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Research Paper

Cancer Stem Cell Gene Variants Predict Disease Recurrence in Patients Treated with Radical Prostatectomy for Prostate Cancer

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Abstract

Background: Cancer stem cells (CSCs) are involved in tumor progression and drug resistance. We hypothesized that variants in CSC marker genes influence treatment outcomes in prostate cancer.

Methods: Ten potentially functional single nucleotide polymorphisms (SNPs) in seven prostate CSC marker genes, *TACSTD2*, *PROM1*, *ITGA2*, *POU5F1*, *EZH2*, *PSCA*, and *CD44*, were selected for analysis of their association with disease recurrence by Kaplan–Meier analysis and Cox regression in a cohort of 320 patients with localized prostate cancer receiving radical prostatectomy.

Results: We identified one independent SNP, rs2394882, in *POU5F1* that was associated with prostate cancer recurrence (hazard ratio 0.32, 95% confidence interval 0.14–0.71, $P = 0.005$) after adjustment for known clinical predictors. Further *in silico* functional analyses revealed that rs2394882 affects *POU5F1* expression, which in turn is significantly correlated with prostate cancer aggressiveness and patient prognosis.

Conclusion: Our results suggest that rs2394882 is prognostically relevant in prostate cancer, possibly by modulating the expression of the CSC gene *POU5F1*.

Key words: prostate cancer; radical prostatectomy; recurrence; cancer stem cell; single nucleotide polymorphism; *POU5F1*

Introduction

Prostate cancer is one of the most frequently diagnosed cancers in men. Treatment options for prostate cancer strongly depend on tumor risk assessment; the most commonly used options are

radical prostatectomy (RP), radiation therapy, endocrine therapy, and chemotherapy with docetaxel [1, 2]. Most patients with prostate cancer respond initially to treatment; however, in numerous cases, eventual recurrence and progression to highly aggressive castration-resistant prostate cancer are observed. Therefore, the identification of biomarkers for diagnosis, monitoring, and therapy of prostate cancer is urgently needed.

Drug resistance and recurrence in prostate cancer may be, at least in part, explained by the existence of cancer stem cells (CSCs); however, this explanation remains controversial. CSCs are a rare subset of the cancer cell population that are capable of self-renewal, giving rise to a hierarchy of proliferative and differentiated tumor cells and leading to tumor progression and recurrence [3, 4]. Putative prostate CSCs were first isolated from human prostate cancer biopsies with $CD44^+/\alpha_2\beta_1$ $integrin^{high}/CD133^+$ markers [5]. These isolated cells exhibit a high potential for self-renewal, are capable of differentiating into heterogeneous cancer cells, and possess the ability to initiate tumor development in immunodeficient mice [6]. Moreover, prostate CSCs are slow growing and highly resistant to chemotherapy and radiotherapy targeting actively dividing cancer cells [7]. These findings suggest a link between CSCs and disease recurrence in patients with prostate cancer. In the present study, we selected 10 potentially functional single nucleotide polymorphisms (SNPs) in prostate CSC marker genes and evaluated their association with disease recurrence in patients with localized prostate cancer receiving RP.

Patients and Methods

Patient recruitment and data collection

In total, 320 patients with localized prostate cancer undergoing initial treatment with RP at the National Taiwan University Hospital, E-Da Hospital, Kaohsiung Medical University Hospital, and Kaohsiung Veterans General Hospital were recruited, as described previously [8-12]. Patient baseline characteristics and treatment outcomes were collected from their medical records. Biochemical recurrence (BCR) was defined as two consecutive prostate-specific antigen (PSA) values of 0.2 ng/mL or more after RP [9, 13]. Written informed consent was obtained from all patients, and the study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital in accordance with the approval procedures.

SNP selection and genotyping

A unique prostate-specific stem cell marker has not yet been identified; however, certain stemness markers that are generally present in stem cells are also expressed in prostate stem cells, including tumor-associated calcium signal transducer 2 (TACSTD2), prominin 1 (PROM1, also termed CD133), integrin subunit alpha 2 (ITGA2), POU class 5 homeobox 1 (POU5F1, also termed OCT4), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), prostate stem cell antigen (PSCA), and CD44 [14-16]. We used the Functional Analysis and Selection Tool for Single Nucleotide Polymorphism (FASTSNP) [17] to predict the functional effects of SNPs in these prostate cancer stem cell marker genes and to estimate their risk scores. SNPs with a risk score lower than 2 and a minor allele frequency of less than 0.02 in the HapMap Han Chinese in Beijing population [18] were excluded, leaving 10 potentially functional SNPs for analysis. Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) and stored at –80°C until analysis. Genotyping was carried out at the National Center for Genome Medicine, Taiwan, using the Agena Bioscience iPLEX matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry technology, as described previously [9]. The genotyping concordance rate among 54 blinded duplicated quality-control samples was 100%. All SNPs conformed to the Hardy–Weinberg equilibrium ($P > 0.05$) and were included for further statistical analyses.

Statistical analysis

Patient clinicopathologic characteristics were summarized as the numbers and percentages of patients. The association of individual SNPs and clinicopathologic characteristics with BCR was assessed using log-rank tests. Multivariate Cox regression was carried out to determine the interdependency of individual SNPs with known prognostic factors such as age, PSA at diagnosis, pathologic Gleason score, pathologic stage, surgical margin, and lymph node metastasis [19, 20]. Trends in *POU5F1* gene expression among genotypes at rs2394882 or prostate tissues were analyzed by Spearman correlation. The Statistical Package for the Social Sciences software version 22.0.0 (IBM, Armonk, NY) was used for other statistical analyses. A two-sided P value of < 0.05 was considered significant.

