Asian Journal of Andrology (2022) 24, 525–532
www.asiaandro.com; www.ajandrology.com

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ORIGINAL ARTICLE

Prostate Cancer

Immunohistochemical analysis of PD-L1 and tumor-infiltrating immune cells expression in the tumor microenvironment of primary signet ring cell carcinoma of the prostate

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Primary signet ring cell carcinoma (SRCC) of the prostate is a rare neoplasm. However, its potential tumorigenic mechanism, clinicopathological features, and prognostic outcome have not been systematically described. To determine the pathogenic mechanism, we detected distributions of programmed cell death-ligand 1 (PD-L1), programmed death 1 (PD-1), and cellular components in the tumor microenvironment, including tumor-infiltrating lymphocytes (CD4 and CD8), tumor-associated macrophages (TAMs; CD163 and CD68), and tumor-associated fibroblasts (vimentin and alpha-smooth muscle actin [α-SMA]), in tumor tissues from four patients with primary prostatic SRCC compared with corresponding adjacent tissues and tumor tissues from 30 patients with prostate adenocarcinoma (PCa) by immunohistochemical staining. We found higher expression of PD-L1, CD163, and CD68 in primary SRCC specimens than that in both corresponding adjacent nontumor specimens and PCa specimens with different Gleason scores, indicating that TAMs may participate in the malignant biological behavior of primary SRCC of the prostate. For further analysis, we searched electronic journal databases and Surveillance, Epidemiology, and End Results (SEER) to identify 200 eligible patients including our four cases. According to Kaplan–Meier survival curve analysis, patients <68 years old, with radical prostatectomy (RP), Gleason score of 7–8, and lower clinical stage had longer overall survival (OS). Moreover, Cox multivariate analysis indicated that race (hazard ratio [HR] = 1.422), surgical approach (HR = 1.654), and Gleason score (HR = 2.162) were independent prognostic factors for OS. Therefore, primary SRCC of the prostate represents a distinct and aggressive subtype of prostate cancer associated with a higher distribution of PD-L1 and TAMs, which warrants further clinical investigation.

Asian Journal of Andrology (2022) **24**, 525–532; doi: 10.4103/aja202186; published online: 14 January 2022

Keywords: clinical features; immunohistochemistry; infiltrating immune cells; primary signet ring cell carcinoma of the prostate; tumor microenvironment

INTRODUCTION

Approximately 1.4 million new cases of prostate cancer, the second most prevalent cancer and fifth leading cause of tumor-related death among men globally, were reported in 185 countries worldwide in 2020, accounting for 14.1% of the 36 cancer diagnoses in men. Incidence rates differ from 6.3 to 83.4 per 100 000 men across regions, with the highest rates in Northern and Western Europe and the lowest rates in Asia and Northern Africa.[1] Moreover, an estimated 248 530 new cases of prostate cancer and 34 130 deaths were reported in the United States in 2021.[2] As a rare histological variant of prostate cancer, primary signet ring cell carcinoma (SRCC) of the prostate is characterized by an intracytoplasmic vacuole that compresses the cell nucleus into a crescent shape.[3,4] Although SRCC mainly occurs in the gastrointestinal (GI) system, it has been detected in the thyroid,[5] breast,[6] pancreas,[7] bladder,[8] and prostate.[9] The diagnosis requires pathological examination, gastrointestinal examination (consisting of an abdominal computed tomography [CT] scan, esophagogastroduodenoscopy, and colonoscopy), and specialized staining processes that facilitate detection of the primary lesion of prostate cancer.[10,11] Patients with primary SRCC of the prostate usually have a poor prognosis, and a standard therapy has not been proposed due to the low number of cases. According to electronic resources, 196 cases of primary SRCC of the prostate have been reported to date. Here, we add four patients in China with primary SRCC of the prostate and summarize similar cases in terms of clinical patient-related and tumor-related pathological characteristics and treatment solutions as well as strategies for monitoring prognosis, based on searching electronic journals and databases. Then, we conducted Kaplan–Meier curves and Cox univariate and multivariate regression analyses to clarify independent prognostic factors for patients with primary prostatic SRCC.

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Received: 01 July 2021; Accepted: 15 October 2021

Moreover, as the tumor microenvironment (TME), including immune effectors, extracellular matrix, and blood vessels, affects the treatment and prognosis of cancer, crosstalk between cancer cells (neck squamous cell carcinomas,[12] pancreatic ductal adenocarcinoma,[13] and colorectal liver metastases[14]) and the surrounding TME has been investigated. On the one hand, immune cells in the TME may modulate the growth and evolution of cancer cells.[15,16] On the other hand, tumors affect the TME by secreting proteins to promote angiogenesis, regulate extracellular signaling, and induce immune tolerance.[17,18] In terms of the mechanism of evasion from host immune responses, programmed cell death-ligand 1 (PD-L1) may interact with programmed death 1 (PD-1) on cytotoxic T lymphocytes, potentially decreasing activation of T cells.[19–21] Therefore, immunohistochemical staining was performed to detect the distributions of PD-L1, PD-1, and cellular components in the TME, such as $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, TAMs, and tumor-associated fibroblasts. We then analyzed the differentially expressed ingredient in tumor tissues from four patients with primary prostatic SRCC compared with corresponding adjacent tissues and tumor tissues from 30 patients with prostate adenocarcinoma (PCa).

PATIENTS AND METHODS

Tissues from case 1 of primary SRCC of the prostate: a 75-year-old male patient presented with a high prostate-specific antigen (PSA) value of 49.73 ng ml^{-1}. Transrectal ultrasound (TRUS; HI VISION, Guangzhou, China) and digital rectal examination (DRE) revealed solid and hard nodules in the prostate. Subsequently, prostate biopsy for histopathological examination showed SRCC with a Gleason score of 7 (4+3). No obvious bone metastases were detected by bone scan. GI examination indicated no evidence of tumor. A normal carcinoembryonic antigen (CEA) level was shown by laboratory examination. The patient had an initial diagnosis of primary SRCC of the prostate and stage T2cNxM0 and received hormonal therapy (250 mg of flutamide administered orally three times a day). Ten months later, the patient underwent transurethral resection of a bladder mass due to urothelial cancer. There was no local recurrent disease after a follow-up of 117 months.

Tissues from case 2 of primary SRCC of the prostate: a 70-year-old man was admitted to the hospital with a PSA value of 10.09 ng ml^{-1} and hematuria that had persisted for 2 weeks. Pelvic magnetic resonance imaging (MRI; GE Healthcare, Waukesha, WI, USA) revealed an enlarged prostate and thickened bladder wall, and computed tomography urography (CTU) showed a bladder filling defect, suggestive of prostate space-occupying lesions. Prostate biopsy revealed primary SRCC of the prostate with a Gleason score of 8 (4+4) on the right side of the prostatic apexes, and immunohistochemical staining was positive for PSA and α-methylacyl coenzyme A racemase (P504S). A bone scan of the body revealed potential bone metastases at stage pT1NxMx. After 2 months of hormone therapy (50 mg of bicalutamide administered orally once a day and a subcutaneous injection of 3.6 mg of Zoladex once a month), the patient underwent laparoscopic radical prostatectomy. Postoperative pathological results showed prostatic hyperplasia with a few disordered local glands (suspicious adenocarcinoma), which indicated that hormone therapy may be effective. The patient was still alive without local recurrence after a follow-up of 106 months.

Tissues from case 3 of primary SRCC of the prostate: a 69-year-old man with PSA >100 ng ml^{-1} presented with hydronephrosis. DRE revealed an enlarged prostate with palpable nodules. TRUS-guided prostate biopsy showed primary SRCC of the prostate with a Gleason score of 7 (4+3). Whole-body bone imaging indicated multiple bone metastases, though the results of GI examination revealed no evidence of tumors. The patient received hormone treatment (250 mg of flutamide administered orally three times a day and a subcutaneous injection of 3.6 mg of Zoladex once a month), and zoledronic acid was used to prevent bone metastasis. In September 2013, palliative transurethral resection of the prostate and bilateral orchiectomy were performed due to progressive dysuria. Postoperative pathological results indicated prostate cancer with a Gleason score of 9 (5+4). Because the patient was unable to tolerate the side effects of paclitaxel monotherapy (PTX: 300 mg dl^{-1} per intravenous drip for 21 days), six cycles of docetaxel chemotherapy (docetaxel: 120 mg dl^{-1} per intravenous drip for 21 days) was administered. He continued subcutaneous injection of 3.6 mg of Zoladex once a month and was still alive after a follow-up of 142 months.

