Role of somatostatin receptor SSRT5-AS1 in predicting biomarkers of primary androgen deprivation therapy on prostate cancer in Indonesian population

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ABSTRACT

Introduction: Androgen deprivation therapy (ADT) has been the pivotal strategy for treating advanced prostate cancers. Despite the high efficacy of ADT in prohibiting tumor growth, >50% cases of prostate cancer will develop into an aggressive variant known as castration resistant prostate cancer (CRPC). This study aimed to evaluate the potential role SSRT5-AS1 expression as a biomarker for response to ADT in prostate cancer.

Materials and Methods: In total, 36 patients diagnosed with prostate cancer at Dr. Sardjito General Hospital, Yogyakarta, Indonesia were enrolled from 2015 and 2019. The expression of SSRT5-AS1 in primary tumors was quantified using quantitative real-time polymerase chain reaction.

Results: The mean age of patients enrolled in this study was 69.07 ± 8.7 years, and the mean of prostate-specific antigen in patients was 141.22 ±112.28 ng/ml. Compared with the median, a higher expression of SSTR5-AS1 had more significant prognostic value than the variable shorter time to CRPC (*p*= 0.043).

Conclusion: This study demonstrated that high expression of SSRT5-AS1 is a promising biomarker to predict response to ADT in patients with prostate cancer.

KEYWORDS:
ADT, prostate cancer, SSRT5-AS1, CRPC, biomarkers

INTRODUCTION

Prostate cancer (PCa) is a tumor pathology with the highest incidence in men and is strongly influenced by the hormonal milieu, especially androgens.[1] The use of hormonal therapy for treating PCa dates back to 1941 when American surgeons initiated endocrine manipulation. Ever since then, androgen deprivation therapy (ADT) has become the gold standard for both locally advanced and metastatic PCas.[1,2] After receiving ADT, PCa progresses into a variant that no longer responds to ADT. This variant is termed castration resistant PCa (CRPC).[3] The time required for this variant to develop is known as the time to CRPC. This time reportedly differs among patients with PCa, which has led to emerging issues to find biomarkers to facilitated treatment selection to PCa patients. ADT is associated with several significant side effects that can affect the quality of a patient's life. Consideration must be given for implementing ADT in patients who had benefited less from this therapy, to avoid morbidity associated with androgen derivation.

CRPC is marked by disease progression despite continuous hormonal manipulation, such as with ADT, with a profile that may present a continuous increase in serum prostate-specific antigen (PSA), clinical and radiological progression of pre-existing disease, and appearance of new metastases. Because CRPC is typically unresponsive to ADT and patients show differing time to develop CRPC, the occurrence of this condition is considerably challenging in the clinical scenario. This is because diagnostic options with current biomarkers often lead to over-diagnosis and over treatment owing to limited specific biomarkers to guide clinical decision-making.[4-6] Indeed, new treatment and diagnostic modalities still need to be developed, and significant efforts are being implemented with advances being reached in basic, translational, and clinical research fields. However, diagnostic approaches are still limited, particularly in advanced disease states owing to the heterogeneity and complexity of the disease. Hence, specific biomarkers are required to help in clinical settings to identify early responses to treatment outcomes and to identify patients who are most likely to benefit from ADT.[6]

Notably, PCa has a marked endocrine nature with other non-sex hormones such as somatostatin, which is also related with normal prostate and PCa development. Several studies have reported the role of somatostatin receptor signaling pathway (SSRT) in different types of tumors with SSRT5 expression, which is more pre-dominant in all tissue samples, especially SSTR5-AS1.[7-9] However, the role of SSTR5-AS1 in PCa is still unclear. Therefore, we evaluated the prognostic significance of SSTR5-AS1 overexpression in patients with PCa and time to develop CRPC. The goal of the study was to increase the knowledge of therapy resistant PCa by incorporating novel biomarkers, which could help improve predictive and prognostic models.

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Table I: Characteristics of patients

Variables	Mean (± SD)/ count (%)
Age, years	69.07(±8.7)
PSA, ng/ml	141.22 (±112.28)
Time to CRPC, month	25.7 (±18.36)
ISUP Groups (%)	
1	5 (13.9%)
2	4 (11.1%)
3	1 (2.8%)
4	9 (25%)
5	17 (47.2%)
Surgical castration (%)	
Yes	16 (44.4%)
No	20 (55.6%)
T staging (%)	
T1a	4 (11.1%)
T1b	2 (5.6%)
T1C	9 (25%)
T2a	2 (5.6%)
T2b	10 (27.8%)
T2C	7 (19.4%)
T3C	2 (5.6%)
N staging (%)	
Nx	29 (80.6%)
N0	4 (11.1%)
N1	3 (8.4%)
M staging (%)	
M0	18 (50%)
M1B	18 (50%)
Comorbidities (%)	
Cerebrovascular	10 (27.8%)
Dyslipidemia	16 (44.4%)
ESRD	7 (19.4%)
T2DM	13 (36.1%)

