Asian Journal of Urology 11 (2024) 569–574

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Original Article

Anillin actin-binding protein expression correlates with poor prognosis for prostate cancer patients

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Received 16 February 2023; accepted 12 July 2023
Available online 1 September 2023

KEYWORDS
Prostate cancer;
Androgen receptor;
Octamer transcription factor 1;
Anillin actin-binding protein

Abstract *Objective:* Octamer transcription factor 1 (OCT1), a transcription factor that interacts with androgen receptor, is involved in prostate cancer (PCa) progression. The OCT1 target gene, Anillin actin-binding protein (*ANLN*), is highly expressed in castration-resistant PCa tissue; however, it remains unclear whether ANLN expression in hormone-sensitive PCa tissue could be used as a predictive biomarker for poor prognosis of patients. We aimed to investigate ANLN expression in PCa tissue obtained via radical prostatectomy and its correlation with clinical parameters.
Methods: Immunohistochemical staining for ANLN was performed on 86 PCa specimens, followed by evaluation using immunoreactivity (IR) scores. Prognosis was analyzed by the log-rank test using the Kaplan–Meier method to generate a cancer-specific survival curve. The correlations between ANLN IR and clinical parameters as well as OCT1 IR were analyzed using the Chi-squared test.

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Peer review under responsibility of Tongji University.
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https://doi.org/10.1016/j.ajur.2023.07.002

Results: The median IR score was 0 for ANLN. Accordingly, given the low median IR score, an IR score of $\geq$3 was defined as positive. There were 17 (19.8%) ANLN-positive cases, and these cases had a significantly poorer prognosis. Multivariate analysis revealed that the Gleason score, pathological tumor and lymph node stages, and positive ANLN expression were significant predictors of poor prognosis. Notably, patients with both positive ANLN and high OCT1 expression had a significantly decreased overall survival ($p=0.001$).
Conclusion: ANLN, which is a OCT1 target gene especially in castration-resistant PCa, is expressed in a small number of hormone-sensitive PCa cases. Both positive ANLN expression and high OCT1 expression are significantly correlated with poor prognosis for PCa patients.

1. Introduction

Androgen and androgen receptor (AR) signaling pathways are crucially involved in prostate cancer (PCa) growth [1,2]. Therefore, androgen deprivation therapy is an effective treatment for PCa. However, some patients can develop resistance to androgen deprivation therapy, leading to castration-resistant PCa (CRPC) [3].

Octamer transcription factor 1 (OCT1) interacts with AR and regulates the expression of its target genes. Moreover, high OCT1 expression is correlated with poor prognosis in patients with PCa [4]. Further, there is higher OCT1 expression in AR-positive CRPC cells than in hormone-sensitive PCa (HSPC) cells. Interestingly, we previously demonstrated that Anillin actin-binding protein (*ANLN*) is regulated by AR with OCT1, and is more highly expressed in CRPC cells compared with that in HSPC cells [5]. *ANLN* is shown to be involved in cell division by binding to actin and myosin, promoting CRPC cell proliferation [5]. However, its clinical significance in early-stage HSPC remains unclear.

Here, we aimed to investigate ANLN expression in PCa tissue obtained through radical prostatectomy and its correlation with clinical parameters.

2. Materials and methods

2.1. Tissue specimens and patient characteristics

PCa tissue was obtained from 86 patients who underwent radical prostatectomy at the Nihon University Itabashi Hospital (Tokyo, Japan). The mean age of the patients was 67.9 (range: 50.0—85.0) years. Further, the mean preoperative prostate-specific antigen level was 27.6 (range: 1.5—218.5) ng/mL. Two pathologists evaluated the specimens and graded malignancy using the Gleason score. There were 36, 23, 15, 8, and 4 patients with Gleason scores of $\leq$6, 7, 8, 9, and 10, respectively. The pathological tumor (pT) stages were 2 ($n=36$), 3 ($n=48$), and 4 ($n=2$), while the pathological lymph node (pN) stages were 0 ($n=72$) and 1 ($n=14$). Written informed consents were obtained from all patients, and the study was approved by the Institutional Ethics Committee of the Nihon University Itabashi Hospital (No. RK-190611-3).