Tissues from case 4 of primary SRCC of the prostate: a 67-year-old man presented with a PSA value of 65.87 ng ml^{-1} and difficulty urinating for 1 week. DRE demonstrated a tenacious prostate with a moderate increase in size. Both ultrasound and CT of a urinary system scan suggested prostate hyperplasia with calcification, and prostate MRI identified multiple diffusely restricted areas of the prostate and vesical diverticulum, which suggested prostate cancer. Subsequently, prostate biopsy revealed primary SRCC of the prostate with a Gleason score of 9 (5+4). Gastrointestinal tumors were not detected using GI or colon fiber endoscopy. The patient refused relevant surgery and medication for economic reasons. The patient was still alive after a follow-up of 20 months.

Immunohistochemical staining

Tumor specimens were obtained from 30 PCa patients, which included 10 cases with Gleason scores of 2–4, 10 cases with Gleason scores of 5–7, and 10 cases with Gleason scores of 8–10. The median age was 73 (range: 63–89) years, and PSA values varied from 0.5 to >100 (median: 12.08) ng ml^{-1}. Most patients ($n = 15$) presented with stage II disease at diagnosis. Twenty-three patients presented with pathological stage T2 cancer, followed by T1/T3 cancer ($n = 3$). Half of the tissue samples ($n = 15$) were obtained from radical prostatectomy; the other half were from transurethral resection of prostate (TUR-P). Two patients had perineural invasion; 2 patients had lymph node involvement; 1 patient had lymphovascular emboli; and 5 patients had distant metastasis. All tissue samples were evaluated by two experienced independent pathologists for confirmation of PCa.

Tissues were fixed in formalin (10%) for 48 h, embedded in paraffin after dehydration with ethanol solution, and cut into longitudinal sections (thickness of 4 μm). The tissues were stained with hematoxylin and eosin (HE; PH0516, Phygene Life Sciences Company, Fuzhou, China), periodic acid Schiff (PAS) stain kit (ab150680, Abcam, Cambridge, UK), mucicarmine stain (ab150677, Abcam), and Alcian blue stain kit (ab150662, Abcam).

Four-micrometer sections were stained by immunohistochemistry using a standard protocol. After dewaxing with xylene overnight and subjecting it to alcohol solutions, the sections were heated in 10 mmol l^{-1} citrate buffer (MVS-0066, Maixin Biological Technology Development Company, Fuzhou, China) for 15 min at 120°C for antigen retrieval. Endogenous peroxidases were intercepted using 3% hydrogen peroxide (SP KIT-A3, Maixin Biological Technology Development Company) for 30 min, and the sections were incubated at 4°C for 24 h with one of the following primary antibodies: rabbit monoclonal anti-PSA (dilution 1: 100; ab224799, Abcam), rabbit monoclonal anti-prostatic–specific acid phosphatase (PSAP; dilution 1: 100; ab166910, Abcam), mouse monoclonal anti-PD-1 (dilution 1: 100; ab52587, Abcam), rabbit

monoclonal anti-PD-L1 (dilution 2 μg ml^{-1}; ab205921, Abcam), rabbit monoclonal vimentin (dilution 1: 300; ab92547, Abcam), rabbit polyclonal anti-alpha-smooth muscle actin (α-SMA; dilution 1: 3000; 14395-1-AP, ProteinTech Group, Chicago, IL, USA), rabbit monoclonal anti-CD163 (dilution 1: 400; ab182422, Abcam), mouse monoclonal anti-CD68 (dilution 1: 3000; ab955, Abcam), rabbit monoclonal anti-CD4 (dilution 1: 500; ab183685, Abcam), and mouse monoclonal anti-CD8 (dilution 1: 6000; 66868-1-Ig, ProteinTech Group). The sections were then incubated with biotinylated secondary antibodies and avidin-conjugated horseradish peroxidase and developed with diaminobenzidine (DAB; DAB-0031, Maixin Biological Technology Development Company). The level of stromal expression was calculated by two experienced investigators who were double blinded. The staining intensity was scored as follows: 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong). Expression of the aforementioned markers was evaluated by multiplying the score for the percentage by the intensity score, which is presented as each individual column in a heatmap.

Search methods for identification of studies and patients

The PubMed, Google Scholar, Embase, Cochrane Library, and Ovid MEDLINE databases were used to search for studies examining primary SRCC of the prostate from database inception until July 2021. The search terms included "signet ring cell", "prostate cancer", "prostate carcinoma", and "primary cancer". Additional eligible studies were searched in the bibliographies of Urological Men's Health: A Guide for Urologists and Primary Care Physicians, Campbell-Walsh Urology, Surgical Procedures for Core Urology Trainees, *etc*. Bibliographies of the retrieved articles were also hand-searched to identify other potentially eligible studies. No filters were applied regarding the date of publication or language. Our study was performed based on the Preferred Reporting Items for Systemic Review and Meta-Analysis (PRISMA) statement. The present study has been registered at the International Prospective Register of Systematic Reviews (PROSPERO; registration No. CRD42021269757). A flowchart of the literature search is presented in **Figure 1**.

Studies were eligible when they satisfied with the following criteria: (i) histopathological examination confirmed the diagnosis of primary prostatic SRCC and (ii) patient-specific and tumor-specific characteristics were reported. The following exclusion criteria were used: (i) patients who had metastasized cancer from other organs or mixed carcinoma; (ii) studies without original data; or (iii) letters to the editor, reviews, or commentaries.

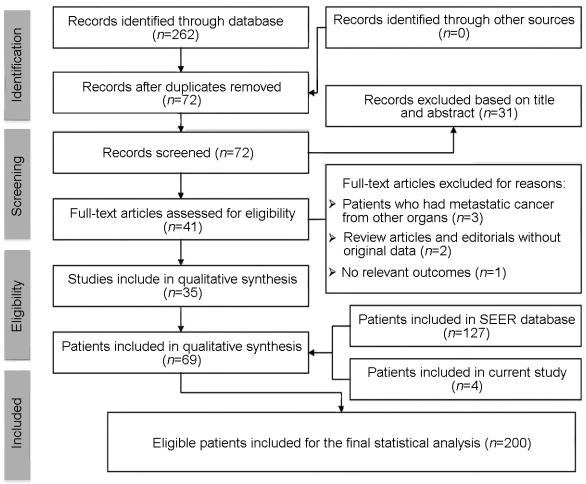

Figure 1: PRISMA flowchart of the selection of studies and patients. PRISMA: Preferred Reporting Items for Systemic Review and Meta-Analysis; SEER: Surveillance, Epidemiology, and End Results.

A meticulous procedure was carried out independently by two investigators (QLT and BF) who selected potentially relevant studies according to the predetermined criteria. Any discrepancies in data extraction were assessed by the reviewer (ZYL), who checked the resulting extractions. Data collected from the studies included study type, year of publication, first author, country, number of patients, recruitment period, PSA level, Gleason score, clinical stage, tumor, node, and metastasis (TNM) stage, treatment modalities, tumor characteristics, histological characteristics, follow-up period, and demographics (age and race).

A quality investigation was performed for our study based on the latest version of the risk assessment tool suggested by the ROBINS-I checklist[22] to evaluate interventions in eligible studies. The studies were investigated for bias regarding selection bias, confounding, intervention measurement, reporting bias, missing data bias, outcome measurement, and other types of bias (**Supplementary Figure 1**).

SEER (Incidence-SEER 18 Regs Custom Data with additional treatment fields, November 2018 Sub, 1975-2016 varying and Incidence-SEER Research Plus Data, 18 Registries, November 2020 Sub, 2000–2018) data were used to review prostate cancer subtypes. SEER*State version 8.3.6.1 was used to achieve a complete case-listing file. The selected criteria for patients included SRCC of the prostate as the first cancer diagnosis who had been histopathologically diagnosed with SRCC (ICD-O-3 8490/3). Patients diagnosed between 1975 and 1999 from SEER database (1975–2016), and between 2000 and 2018 from SEER database (2000–2018) were selected.

