancer Res Clin Oncol*. 2020;146(8):1953-1969.
22. Zhao B, Wei X, Li W, et al. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev*. 2007;21(21):2747-2761.
23. Liu JJ, Zhang X, Wu XH. miR-93 Promotes the Growth and Invasion of Prostate Cancer by Upregulating Its Target Genes TGFBR2, ITGB8, and LATS2. *Mol Ther Oncolytics*. 2018;11:14-19.
24. Mitsunari K, Miyata Y, Asai A, et al. Human antigen R is positively associated with malignant aggressiveness via upregulation of cell proliferation, migration, and vascular endothelial growth factors and cyclooxygenase-2 in prostate cancer. *Transl Res*. 2016;175:116-128.
25. Yuno T, Miyata Y, Matsuo T, et al. Relationship Between Stage-specific Embryonic Antigen-4 and Anti-cancer Effects of Neoadjuvant Hormonal Therapy in Prostate Cancer. *Anticancer Res*. 2020;40(10):5567-5575.
26. Miyata Y, Sakai H, Kanda S, Igawa T, Hayashi T, Kanetake H. Expression of insulin-like growth factor binding protein-3 before and after neoadjuvant hormonal therapy in human prostate cancer tissues: correlation with histopathologic effects and biochemical recurrence. *Urology*. 2004;63(6):1184-1190.
27. Son MW, Song GJ, Jang SH, et al. Clinicopathological Significance of Large Tumor Suppressor (LATS) Expression in Gastric Cancer. *J Gastric Cancer*. 2017;17(4):363-373.
28. Powzaniuk M, McElwee-Witmer S, Vogel RL, et al. The LATS2/KPM tumor suppressor is a negative regulator of the androgen receptor. *Mol Endocrinol*. 2004;18(8):2011-2023.
29. Li W, Wang L, Katoh H, Liu R, Zheng P, Liu Y. Identification of a tumor suppressor relay between the FOXP3 and the Hippo pathways in breast and prostate cancers. *Cancer Res*. 2011;71(6):2162-2171.
30. Cao H, Feng Y, Chen L. Repression of MicroRNA-372 by Arsenic Sulphide Inhibits Prostate Cancer Cell Proliferation and Migration through Regulation of large tumour suppressor kinase 2. *Basic Clin Pharmacol Toxicol*. 2017;120(3):256-263.
31. Zhao B, Li L, Wang L, Wang CY, Yu J, Guan KL. Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis. *Genes Dev*. 2012;26:54-68.
32. Li Y, Zhuang W, Huang M, Li X. Long noncoding RNA DDX11-AS1 epigenetically represses LATS2 by interacting with EZH2 and DNMT1 in hepatocellular carcinoma. *Biochem Biophys Res Commun*. 2019;514(4):1051-1057.
33. Montavon C, Stricker GR, Schoetzau A, Heinzelmann-Schwarz V, Jacob F, Fedier A. Outcome in serous ovarian cancer is not associated with LATS expression. *J Cancer Res Clin Oncol*. 2019;145(11):2737-2749.
34. Marsola APZC, Simões BP, Palma LC, Berzoti-Coelho MG, Burin SM, de Castro FA. Expression of Hippo signaling pathway and Aurora kinase genes in chronic myeloid leukemia. *Med Oncol*. 2018;35(3):26.
35. Mohamed Z, Hassan MK, Okasha S, et al. miR-363 confers taxane resistance in ovarian cancer by targeting the Hippo pathway member, LATS2. *Oncotarget*. 2018;9(53):30053-30065.
36. Furth N, Pateras IS, Rotkopf R, et al. LATS1 and LATS2 suppress breast cancer progression by maintaining cell identity and metabolic state. *Life Sci Alliance*. 2018;1(5):e201800171.
37. Guo Y, Cui J, Ji Z, et al. miR-302/367/LATS2/YAP pathway is essential for prostate tumor-propagating cells and promotes

the development of castration resistance. *Oncogene*. 2017;36(45):6336-6347.
38. Pignot G, Maillet D, Gross E, et al. Systemic treatments for high-risk localized prostate cancer. *Nat Rev Urol*. 2018;15(8):498-510.
39. Enokida H, Yamada Y, Tatarano S, et al. Oncological outcome of neoadjuvant low-dose estramustine plus LHRH agonist/antagonist followed by extended radical prostatectomy for Japanese patients with high-risk localized prostate cancer: a prospective single-arm study. *Jpn J Clin Oncol*. 2020;50(1):66-72.
40. Tafuri A, Cerruto MA, Antonelli A. Neoadjuvant strategies before radical prostatectomy for high risk prostate cancer in the era of new hormonal agents. *Curr Drug Targets*. 2021;22(1):68-76.
41. Liu XL, Zuo R, Ou WB. The hippo pathway provides novel insights into lung cancer and mesothelioma treatment. *J Cancer Res Clin Oncol*. 2018;144(11):2097-2106.

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