2.2. Immunohistochemistry

Immunohistochemical analysis was conducted using the streptavidin-biotin amplification method as previously described [4,6]. Briefly, an anti-ANLN polyclonal antibody (Abcam, Cambridge, UK, ab211872, 1:200 dilution) and an anti-OCT1 polyclonal antibody (Abcam, Cambridge, UK, ab15112, 1:200 dilution) were applied overnight to the samples, followed by Histofine Simple Stain MAX-PO (Nichirei, Tokyo, Japan). We have previously reported the specificity of these antibodies by PCa cell treatment with siRNAs that specifically suppress the expression of each gene, then performing Western blotting with these antibodies [5,7]. Antigen-antibody complexes were visualized using 3,3′-diaminobenzidine (DAB) solution (Nichirei biosciences, Tokyo, Japan). Immunoreactivity (IR) scores were calculated by the sum of the proportion (0, none; 1, $<$1%; 2, 1%—10%; 3, 11%—33%; 4, 34%—66%, and 5, $\geq$67%) and intensity (0, none; 1, weak; 2, moderate; and 3, strong) of the IR. IR scores of $\geq$3 and $\geq$7 were defined as positive ANLN expression and high OCT1 expression, respectively.

2.3. Statistical analyses

The correlations between the IR score and clinicopathological characteristics (age, serum prostate-specific antigen level before treatment, pathological stage, OCT1 IR [4], and Gleason score) were evaluated using the *t*-test or Chi-square test. The validity of the sample size required for the *t*-test was evaluated using G*power (Düsseldorf, Germany) [8]. Setting the effect size to 0.5, alpha error to 0.05, power to 0.8, and two-sided test, the required sample size was 64. Cancer-specific survival curves were obtained using the Kaplan—Meier method and validated using the log-rank test. Statistical analyses were performed using GraphPad Prism (Boston, MA, USA) for Mac 8.0 and JMP 11.0 software (Tokyo, Japan). The statistical significance was set at $p<0.05$.

3. Results

3.1. Immunohistochemical analysis of ANLN in PCa specimens

PCa specimens were analyzed through immunohistochemical staining to visualize the ANLN and OCT1 proteins. Further, both ANLN and OCT1 expression were evaluated using the IR score. Since the median IR score of ANLN was 0, positive IR was indicated by an IR score of $\geq$3. IR of ANLN was observed in cancer cell nuclei, but not in non-cancerous lesions (Fig. 1). While the high OCT1 expression was assigned to be IR scores of 7 or higher according to the previous report [4], OCT1 was strongly expressed in the high Gleason grade area (Supplementary Fig. 1), which is consistent with the previous report [4]. Distribution of IR scores of ANLN is shown in Fig. 2. Table 1 shows the correlation between ANLN expression and clinicopathological features. There were no significant correlations between ANLN IR and any of these parameters (Table 1).

3.2. Clinical significance of ANLN expression in PCa

Table 2 shows the results of univariate and multivariate analyses using the Cox proportional hazard model. Univariate analysis indicated that prognosis was correlated with the Gleason score, pT stage, pN stage, and ANLN (p-values of $<$0.0001, 0.038, 0.0002, and 0.036, respectively). Multivariate analysis revealed that the Gleason score, pT stage, pN stage, and ANLN were significant prognostic factors (hazard ratios of 7.37, 9.71, 12.73, and 12.11, respectively). Fig. 3 shows the Kaplan–Meier cancer-specific survival curves for the 86 localized PCa cases. Among them, 11 patients died during the follow-up period. Overall survival was significantly worse in patients with positive ANLN expression compared to those with negative ANLN expression (Fig. 3A). Of note, the overall survival of patients with both positive ANLN expression and high OCT1 expression was significantly worse than in the other patients (Fig. 3B and Supplementary Fig. 2). Furthermore, both positive ANLN expression and high OCT1 expression were independent prognostic factors (Supplementary Table 1).