Statistical analyses

SPSS version 19.0 (SPSS Corp., Armonk, NY, USA) and R version 3.6.3 software (R Foundation for Statistical Computing, Vienna, Austria) were used to perform statistical analyses. OS was defined as the interval between the diagnosis and the last follow-up or death. Kaplan–Meier curves were drawn to estimate the effects of race, age, PSA, Gleason score, clinical stage, surgery therapy, hormone therapy, and radiation therapy on OS using a log-rank test. Forest plots for the results of multivariate and univariate Cox regression were completed by the visualization package ggplot2 using R software.[23] In addition, a heatmap for immunohistochemical staining results for PCa tissues with different Gleason scores, primary SRCC specimens and corresponding adjacent nontumor specimens was generated with the visualization package ComplexHeatmap of R software under the clustering method of Euclidean.[24] $P < 0.05$ represented a statistically significant difference.

RESULTS

Immunohistochemistry for identifying the histologic source of primary SRCC of the prostate

Microscopic examination of specimens from the four patients using HE staining revealed that tumor cells were arranged in sheets, with nest-like adenoids and single cells with hyperplastic fibrous tissue between these structures. The tumor cytoplasm was vacuolated, and the nucleus was located on the side of the cell, resembling a signet ring (**Figure 2a**). However, the surrounding normal prostate tissue exhibited atrophy. Immunohistochemical staining for PSA and PSAP was performed to ensure the prostatic source (**Figure 2b** and **2c**). Positive staining for PAS, mucicarmine, and Alcian blue revealed the presence of intracytoplasmic mucin, which confirmed that the histologic source was signet ring cells of the prostate (**Figure 2d**–**2f**).

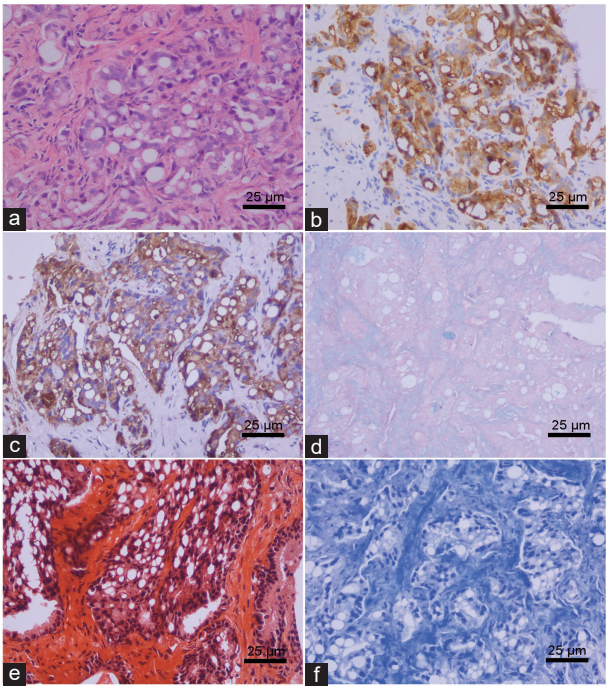

Figure 2: Staining for HE, PSA, PSAP, and mucin at 400× magnification. (**a**) Representative HE staining for primary SRCC of the prostate. Representative immunohistochemical staining for (**b**) PSA and (**c**) PSAP in tumor cells. Representative mucin staining of (**d**) PAS, (**e**) mucicarmine, and (**f**) Alcian blue for primary SRCC of the prostate. HE: hematoxylin and eosin; PSA: prostate-specific antigen; PSAP: prostatic-specific acid phosphatase; SRCC: signet ring cell carcinoma; PAS: periodic acid Schiff.

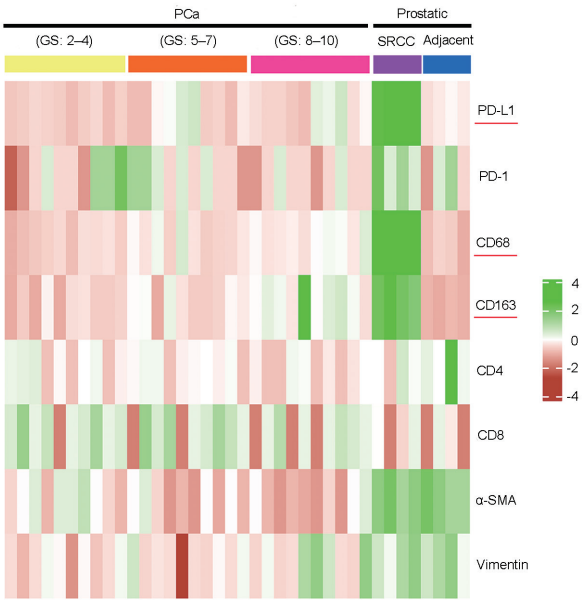

Figure 3: Heatmap for the immunohistochemical staining results among PCa tissues with different Gleason scores, primary SRCC specimens and corresponding adjacent non-tumor specimens. PCa: prostate adenocarcinoma; PD-L1: programmed cell death-ligand 1; PD-1: programmed death 1; α-SMA: alpha-smooth muscle actin; SRCC: signet ring cell carcinoma; GS: Gleason score.

Immunohistochemistry for detecting components of the tumor microenvironment

Immunohistochemical staining was also applied to analyze the distributions of PD-1 and PD-L1, markers of tumor-associated fibroblasts (vimentin and α-SMA), markers of TAMs (CD163 and CD68), and markers of $CD4^+$ (CD4) and $CD8^+$ T lymphocytes (CD8). As shown in **Figure 3** and **4**, we found higher expression of PD-L1, CD163, and CD68 in primary SRCC specimens than those in both corresponding adjacent nontumor specimens and conventional PCa specimens with different Gleason scores, indicating that TAMs may participate in the malignant biological behavior of primary SRCC of the prostate. Nevertheless, an obvious difference in the staining distribution of low PD-1, CD4, and CD8 expression was not observed among PCa specimens with different Gleason scores, primary SRCC specimens, and corresponding adjacent nontumor specimens. In contrast, higher levels of vimentin and α-SMA were detected in both primary SRCC specimens and corresponding adjacent nontumor specimens, with no difference in the distribution of cancer-associated fibroblasts. Moreover, levels of vimentin and α-SMA were lower in PCa specimens with different Gleason scores than those in primary SRCC specimens.

Patient-specific characteristics in primary prostatic SRCC

After an initial search of electronic databases to identify studies examining primary SRCC of the prostate based on the title/abstract and full text, thirty-five published articles including 69 cases with primary SRCC of the prostate were identified. A total of 127 eligible patients were selected from the SEER database according to the screening criteria. A summary of the characteristics of all 200 patients with our four cases is shown in **Supplementary Table 1**. All patients were aged between 44 years and 91 years. Of the eligible patients, 70.4% (102/145) were White, 18.6% (27/145) were Black, and 11.0% (16/145) were Asian or Pacific Islander. PSA values varied substantially between 0.19 ng ml^{-1} and 1990.00 ng ml^{-1}. PSA levels of 72 (92.3%, 72/78) individuals were greater than 4.00 ng ml^{-1}, and PSA levels of 44 (56.4%, 44/78) individuals were greater than 10.00 ng ml^{-1}. It should be noted that the SEER database does not provide information about DRE and GI examination. Based on 73 patients in the included studies, 37.0% (27/73) had an abnormal DRE result (**Supplementary Figure 2a**), and 49.3% (36/73) had a negative GI result (**Supplementary Figure 2b**).

Tumor-specific characteristics of primary prostatic SRCC

Most patients (30.0%, 60/200) presented with stage T2 cancer, followed by T3 (13.5%, 27/200), T1 (10.0%, 20/200), and T4 (6.0%, 12/200), as shown in **Supplementary Figure 3a**. The most frequent Gleason score was 9–10 (23.5%, 47/200), followed by 7–8 (12.0%, 24/200), as shown in **Supplementary Figure 3b**. Most patients (38.0%, 76/200) presented with stage II–III disease at diagnosis. However, the SEER dataset lacks information on immunohistochemical staining. According to the included studies of 73 cases, the most widely used staining method was PSA or PSAP staining (84.2%, 48/57), followed by PAS (63.6%, 28/44) and Alcian blue (50.0%, 17/34) staining (**Supplementary Figure 3c**).