ESRD=end-stage renal disease; T2DM:type 2 diabetes mellitus

Table II: Predictive value time to CRPC

Variable	Time to CRPC	*p* value
High expression of SSTRS-AS1	19.40 ± 5.9.55	0.043
Low expression ofSSTRS-AS1	30.96 + 3.18	
Surgical castration	32.12 + 3.9	0.132
Medical castration	22.63 + 4.17	
High volume diseases	26.75 + 3.48	0.728
Low volume diseases	28.67 + 4.7	
PSA > 20 ng/ml	24.85 + 3.13	0.072
PSA < 20 ng/ml	37.88 + 6.25	

p values were calculated using Tarone-ware test

MATERIALS AND METHODS

Patients

In total, 36 patients who were diagnosed with PCa from RSUP Dr. Sardjito General Hospital(SGH), Yogyakarta, Indonesia between 2015 and 2019 were enrolled. Patients who received hormonal therapy as the primary treatment were enrolled. All clinical and demographic data were gathered from electronic medical records of SGH. Patients who received local therapy were excluded. This study received approval from the Medical and Health Research Ethics Committee, Universitas Gadjah Mada (KE/0158/02/2020).

The primary endpoint of this study was response to hormonal therapy, described as time to achieve CRPC. CRPC was defined as secondary radiographic or clinical progress of metastases during hormonal therapy or/and increase of PSA values during hormonal therapy after achieving nadir values with a testosterone level of <50 ng/ml. Clinical staging was determined by unified tumor, node, and metastases criteria according to the EAU 2021 guidelines[5] and using the digital rectal examination, magnetic resonance imaging, computed tomography with contrast, or bone survey.

The pathological results on this study were categorized according to the 2005 International Society of Urological Pathology (ISUP) score that is currently being used as a risk stratification parameter in the 2021 EAU guidelines of prostate cancer.[5]

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Methods previously described by Indrawarman et al. were used.[10] In addition, this study was conducted in compliance Helsinki Declaration, and the study was registered with International Standard Randomized Controlled trial register (ISRCTN) under the reference no 24834343.[11]

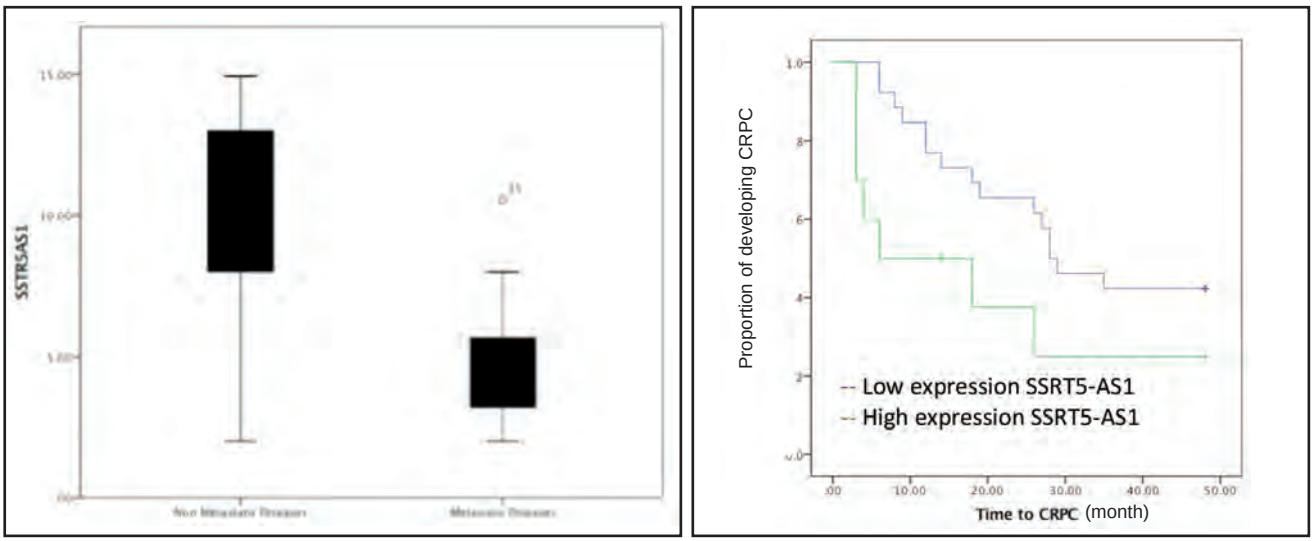

Fig. 1: The expression of SSRT5-AS1 in patients with prostate cancer with no metastases and bone metastases at diagnoses (p< 0.001) (left) and Kaplan-Meier estimates of time to CRPC in patients with prostatic cancer who received ADT for expressions of SSRT5-AS1 (right).