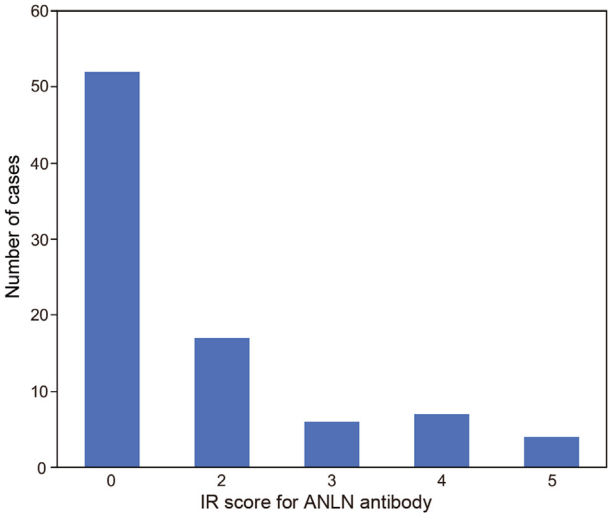

Figure 2 Distribution of IR scores for ANLN antibody. ANLN expression was negative in 80% of the localized prostate cancer cases (52 cases with an IR score of 0 and 17 cases with an IR score of 2), while 17 cases have positive ANLN expression with an IR score of 3 or higher. IR, immunoreactivity; ANLN, Anillin actin-binding protein. Note: there were no cases with an IR score of 1.

4. Discussion

The AR is crucially involved in PCa by facilitating cancer progression through the expression of target genes. Various AR collaborative transcription factors regulate PCa growth and progression, with their abnormalities contributing to the resistance of PCa to therapy [9]. AR collaborative transcription factors change with the disease stage. Accordingly, AR target genes vary across the different disease stages. Specifically, the distribution of AR-binding regions and the types of AR collaborative transcription factors differ between HSPC and CRPC. The canonical AR collaborative transcription factors are not mobilized in the CRPC-specific AR binding region. Instead, AR-independent transcription factors, including MYC proto-oncogene, are recruited to these sites [10].

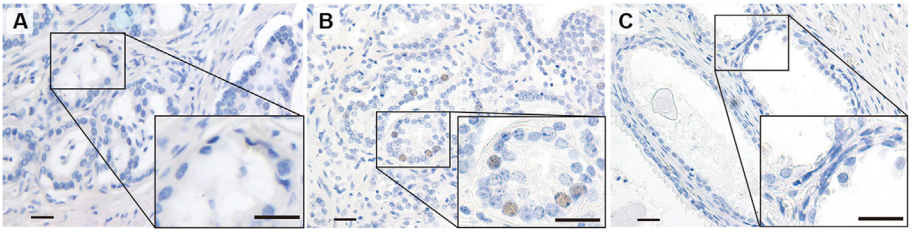

Figure 1 Representative immunohistochemical staining images. (A) Negative immunoreactivity in cancerous region; (B) Positive immunoreactivity in cancerous region; (C) Negative immunoreactivity in non-cancerous region. The scale bar indicates 25 μm.

Table 1 Correlation between ANLN expression and clinicopathological findings in patients with prostate cancer ($n=86$).

Variable	ANLN expression		*p*-Value
	Negative ($n=69$)	Positive ($n=17$)	
Age, year	67.9±6.2	67.8±3.77	0.96
PSA, ng/mL	27.3±37.8	28.8±23.8	0.89
Gleason score			
5 or 6	32	4	0.32
7	18	5	
8	9	6	
9	7	1	
10	3	1	
pT stage			0.55
2	30	6	
3	38	10	
4	1	1	
pN stage			0.56
N0	57	15	
N1	12	2	
OCT1 expression			0.30
Low	42	8	
High	27	9	

ANLN, anillin actin-binding protein; IR, immunoreactivity; PSA, prostate-specific antigen; OCT1, octamer transcription factor 1; pT, pathological tumor; pN, pathological lymph node.
Note: values are presented as mean±standard deviation or *n*, unless otherwise stated; IR scores were obtained as the sum of the proportion (0, none; 1, <1%; 2, 1% to 10%; 3, 11% to 33%; 4, 34% to 66%; and 5, ≥67%) and the intensity (0, none; 1, weak; 2, moderate; and 3, strong) of IR; IR scores of 0–2 and 3–5 were defined as negative and positive ANLN expression, while 0–6 and 7, 8 as low and high OCT1 expression [4,6], respectively.