Treatment-specific characteristics of primary prostatic SRCC

In general, the therapeutic strategy for this malignancy is a combination of surgical, hormone, and radiation treatments. For 192 patients with available treatment data, the adjuvant therapy was mainly hormone therapy alone (15.1%, 29/192), followed by hormone therapy with radiotherapy (9.9%, 19/192). Notably, radical prostatectomy was performed in 30.7% of patients (59/192), and 8.9% of patients (17/192) occasionally underwent TUR-P. The median OS time was 47.5 months after diagnosis. Among 200 eligible patients, 188 had survival time and outcome after follow-up data available, which was assessed

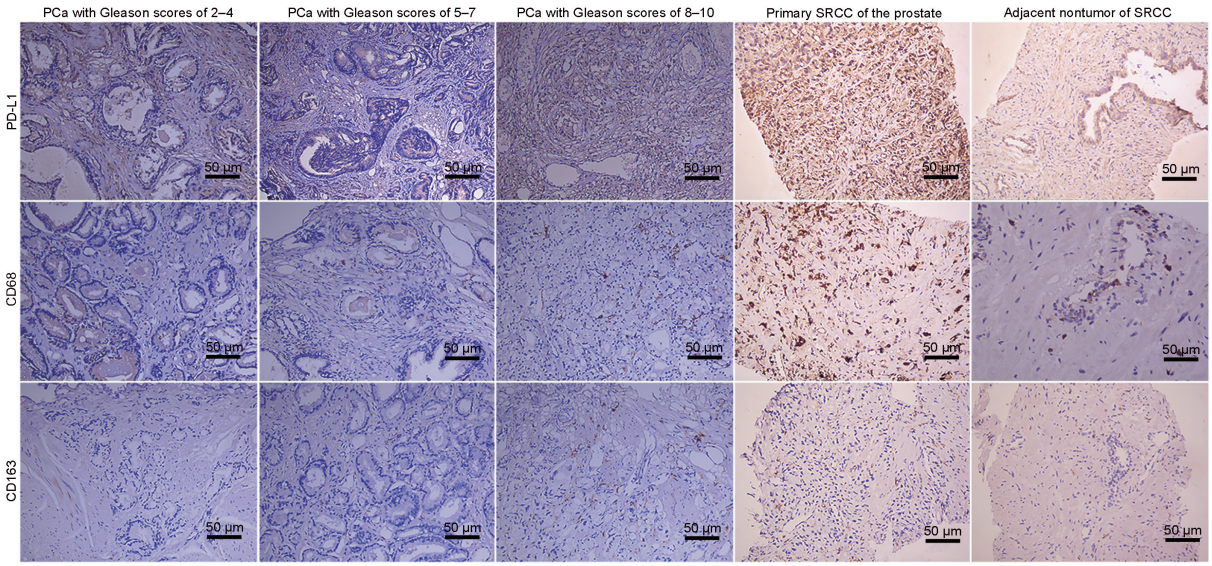

Figure 4: Representative images of PD-L1, CD68, and CD163 immunohistochemical staining in primary SRCC specimens and negative controls (corresponding adjacent nontumor specimens and PCa specimens with different Gleason scores) at 200× magnification. High PD-L1, CD68 and CD163 expression levels appear as brown or brown–yellow staining. PCa: prostate adenocarcinoma; SRCC: signet ring cell carcinoma.

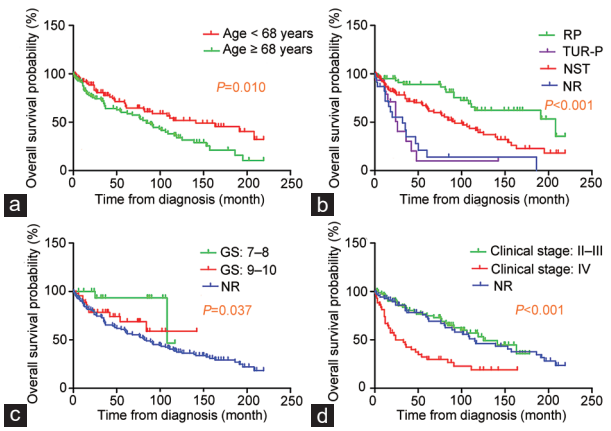

Figure 5: Kaplan–Meier curves for OS of patients with primary prostatic SRCC stratified according to (**a**) age, (**b**) surgical approach, (**c**) GS, and (**d**) clinical stage. NST: nonsurgical treatment; NR: not reported; RP: radical prostatectomy; SRCC: signet ring cell carcinoma; TUR-P: transurethral resection of prostate; GS: Gleason score.

by Kaplan–Meier curves and Cox univariate and multivariate regression analyses. The Kaplan–Meier curve showed that patients younger than 68 years had a longer OS time than patients older than or equal to 68 years ($P = 0.010$; **Figure 5a**). Patients who underwent radical prostatectomy had a longer OS time than those treated with TUR-P or no surgery ($P < 0.001$) (**Figure 5b**). Moreover, patients with a Gleason score of 9–10 and higher clinical stage (IV) experienced a shorter OS than those with a Gleason score of 7–8 ($P = 0.037$) and lower clinical stage (II–III; $P < 0.001$), as shown in **Figure 5c** and **5d**. Survival analysis using the multivariate Cox regression model adjusted for age, race, PSA, Gleason score, clinical stage, surgery therapy, hormone therapy, and radiation therapy suggested that race (hazard ratio [HR] = 1.422, 95% confidence interval [CI]: 1.186–1.706; $P < 0.001$), Gleason score (HR = 2.162, 95% CI: 1.332–3.509 ; $P = 0.002$), and surgical approach (HR = 1.654, 95% CI 1.286–2.128; $P < 0.001$) were independent prognostic factors for OS for patients with primary prostatic SRCC, as illustrated in **Figure 6**.

DISCUSSION

As a component of the TME in many cancers, TAMs are divided into two main subsets. The first is called proinflammatory (M1) macrophages, which produce a mutagenic microenvironment to participate in the initial process of tumorigenesis.[25,26] TAMs often differentiate into the second subset called anti-inflammatory (M2) macrophages, which create an immunosuppressive TME for tumor growth promotion.[27,28] Immunohistochemistry of tissue samples from our four patients with primary prostatic SRCC showed an increase in the staining distribution of CD163 and CD68 in tumor tissues compared with corresponding adjacent non-tumor tissues and PCa tissues. As a marker of TAMs, CD68, a scavenger receptor, is expressed at high levels in tissue macrophages and is considered to be a panmacrophage marker. CD163 is a highly specific marker of the M2 macrophages subset, and TAMs in most tumors present the M2 macrophages phenotype, which produces immunosuppressive factors and expresses PD-L1 to directly inhibit T-cell function to promote tumor invasion, angiogenesis and metastasis.[29–31] As high CD68 and/or CD163 expression on macrophages is thought to be associated with advanced tumor stage and worse prognosis in breast cancer,[32] ovarian cancer,[33] and cutaneous melanoma,[34] TAMs may participate in the malignant progression of tumors or affect the malignant biological behavior of primary SRCC of the prostate.

In our study, we found higher expression of PD-L1 in tumor samples from four patients with primary SRCC of the prostate than that in corresponding adjacent nontumor tissues or prostate adenocarcinoma tissues. Many tumor and immune cells express PD-L1, which is thought to play an essential role in decreasing cancer immunity by binding PD-1 and B7.1 (CD80), which are positive regulators of T lymphocyte inactivation. Binding of PD-L1 to PD-1/B7.1 blocks T-cell proliferation, migration and cytotoxic mediator secretion and reduces the killing of tumor cells.[35–37] In primary gastric SRCC, Jin *et al.*[38] evaluated PD-1 and PD-L1 expression and infiltration by $CD3^+$ T cells in advanced gastric SRCC and found that PD-1 expression on tumor-infiltrating lymphocytes was significantly related to PD-L1 expression. Another study by Huang *et al.*[39] found that gastric SRCC

patients with the AT-rich interacting domain containing protein 1A (*ARID1A*) mutations had higher PD-L1 expression than counterparts without based on the Cancer Genome Atlas (TCGA). These authors proposed a hypothesis that Epstein–Barr virus (EBV) infection may have an influence on *PIK3CA* and *ARID1A* mutations, which may contribute to elevation of PD-L1 expression. For SRCC in the colon, Alvi *et al.*[40] observed higher CD3 and PD-L1 levels were microsatellite instability cases associated with the hypermethylated genotype, and Tai *et al.*[41] detected that PD-L1 expression was associated with an improved prognosis, representing a possible ideal target for immune checkpoint-based therapy. Therefore, we speculate that patients with primary SRCC of the prostate presenting with high PD-L1 expression may benefit from treatment with anti-PD-1/PD-L1 antibodies, including nivolumab, pembrolizumab, and durvalumab, though clinical trials with a large sample size are required for verification.