RNAs were extracted from formalin-fixed and paraffin-embedded biopsied samples of PCa tissues and two additional samples of benign prostatic hyperplasia (normal references). Hybrid-R™ Isolation Kit (GeneAll, Seoul-South Korea) was used to extract total RNAs, and NEXpro™qRT-PCR Kit (NextPro, Seoul-South Korea) was used to assess SSRT5-AS1 expression. The primer pair sequences used for the quantification were as follows: 5'-ACTACAGGTGCCATCAGACC-3' (forward) and 5'-GGTGTGCTGGAAAAGGGTCC (reverse). Amplification conditions comprised an initial denaturation step at 95°C for 10 min, followed by 40 cycles at 95°C for 20 s, at 55°C for 40 s, and at 72°C for 60 s. The extension step was conducted at 72°C for 5 min. Amplification of samples using q-PCR was performed using BiONEERExicycle™ 96 (BioNEER, Daejeon, South Korea). RB1 and TP53 expression was determined by the cycle threshold values and was normalized using GADPH, as previously described.

RESULTS

The mean age of patients was 69.07 ± 8.7 years, and the mean PSA was 141.22 ± 112.28 ng/ml. Most patient who was enrolled were classified to have high risk, with 47.2% patients being in ISUP 5 group and 25% being in ISUP 4 group. In this study, patients were castrated using LHRH agonist approximately 55.6% of enrolled patients, and 44.4% patient were surgical castrated.

To further examine the association of SSTR5-AS1 with tumor spread, we evaluated SSTR5-AS1 expression in both patients with non-metastatic disease and patients with metastatic diseases.

At diagnosis, the expression SSTR5-AS1 in the metastatic disease group was significantly lower than that in the non-metastatic disease group (p<0.001).

However, the metastatic status in this study showed similar time to CRPC after receiving castration (Tarone-ware p= 0.728). The expression of SSTR5-AS1 was also similar between patients with PSA value at diagnosis >20 ng/ml and those with PSA value <20 ng/ml. However, time to CRPC in patients with PSA > 20 ng/ml reached faster than in other groups. However, results showed no statistically significant correlation in PSA level and SSTR5-AS1 expression.

In addition, compared with median, higher expressions of SSTR5-AS1 had more significant prognostic value than shorter time to CRPC (Figure 1) (mean: 19.40 + 5.95, p = 0.043) compared to patient with lower expression of SSTRS-AS1 (Figure 1). Meanwhile, the method of castration, diseases volume according CHAARTED study, and PSA value >20 were not statistically significant predictors of time to develop CRPC in this pilot study (Table II).

DISCUSSION

Somatostatin receptors (SSTRs) are commonly expressed on neuroendocrine tumors (NEDs), and their expression has been correlated with disease prognosis in various types of cancer.[11-13] To function biologically and activate it's signaling pathway, somatostatin needs to bind with five somatostatin receptor family gene products (SSTR1 to SSTR5).[11-12] The development of NED and SSTRs over expression by CRPC has been associated with negative or worse prognosis.[14-16] However, the context of NED in PCa is still debatable owing to the mechanism by which it develops and the clinical significance. However, some studies have reported the presence of neuroendocrine cells in PCa.[17-18]

The methods of castration did not show statistical significance. This finding was consistent with that reported by a previous study.[5-6,19] In addition, this study also revealed that metastatic volume by charted study and the initial PSA value did not show statistical significance. This result may be attributed to small sample power.

However, patients with high SSTR5-AS1 expression had shorter time to CRPC after receiving ADT as single therapy. Thus, this indicated that these patients benefit less from single ADT. Our study is the first to evaluate SSRT5-AS1 in a clinical setting for predicting the time CRPC in Asian population. Previously, Ramnarine et al. reported that patients with high expression who underwent prostatectomy plus adjuvant ADT had worse metastasis-free survival than those with low expression of SSTR5-AS1. However, for patient who did not receive ADT as adjuvant therapy had similar metastasis-free survival.[20]

Remarkably, the overexpression of SSTR5 has been indicated as a potential oncogenic factor, presenting as either aggressiveness or high proliferation rate in adenocarcinomas of the lung, squamous cell carcinomas of the lung, and small lung cancer.[21] Mass et al. reported a decrease in SSTR1–4 expression and overexpression of SSTR5, which possibly resulted from the evolution of tumor cells to elude cell cycle control by somatostatin, which can subsequently act as a growth advantage. However, the knowledge about the role of SSTR5 in PCa is still very limited and its therapeutic potential remains unknown.[20-21]

CONCLUSION

To investigate the prognostic value of SSTR5, we measured the expression of SSTR5-AS1 in patients with PCa treated with ADT. Our results showed no apparent correlation between the expression of SSTR5-AS1 with disease metastasis and PSA score. However, our results illustrated a correlation between the expression of SSTR5-AS1 and time to CRPC, which indicated that the expression of SSTR5-AS1 could be considered as a biomarker to predict response to therapy in PCa.

Currently, there is no standard for the cut-off of overexpression of SSTR5-AS1 that can be used as a biomarker for disease progression. Future studies with larger populations are needed to validate and conform our findings. Additionally, further population studies should consider ethnicity in sample characteristics to determine if this biomarker is limited to the Javanese population.

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