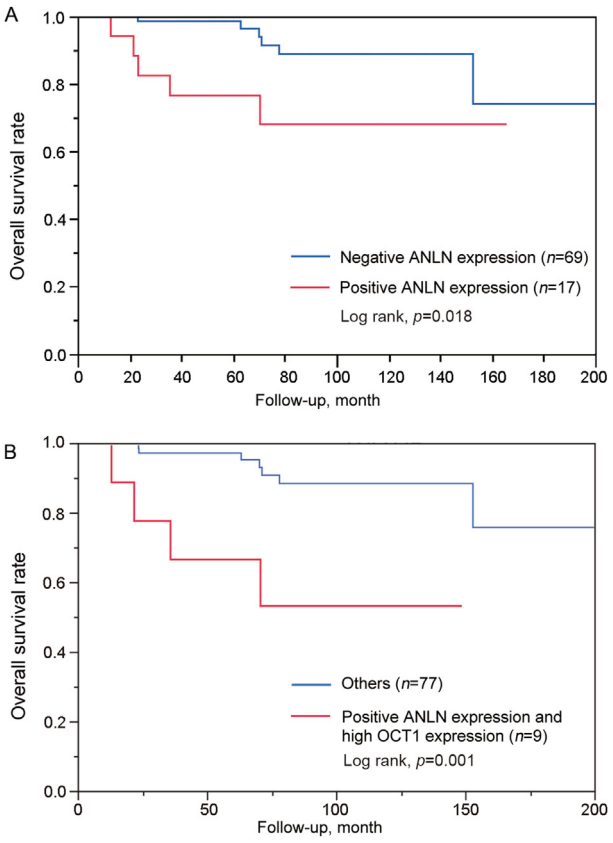

Figure 3 The Kaplan–Meier cancer-specific survival curves for predicting prognosis of the 86 localized PCa cases. (A) According to ANLN expression. (B) According to both ANLN and OCT1 expression. ANLN, Anillin actin-binding protein; IR, immunoreactivity; OCT1, octamer transcription factor 1. IR scores were obtained as the sum of the proportion (0, none; 1, <1%; 2, 1%–10%; 3, 11%–33%; 4, 34%–66%, and 5, ≥67%) and the intensity (0, none; 1, weak; 2, moderate; and 3, strong) of IR; IR scores of ≥3 and ≥7 were defined as positive ANLN and high OCT1 expression, respectively [4,6].

OCT1 is a poor prognostic factor in localized PCa and promotes HSPC cell proliferation by expressing various OCT1 target genes [5,7,9,11–13]. We have previously demonstrated that there is a positive relationship between AR and OCT1 expression [4]. IR score of 6 or higher for AR expression was observed in 90/102 cases, with 47 cases scoring 7 or higher. On the other hand, OCT1 had a very similar pattern of expression to AR, with an IR score of 6 or higher of 77/102 and a strong positive of 40/102 [4]. Although both OCT1 high expression and ANLN positive cases showed poor prognosis (Fig. 3 and supplementary Fig. 2), we could not find a significant correlation between OCT1 expression and ANLN expression ($p=0.30$, Table 1), possibly due to the small number of positive cases in this study. We have shown that *ANLN* is a

Table 2 Univariate and multivariate Cox proportional hazard analyses of overall survival ($n=86$).