"Signet ring cell" is a term describing the histological pattern of a tumor cell that is identified by compression of the nucleus into a crescent shape and displacement by an intracytoplasmic vacuole.[42] The first signet ring cell change of the prostate was described in 1981,[43] and 2.5% of patients with adenocarcinoma of the prostate were estimated to have this condition.[44] As SRCC is commonly present with other patterns of high-grade prostate cancer, Guerin *et al.*[42] suggested that SRCC is a variant of high-grade adenocarcinoma rather than a histological type. Currently, pathological diagnosis of primary prostatic SRCC must consider these two aspects. (1) The signet ring cell proportion of 20% to 50% of the entire number of tumor cells will usually help establish an SRCC diagnosis.[42,44] A relatively persuasive indication of the prostatic source is the presence of more typical prostatic adenocarcinoma cells in the specimen.[45] (2) Immunohistochemistry for PSA and PSAP may assist in diagnosis.[46] In this investigation, 84.2% of patients presented positive staining for PSA and PSAP. Other types of positive staining included PAS in approximately 63.6% of patients and Alcian blue in approximately 50.0% of patients.

Based on our four cases and cases identified in the literature, 68 years is the average age at diagnosis (range: 44–91 years), the same as that in a previous report.[47] As signet ring cells can more commonly be detected in the gastrointestinal tract, gastrointestinal endoscopy may be useful for distinguishing primary SRCC from metastatic SRCC. Among the included patients, only 49.3% underwent a recorded GI examination with a negative result. In terms of the clinicopathological characteristics of the tumor, most patients presented with T2 stage disease and a Gleason score of 9–10. The multivariate Cox model identified race (HR = 1.422; $P < 0.001$), Gleason score (HR = 2.162; $P = 0.002$), and surgical approach (HR = 1.654; $P < 0.001$) as independent prognostic factors for OS. To date, there is no standard treatment for the management of primary SRCC of the prostate. As clinical cases are limited and scattered worldwide, urologists often adopt treatment options for adenocarcinoma of the prostate comprising various combinations of hormone therapy, radiotherapy, and surgery. In terms of chemotherapy, one patient accepted chemotherapy with leucovorin, fluorouracil and oxaliplatin (FOLFOX) and Erbitux, which produced a near-complete response.[48] In addition, combination therapy for primary SRCC of the prostate has received increasing attention. Yoshimura *et al.*[49] reported a survival period of 100 months for a patient treated with the combination of hormone therapy and radiotherapy, and Lilleby *et al.*[50] reported a favorable response of a patient with primary SRCC of the prostate to neoadjuvant hormone therapy and radiotherapy after 12 months of follow-up. In terms of controlling increases in PSA level, neoadjuvant hormonal therapy and radical prostatectomy show good therapeutic effects.[51,52] Regarding surgical treatment, except for radical prostatectomy, which was performed in 30.7% of patients (59/192), 8.9% of patients (17/192) underwent TUR-P. Patients with a normal total PSA level received TUR-P treatment for severe prostatic hyperplasia, but the pathological diagnosis unexpectedly indicated a rare type of cancer. In addition, patients with advanced prostate cancer had a main complaint of acute urinary retention and underwent palliative TUR-P. Of the patients included in our study, the 15 patients who underwent hormone therapy and radiotherapy presented the highest survival rate of 36 months. Overall, based on the treatment characteristics of included studies[3,4,9,10,42-44,46-73] and patients, aggressive and comprehensive treatment should be considered, which likely will rely on early invasive surgery combined with hormone treatment and adjuvant radiation therapy.

Our study also has some limitations. First, the number of available cases was very limited as a result of the scarcity of this disease, with research spanning almost 30 years. Hence, several patients were probably diagnosed at an advanced stage in the pre-PSA era. Second, although we found that PD-L1 and M2 macrophages were expressed at higher levels in tumor tissues from four patients with primary prostatic SRCC than those in corresponding adjacent non-tumor tissues and prostate adenocarcinoma tissues, the precise mechanism warrants further investigation based on multicentre research, which will help investigate the clinical profile and primary cell culture technology. Genetic alterations of SRCC in prostate cancer should thus be revealed. Furthermore, the staining methods did not use rigorous criteria to diagnose early cases and were different from the methods used currently to exclude a GI source. To date, systematic reviews of primary SRCC of the prostate are limited, and the classification criteria for some clinical information also differ, requiring a careful interpretation

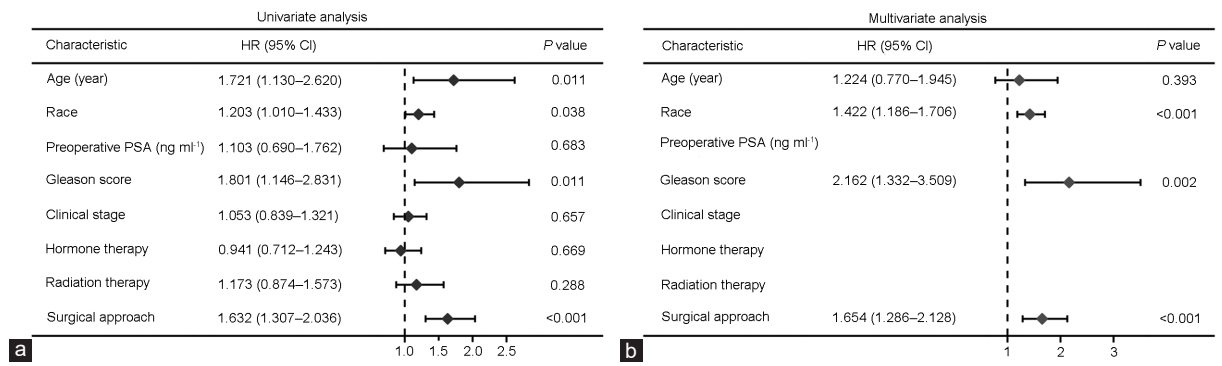

Figure 6: (**a**) Univariate and (**b**) multivariate analyses of factors associated with overall survival in patients with primary SRCC of the prostate. CI: confidence interval; HR: hazard ratio; PSA: prostate-specific antigen; SRCC: signet ring cell carcinoma.

of the results and reference to the original data for clinical purposes. By evaluating cases in the literature, we hope that more available clinical data will be added to solve the treatment dilemma.

CONCLUSIONS

Primary SRCC of the prostate represents a distinct subtype of prostate cancer associated with a high distribution of PD-L1 and TAMs by immunohistochemical findings. Clinically, primary SRCC of the prostate may show aggressive behavior with a high rate of recurrent disease, which warrants further clinical investigation.

AUTHOR CONTRIBUTIONS

ZYL, BF, QLT, and SW contributed to the study concept and design. ZYL and BF undertook project leadership and guaranteed this work. XRY, SW, and HLW carried out the immunohistochemical staining. QLT and XRY collected the clinical data, performed the public databases search and data extraction. QLT, ZHD, and TQL carried out the statistical analysis. QLT and BF wrote the manuscript. ZYL, BF, LW, and XRY revised and reviewed the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation of China (No. 31800787 and No. 81772739), the Natural Science Foundation of Liaoning Province (No. LQ2017025), the Doctoral Research Startup Foundation of Liaoning Province (No. 20180540020), the Medical Scientific Research Project of Dalian City (No. 1812038), and the United Fund of the Second Hospital of Dalian Medical University and Dalian Institute of Chemical Physics, Chinese Academy of Sciences (UF-QN-202004).

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Patient-specific and tumor-specific characteristics of reported cases of signet ring cell carcinoma of the prostate

Reference	*Age (year)*	*Race*	*PSA at diagnosis (ng ml^{-1})*	*Treatment*	*TNM stage*	*Clinical stage*	*Gleason score*	*Follow-up (month)*	*Outcome*
Gupta and Gulwani 2020[53]	72	NR	6.5	TURP[b]	NR	NR	5+5=10	6	Alive
Blas *et al*. 2019[4]	82	NR	331	H	$T_{2c}N_1M_1$	4	5+5=10	11	Dead
	75	NR	10.6	H+R	$T_{2b}N_xM_x$	NR	5+4=9	23	Alive
	79	NR	18	H	$T_{2c}N_xM_x$	NR	5+5=10	21	Alive
	79	NR	11	RP+H	$T_{2c}N_0M_x$	NR	4+5=9	37	Alive
	74	NR	43	H+R	$T_{2c}N_0M_1$	4	5+4=9	84	Dead
Gök *et al*. 2018[3]	70	NR	7.26	H+R	NR	NR	5+5=10	16	Alive
Xiao and Unger 2017[54]	74	White	6.1	NR	NR	NR	NR	NR	Alive
Tiwari *et al*. 2017[55]	65	NR	1990	H	NR	NR	5+5=10	24	Alive
Sáez Barranquero and Herrera Imbroda 2017[9]	74	NR	10.3	H	NR	NR	NR	6	Alive
Kim *et al*. 2016[56]	56	Asian	0.64	RP+R+C	NR	NR	NR	24	Dead
Celik *et al*. 2014[57]	66	NR	66.58	TURP+H+R+C	$T_xN_xM_{1b}$	4	5+5=10	42	Dead
Haddad *et al*. 2014[58]	63	NR	10	H+R	$T_{2b}N_0M_0$	2	3+4=7	NR	NR
Kwon *et al*. 2013[59]	61	Asian	14.7	H+C	$T_{2b}N_0M_{1b}$	4	NR	11	Dead
Roldán *et al*. 2012[48]	65	NR	6.6	RP+C	$T_{3b}N_0M_0$	3	4+5=9	23	Alive
Bonetti *et al*. 2011[60]	70	NR	NR	Hc	NR	NR	NR	11	Dead
Hashimoto *et al*. 2011[61]	61	Asian	0.19	C	$T_3N_2M_2$	4	NR	16	Dead
Warner *et al*. 2010[47]	58	NR	NR	TURP+R	$T_1N_xM_x$	NR	NR	24	Dead
	82	NR	NR	H	$T_4N_xM_1$	4	NR	5	Dead
	68	NR	NR	H	$T_4N_xM_1$	4	NR	12	Dead
	65	NR	NR	TURP+H	$T_3N_xM_1$	4	NR	24	Dead
	67	NR	NR	RP	$T_{3a}N_xM_x$	NR	3+5=8	108	Dead
	67	NR	1.9	H+R	$T_{2a}N_xM_x$	NR	4+5=9	48	Alive
	79	NR	5.9	RP	$T_{3b}N_xM_x$	NR	NR	4	Dead
	51	NR	NR	RP+R	$T_{3b}N_xM_x$	NR	NR	36	Alive
	59	NR	4.8	RP+H	$T_{2b}N_xM_x$	NR	4+4=8	12	Alive
Matsuoka *et al*. 2007[62]	62	NR	364.7	H	$T_4N_1M_1$	4	5+4=9	15	Dead
Derouiche *et al*. 2007[63]	85	NR	9.1	TURP+R	$T_2N_xM_x$	2	NR	18	Alive
Lilleby *et al*. 2007[50]	70	NR	27	H+R	$T_{3b}N_0M_0$	3	4+4=8	12	Alive
Fujita *et al*. 2004[64]	75	NR	9.3	RP+H	$T_{2a}N_0M_0$	2	NR	12	Alive
Akagashi *et al*. 2003[52]	72	NR	470	H	$T_4N_0M_0$	4	NR	20	Alive
Kuroda *et al*. 1999[10]	81	Asian	>100	H	$T_2N_xM_x$	2	NR	2	Alive
Torbenson *et al*. 1998[44]	77	NR	92.6	NR	$T_{1c}N_xM_0$	NR	7	NR	NR
	69	NR	NR	H+R	$T_xN_xM_1$	4	9	18	Dead
	82	NR	5.2	H+R	$T_{1c}N_xM_0$	NR	7	NR	NR
	54	NR	24	H	$T_{3a}N_0M_0$	3	7	34	Alive
	64	NR	8.8	H+R	$T_2N_0M_0$	2	9	NR	NR
	67	NR	4.4	NR	$T_{2c}N_0M_0$	2	7	32	Alive
	63	NR	16.7	R	$T_{2c}N_0M_0$	2	7	24	Alive
	76	NR	NR	NR	NR	NR	9	NR	NR
	67	NR	19.9	H	$T_{2c}N_0M_0$	2	8	21	Alive
	67	NR	15.8	RP+H+R	$T_{2c}N_xM_0$	NR	8	27	Alive
	66	NR	29.6	NR	$T_{2c}N_0M_0$	2	6	NR	NR
	71	NR	20	NR	$T_{2c}N_0M_0$	2	8	NR	NR
Kanematsu and Hiura 1997[51]	76	NR	237	RP+H	$T_{2c}N_xM_x$	NR	NR	36	Alive
Leong *et al*. 1996[65]	71	NR	536	TURP	$T_4N_xM_x$	4	5+4=9	11	Dead
Yoshimura *et al*. 1996[49]	65	NR	NR	H+R	$T_3N_0M_0$	3	NR	100	Alive
Segawa and Kakehi 1993[66]	61	Asian	Normal	TURP+H+R	NR	3	NR	26	Dead
Skodras *et al*. 1993[67]	57	Black	Normal	TURP+H+R	$T_4N_xM_x$	4	NR	NR	NR
Guerin *et al*. 1993[42]	84	NR	NR	TURP+TURBT	NR	≤2	NR	13	Alive
	81	NR	NR	TURP	NR	≤2	NR	15	Alive
	70	NR	NR	TURP+H	NR	4	NR	1	Dead
	83	NR	NR	TURP+H	NR	4	NR	12	Dead
	78	NR	NR	TURP	NR	≤2	NR	15	Dead
Ben-Izhak and Lichtig 1992[68]	70	NR	NR	TURP+H+R	NR	4	NR	36	Dead

Contd...

Supplementary Table 1: Contd...

Reference	*Age (year)*	*Race*	*PSA at diagnosis (ng ml^{-1})*	*Treatment*	*TNM stage*	*Clinical stage*	*Gleason score*	*Follow-up (month)*	*Outcome*
Catton *et al*. 1992[69]	63	NR	NR	H	NR	4	NR	24	Dead
Alline and Cohen 1992[70]	53	NR	5.2	TURP+H	NR	4	5+5=10	NR	NR
Hejka and England 1989[71]	57	White	NR	NR	NR	NR	NR	NR	NR
Remmele *et al*. 1988[72]	67	White	NR	H	NR	4	NR	2	Dead
Ro *et al*. 1988[46]	50	White	NR	d	NR	4	NR	6	Alive
	65	White	NR		NR	3	NR	12	Alive
	78	White	NR		NR	3	NR	36	Dead
	68	White	NR		NR	4	NR	50	Dead
	72	Black	NR		NR	3	NR	32	Dead
	63	White	NR		NR	4	NR	47	Dead
	64	White	NR		NR	3	NR	60	Dead
	80	White	NR		NR	3	NR	3	Alive
Kums and van Helsdingen 1985[73]	63	NR	NR	TURP+R	NR	2	NR	48	Dead
Giltman 1981[43]	77	white	NR	NR	NR	4	NR	0.5	Dead
Patient 1[a]	62	White	NR	RP	NR	NR	NR	192	Alive
Patient 2	67	White	NR	RP+H	$T_2N_1M_0$	4	NR	111	Dead
Patient 3	60	Black	NR	RP	$T_{3a}N_0M_0$	3	NR	127	Alive
Patient 4	69	Black	12.3	H+R[e]	$T_2N_0M_{1b}$	4	5+4=9	3	Dead
Patient 5	44	White	≥98	H+R+C[e]	$T_{1b}N_1M_0$	4	5+5=10	18	Dead
Patient 6	84	Black	NR	H[e]	$T_{1b}N_0M_{1b}$	4	5+4=9	12	Dead
Patient 7	76	White	NR	NR[f]	NR	NR	NR	195	Dead
Patient 8	60	White	NR	RP	NR	NR	NR	209	Alive
Patient 9	74	White	NR	NR[f]	NR	NR	NR	55	Dead
Patient 10	84	White	NR	NR[f]	NR	NR	NR	1	Dead
Patient 11	68	White	NR	NR[f]	NR	NR	NR	157	Dead
Patient 12	69	White	NR	NR[f]	$T_{1c}N_0M_0$	2	NR	178	Alive
Patient 13	81	White	NR	NR[f]	$T_{1c}N_0M_0$	2	NR	125	Dead
Patient 14	75	White	NR	NR[f]	$T_{1c}N_0M_0$	2	NR	84	Dead
Patient 15	66	White	8.7	RP	$T_{2c}N_0M_0$	2	5+4=9	74	Alive
Patient 16	53	Asian	≥98	C[f]	NR	NR	NR	35	Dead
Patient 17	57	Black	NR	RP	NR	NR	NR	219	Alive
Patient 18	61	Black	NR	RP	NR	NR	NR	191	Dead
Patient 19	79	White	NR	NR[f]	NR	NR	NR	37	Dead
Patient 20	82	White	NR	NR[f]	NR	NR	NR	89	Dead
Patient 21	91	White	NR	R[e]	NR	NR	NR	20	Dead
Patient 22	65	White	NR	NR[e]	$T_2N_0M_0$	2	NR	11	Alive
Patient 23	73	White	NR	NR[f]	$T_{2a}N_0M_0$	2	NR	77	Dead
Patient 24	69	White	NR	RP+H	$T_{3b}N_0M_0$	3	NR	99	Dead
Patient 25	81	Black	≥98	NR[f]	$T_2N_0M_0$	2	5+3=8	25	Dead
Patient 26	65	White	6.5	NR[f]	$T_{1c}N_0M_0$	2	4+4=8	37	Alive
Patient 27	83	White	NR	NR[f]	$T_2N_0M_0$	2	NR	13	Dead
Patient 28	84	White	NR	NR[f]	$T_2N_xM_{1c}$	4	NR	NR	Dead
Patient 29	58	NR	NR	RP+H+R	$T_4N_0M_0$	4	NR	134	Alive
Patient 30	63	White	NR	RP	$T_{2a}N_0M_0$	2	NR	118	Alive
Patient 31	64	White	32.2	RP	$T_2N_0M_0$	2	5+5=10	53	Alive
Patient 32	63	White	28	NR[f]	$T_2N_xM_x$	NR	5+4=9	39	Alive
Patient 33	49	White	19	RP	NR	NR	4+5=9	11	Alive
Patient 34	79	White	NR	NR[f]	NR	NR	NR	219	Alive
Patient 35	71	NR	NR	NR[f]	NR	NR	NR	116	Dead
Patient 36	72	White	NR	NR[f]	$T_2N_0M_0$	2	4+4=8	90	Alive
Patient 37	83	Asian	NR	NR[f]	$T_2N_0M_0$	2	NR	10	Dead
Patient 38	80	White	NR	NR[f]	$T_{1c}N_0M_0$	2	NR	68	Dead
Patient 39	65	White	NR	RP	$T_4N_0M_0$	4	NR	91	Dead
Patient 40	73	White	NR	NR[f]	$T_{1c}N_0M_0$	2	NR	119	Dead
Patient 41	76	White	NR	NR[f]	$T_{1c}N_0M_0$	2	NR	118	Alive