Variable	Univariate			Multivariate		
	HR	95% CI	*p*-Value[a]	HR	95% CI	*p*-Value[a]
PSA (>20 ng/mL *vs.* ≤20 ng/mL)	1.13	0.13–9.49	0.896			
Gleason score (high *vs.* low)[b]	24.65	4.68–453.23	**<0.0001**	7.37	1.15–144.18	**0.033**
pT stage (>2 *vs.* ≤2)	5.67	1.07–104.41	**0.038**	9.71	1.17–240.14	**0.032**
pN stage (1 *vs.* 0)	10.77	3.23–41.34	**0.0002**	12.73	2.34–112.37	**0.002**
ANLN (positive *vs.* negative)	3.79	1.09–12.64	**0.036**	12.11	2.15–98.43	**0.004**

HR, hazard ratio; CI, confidence interval; PSA, prostate-specific antigen; pT, pathological tumor; pN, pathological lymph node; ANLN, Anillin actin-binding protein.
[a] Significant *p*-values (<0.05) are expressed in bold.
[b] High Gleason score: 8–10; low Gleason score: 5–7.

representative target gene of AR and OCT1 in CRPC tissue [5,7]. However, the present finding suggests that ANLN might be regulated by transcription factors other than AR and OCT1 in the early stage of PCa. For example, the transcription factors that regulate prostate-specific membrane antigen expression, a protein specific to CRPC, vary during the disease progression, resulting in altered expression levels [14]. To gain a better understanding of ANLN-positive cases as a predictive biomarker in the early-stage of PCa, further research using a larger sample size with expression evaluation of transcription factors that regulate ANLN, including OCT1 and AR, will be required in the future. Notably, it is abundantly expressed in CRPC cells [5]. Furthermore, OCT1 targets different genes depending on the cancer status, with *ACSL3* being highly expressed in LNCaP cells (HSPC cells), *ANLN* and *DLGAP5* being highly expressed in 22Rv1 cells (CRPC model cells), and *PFN2* being highly expressed in AR-negative PCa as OCT1 target genes [5,7,11,13]. ANLN is implicated in the cytoskeletal dynamics for cell cycle progression. In the telophase, ANLN is relocated to the cytoplasm, where it accumulates in the contractile ring and cleavage furrow. Therefore, ANLN promotes G2/M transition for cell proliferation in CRPC [5].

In the present study, 17 out of 86 (19.8%) cases were positive for ANLN, with most of them ($n=13$) having an IR score of 3 or 4. Contrastingly, previous studies on CRPC showed that all ANLN-positive cases had an IR score of ≥ 5 [7]. Although a study using a public microarray database reported a positive correlation between OCT1 and ANLN expression in CRPC tissue [5], it was not found in our histological analysis using HSPC tissue. Our findings suggest that ANLN expression may be regulated by other factors than OCT1 in early-stage PCa even though it is regulated by increased OCT1 expression in CRPC.

ANLN expression is an independent poor prognostic factor for patients with HSPC. Moreover, we found that patients who had positive ANLN expression and high OCT1 expression had a markedly poor prognosis. Considering that the combination of OCT1 and ANLN is a poor prognostic factor for HSPC, evaluating these immune activities may also be useful in selecting patients who need early multimodal therapy.

5. Conclusion

Positive ANLN expression in PCa tissue is correlated with poor prognosis in patients with localized PCa. Although ANLN is expressed in a small number of HSPC cells, a worse outcome was observed in PCa patients with both positive ANLN expression and high OCT1 expression. These results suggest that ANLN expression increases with PCa progression and is a poor prognostic factor.

Author contributions

Study concept and design: Kenichi Takayama, Satoru Takahashi, Satoshi Inoue.
Data acquisition: Shinichiro Yamamoto, Daisuke Obinata, Daigo Funakoshi, Kyoko Fujiwara.
Data analysis: Daisuke Obinata, Makoto Hara.
Drafting of manuscript: Daisuke Obinata, Kenichi Takayama.
Critical revision of the manuscript: Satoshi Inoue.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

We acknowledge the technical assistance from Emiko Kuriki, Asako Oguni, Toyoharu Jike, Isamu Isahai, and Naoko Abe. The authors are supported by funding from Takeda Science Foundation, and JSPS KAKENHI (grant numbers JP19H03793 to Obinata D, JP20K07350 to Takayama K, JP19K09740 to Takahashi S, JP20K07875 to Hara M, and JP20K21667 and JP21H04829 to Inoue S).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajur.2023.07.002.

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