Contd...

Supplementary Table 1: Contd...

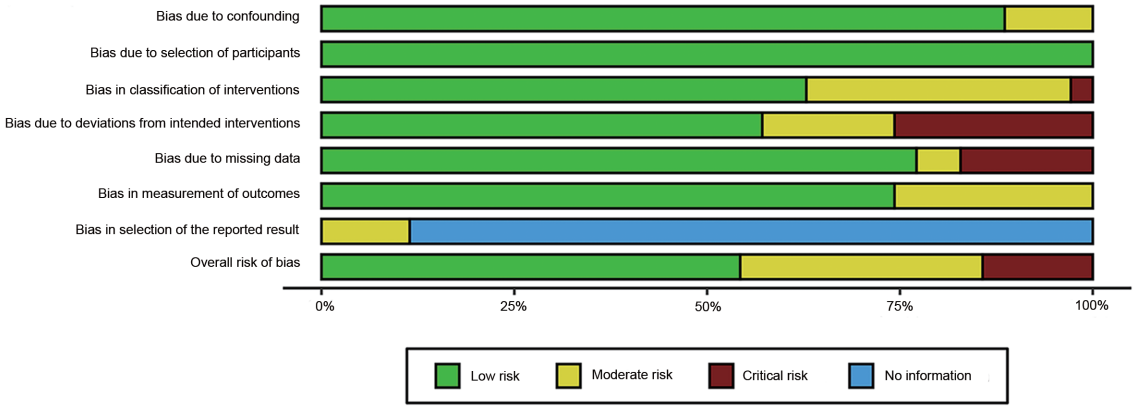
Reference	*Age (year)*	*Race*	*PSA at diagnosis (ng ml^{-1})*	*Treatment*	*TNM stage*	*Clinical stage*	*Gleason score*	*Follow-up (month)*	*Outcome*
Patient 42	82	White	20	NR[f]	$T_{1c}N_0M_0$	2	5+4=9	106	Alive
Patient 43	50	White	NR	NR[f]	NR	NR	NR	35	Dead
Patient 44	65	Black	NR	NR[f]	$T_xN_0M_{1c}$	4	NR	3	Dead
Patient 45	68	White	NR	RP	$T_{2c}N_0M_0$	2	NR	123	Alive
Patient 46	75	White	NR	NR[f]	NR	NR	NR	101	Dead
Patient 47	69	White	4.4	NR[f]	$T_{1c}N_xM_0$	NR	5+4=9	46	Alive
Patient 48	59	Asian	NR	RP	NR	NR	NR	208	Dead
Patient 49	83	White	NR	NR[e]	NR	NR	NR	35	Dead
Patient 50	63	White	NR	NR[f]	NR	NR	NR	61	Dead
Patient 51	85	Black	NR	NR[f]	NR	NR	NR	153	Dead
Patient 52	82	White	NR	NR[f]	NR	NR	NR	109	Dead
Patient 53	48	White	NR	RP	NR	NR	NR	197	Alive
Patient 54	54	Black	NR	NR[f]	NR	NR	NR	59	Dead
Patient 55	68	White	NR	RP	$T_{3a}N_1M_0$	4	NR	33	Dead
Patient 56	74	White	NR	NR[f]	$T_xN_0M_0$	NR	NR	62	Dead
Patient 57	72	White	NR	NR[f]	$T_{1c}N_0M_0$	2	NR	154	Alive
Patient 58	58	Black	NR	NR[f]	$T_4N_0M_0$	4	NR	60	Dead
Patient 59	67	White	NR	RP	$T_{3a}N_0M_0$	3	NR	82	Dead
Patient 60	73	White	NR	NR[f]	$T_2N_0M_x$	NR	NR	145	Alive
Patient 61	68	White	NR	NR[f]	$T_4N_0M_0$	4	NR	125	Alive
Patient 62	72	White	9.2	RP	$T_{2c}N_0M_0$	2	3+5=8	90	Alive
Patient 63	59	White	NR	RP	$T_{3a}N_0M_0$	3	NR	160	Alive
Patient 64	74	NR	NR	NR[f]	NR	NR	4+5=9	102	Alive
Patient 65	54	Black	7	RP	$T_{2c}N_0M_0$	2	3+4=7	103	Alive
Patient 66	62	White	≥98	NR[f]	$T_xN_0M_{1b}$	4	NR	8	Dead
Patient 67	66	White	4.1	NR[f]	$T_{1c}N_0M_0$	2	4+4=8	85	Alive
Patient 68	72	White	12.7	RP	$T_{2c}N_0M_0$	2	4+5=9	34	Alive
Patient 69	67	Black	≥98	RP+H+R	$T_{3b}N_1M_0$	4	4+5=9	74	Alive
Patient 70	61	Black	5.2	RP	$T_{2c}N_0M_0$	2	5+4=9	40	Alive
Patient 71	79	Black	37.4	H[e]	NR	NR	5+4=9	16	Dead
Patient 72	59	NR	NR	NR[f]	NR	NR	5+4=9	18	Alive
Patient 73	68	White	9	RP	NR	NR	5+4=9	14	Alive
Patient 74	72	Black	4.1	RP+H	NR	NR	4+4=8	27	Alive
Patient 75	67	Black	NR	RP	NR	NR	NR	81	Dead
Patient 76	71	White	NR	NR[f]	NR	NR	NR	139	Dead
Patient 77	66	Asian	NR	NR[f]	NR	NR	NR	211	Alive
Patient 78	63	White	NR	RP	NR	NR	NR	115	Dead
Patient 79	69	White	NR	RP	$T_{2c}N_0M_0$	2	NR	76	Dead
Patient 80	62	White	NR	NR[f]	$T_2N_0M_0$	2	NR	163	Dead
Patient 81	59	White	NR	RP	$T_2N_0M_0$	2	NR	170	Alive
Patient 82	60	White	NR	RP	$T_{3a}N_1M_0$	4	NR	164	Alive
Patient 83	55	Asian	NR	RP	$T_2N_0M_0$	2	NR	167	Alive
Patient 84	69	Black	NR	NR[f]	$T_2N_0M_0$	2	NR	106	Dead
Patient 85	84	Asian	NR	NR[f]	$T_2N_0M_0$	2	NR	8	Dead
Patient 86	65	White	NR	NR[f]	$T_2N_0M_0$	2	NR	45	Dead
Patient 87	57	White	NR	RP+R	$T_{3a}N_0M_0$	3	NR	154	Alive
Patient 88	63	White	NR	NR[f]	$T_2N_xM_{1b}$	4	NR	7	Dead
Patient 89	66	White	NR	RP	$T_{2c}N_0M_0$	2	NR	149	Alive
Patient 90	57	White	NR	RP	$T_{3b}N_0M_0$	3	NR	27	Dead
Patient 91	68	White	NR	C[f]	$T_3N_0M_{1b}$	4	NR	22	Dead
Patient 92	68	White	NR	NR[f]	$T_2N_0M_0$	2	NR	15	Dead
Patient 93	72	White	NR	NR[f]	NR	NR	NR	76	Dead
Patient 94	74	White	NR	NR[f]	$T_3N_1M_0$	4	NR	121	Alive
Patient 95	66	White	NR	RP+R	$T_{3a}N_0M_0$	3	NR	110	Alive
Patient 96	64	White	NR	RP	$T_{2c}N_0M_0$	2	NR	116	Alive
Patient 97	71	White	30.3	NR[f]	$T_2N_1M_0$	4	5+4=9	54	Dead

Contd...

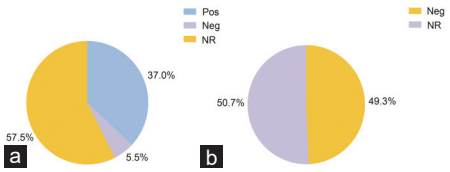
Supplementary Table 1: Contd...

Reference	*Age (year)*	*Race*	*PSA at diagnosis (ng ml^{-1})*	*Treatment*	*TNM stage*	*Clinical stage*	*Gleason score*	*Follow-up (month)*	*Outcome*
Patient 98	69	Asian	13.4	RP	$T_{3a}N_0M_0$	3	5+4=9	102	Alive
Patient 99	69	White	27.3	NR[f]	$T_4N_1M_0$	4	5+5=10	62	Alive
Patient 100	67	White	14.9	NR[f]	NR	NR	5+4=9	28	Alive
Patient 101	62	White	5.4	RP	NR	NR	4+3=7	6	Alive
Patient 102	77	White	NR	NR[e]	NR	NR	NR	186	Dead
Patient 103	57	White	NR	RP	$T_{3b}N_0M_0$	3	NR	4	Dead
Patient 104	80	White	NR	NR[e]	$T_{2c}N_0M_0$	2	NR	12	Dead
Patient 105	73	Black	NR	RP	$T_{3b}N_0M_0$	3	NR	91	Dead
Patient 106	81	White	NR	NR[f]	$T_{1c}N_xM_x$	NR	NR	117	Alive
Patient 107	71	White	≥98	NR[f]	$T_2N_0M_0$	2	5+5=10	77	Alive
Patient 108	61	White	5.5	RP	NR	NR	4+5=9	6	Dead
Patient 109	64	White	64.7	RP+H+R+C	NR	NR	5+5=10	21	Alive
Patient 110	77	White	NR	NR[f]	NR	NR	NR	202	Alive
Patient 111	73	White	NR	NR[f]	$T_{2c}N_0M_{1b}$	4	NR	35	Dead
Patient 112	82	Black	NR	NR[e]	NR	NR	NR	2	Dead
Patient 113	75	White	NR	NR[f]	$T_{2c}N_0M_0$	2	NR	37	Dead
Patient 114	66	White	4.3	RP	$T_{3a}N_0M_0$	3	4+5=9	88	Alive
Patient 115	49	Black	8	RP+H+R	$T_{3a}N_0M_0$	3	3+5=8	86	Alive
Patient 116	58	Black	4.6	NR[f]	$T_{1c}N_0M_0$	2	4+5=9	40	Alive
Patient 117	79	White	NR	NR[f]	NR	NR	NR	92	Dead
Patient 118	75	White	NR	NR[f]	$T_{1c}N_0M_0$	2	NR	150	Alive
Patient 119	67	White	NR	NR[f]	$T_2N_0M_{1b}$	4	4+4=8	88	Alive
Patient 120	57	White	NR	R[f]	NR	NR	NR	209	Alive
Patient 121	52	Black	NR	RP+R	NR	NR	NR	192	Alive
Patient 122	75	White	NR	NR[f]	NR	NR	NR	80	Dead
Patient 123	58	White	NR	NR[f]	$T_{2a}N_0M_0$	2	NR	173	Alive
Patient 124	60	Black	NR	NR[f]	$T_{2c}N_0M_0$	2	NR	141	Dead
Patient 125	74	Black	NR	NR[e]	$T_2N_0M_0$	2	4+5=9	85	Alive
Patient 126	50	Black	6	RP	$T_4N_0M_{1c}$	4	5+5=10	76	Alive
Patient 127	65	White	3.9	RP+H+R	$T_{3b}N_0M_0$	3	5+4=9	75	Alive
Our study	75	Asian	49.73	H	$T_{2c}N_xM_0$	NR	4+3=7	117	Alive
	70	Asian	10.09	RP+H	$T_1N_xM_x$	NR	4+4=8	106	Alive
	69	Asian	>100	TURP+H+R+C	$T_xN_xM_{1b}$	4	5+4=9	142	Alive
	67	Asian	65.87	NR	NR	NR	5+4=9	20	Alive

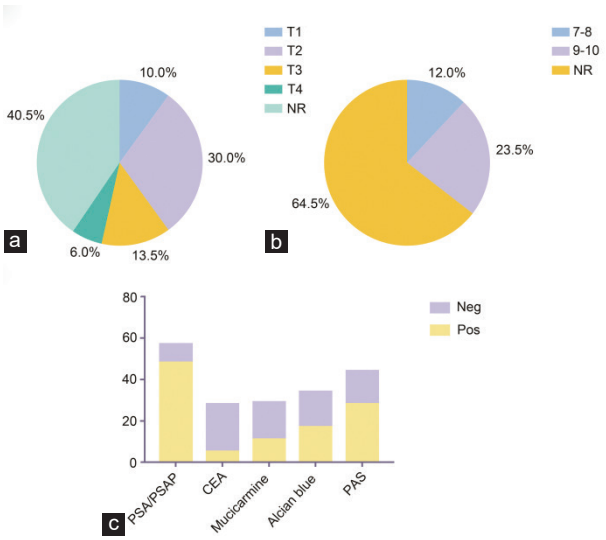
[a]Data were available for 127 patients from SEER database; [b]The patient underwent TURP but the study did not specify whether postoperative treatment was radiation, hormonal treatment, or both; [c]The patient underwent hormonotherapy but the study did not specify the surgical approach; [d]This study did not specify the individual treatment; [e]The specific surgical approach information of these patients was not available of SEER database; [f]These patients did not undergo surgery. C: chemotherapy; H: hormonotherapy; R: radiotherapy; RP: radical prostatectomy; NR: not reported; PSA: prostate-specific antigen; TURBT: transurethral resection of bladder tumor; TURP: transurethral resection of the prostate



Supplementary Figure 1: Methodological quality graph for our study of the incidence. The authors' judgments about each methodological quality item of ROBINS-I are presented as different colors across all included studies. The red, yellow, and green colors represent critical, moderate, and low bias, respectively.



Supplementary Figure 2: (**a**) Pie chart showing an abnormal result of digital rectal examination in 37.0% of patients with SRCC of the prostate. (**b**) Pie chart showing the proportion of patients undergoing gastrointestinal endoscopy. SRCC: signet ring cell carcinoma; NR: not reported.



Supplementary Figure 3: (**a**) Pie chart showing the distribution of the clinical stages of patients with primary prostatic SRCC. The proportions of patients with clinical stage T_1–T_4 disease were 10.0%, 30.0%, 13.5%, and 6.0%, respectively. (**b**) Pie chart showing the distribution of the Gleason scores of patients with SRCC of the prostate. The proportions of patients with Gleason scores of 7–8 and 9–10 were 12.0% and 23.5%, respectively. (**c**) Bar chart showing the proportions of patients with positive immunohistochemical staining for PSA or PSAP, CEA, mucicarmine, Alcian blue, and PAS, which may assist in determining a definitive diagnosis of primary prostatic SRCC. SRCC: signet ring cell carcinoma; NR: not reported; PSA: prostate-specific antigen; PSAP: prostatic-specific acid phosphatase; CEA: carcinoembryonic antigen; PAS: periodic acid Schiff.