Table 1. Genotyped SNPs and the *P* values of their association with BCR in patients with localized prostate cancer treated with RP

Gene	SNP ID	Chromosome	Position	Possible Functional Effects	Log-rank *P*		
					Additive	Dominant	Recessive
TACSTD2	rs14008	1	59042181	Missense (conservative); Splicing regulation	0.809	0.811	0.909
PROM1	rs6449209	4	15982166	Splicing site	0.924	0.932	0.803
PROM1	**rs2078622**	4	16037352	Splicing site	0.072	**0.045**	–
ITGA2	rs1062535	5	52351413	Sense/synonymous; Splicing regulation	0.279	0.642	0.101
ITGA2	rs1801106	5	52358757	Splicing site	0.646	0.646	–
POU5F1	**rs2394882**	6	31132649	Splicing site	**0.012**	0.184	**0.003**
EZH2	rs2302427	7	148525904	Missense (conservative)	0.321	0.160	0.555
PSCA	rs2294008	8	143761931	Missense (conservative); Splicing regulation	0.181	0.217	0.344
PSCA	rs3736001	8	143762807	Missense (conservative); Splicing regulation	0.057	0.090	0.158
CD44	rs1071695	11	35201842	Sense/synonymous; Splicing regulation	0.905	0.934	0.656

Abbreviations: SNP, single nucleotide polymorphism; BCR, biochemical recurrence; RP, radical prostatectomy.
–, not calculated due to insufficient numbers.
P < 0.05 are in boldface.

Bioinformatics analysis

The association of rs2394882 with *POU5F1* expression was evaluated using mRNA data from lymphoblastoid cell lines derived from 270 HapMap individuals from four worldwide populations [18]. The GI_42560247-A probe was used for *POU5F1* expression analysis. Publicly available transcriptome data from the Memorial Sloan-Kettering Cancer Center (MSKCC) Prostate Oncogenome [21], Lapointe *et al*. [22], The Cancer Genome Atlas (TCGA) dataset for prostate adenocarcinoma [23], and the SurvExpress database [24] were utilized to analyze *POU5F1* gene expression and clinical outcomes.

Results

The clinicopathologic characteristics of the 320 patients with localized prostate cancer after RP and their associations with BCR are presented in Table S1. One hundred and sixteen (36.3%) patients exhibited BCR during the median follow-up of 26 months, and the median BCR-free survival was 53 months. BCR was significantly related to PSA varieties at diagnosis, as well as to pathologic Gleason score, stage, surgical margin, and lymph node metastasis (all $P < 0.001$).

Among the 10 genotyped potentially functional SNPs in the seven prostate CSC marker genes, *TACSTD2*, *PROM1*, *ITGA2*, *POU5F1*, *EZH2*, *PSCA*, and *CD44*, *PROM1* rs2078622, and *POU5F1* rs2394882 were significantly associated with BCR (log-rank $P \leq 0.045$, Table 1 and Figure 1). The effects of *PROM1* rs2078622 and *POU5F1* rs2394882 on BCR in patients with prostate cancer treated with RP were further assessed by univariate and multivariate Cox regression analyses (Table 2). Patients who carried the homozygous variant AA genotype of *POU5F1* rs2394882 had a significantly decreased risk of recurrence (hazard ratio 0.37, 95% confidence interval 0.19–0.74, $P = 0.005$). This association remained significant ($P = 0.005$) after adjustment for known predictors, including age, PSA at diagnosis, pathologic Gleason score, pathologic stage, surgical margin, and lymph node metastasis. However, *PROM1* rs2078622 did not reach significance in multivariate Cox regression analysis. These results indicated that, in addition to clinical features, *POU5F1* rs2394882 represents an independent prognostic factor for prostate cancer recurrence after RP.

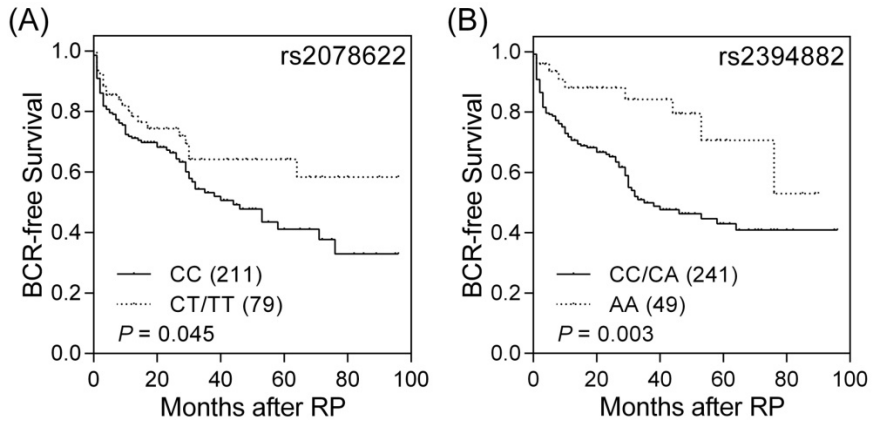

Figure 1. Impact of *PROM1* rs2078622 and *POU5F1* rs2394882 on prostate cancer prognosis. Kaplan–Meier analysis of BCR after RP, stratified by genotypes at (A) *PROM1* rs2078622 and (B) *POU5F1* rs2394882. Numbers in parentheses indicate numbers of patients.

Next, we investigated whether rs2394882 affects *POU5F1* expression using the HapMap data. Individuals carrying a homozygous for the protective A allele of rs2394882 showed lower *POU5F1* expression than those carrying a risk allele C ($P = 0.027$, Figure 2A). To assess the effect of *POU5F1* on prostate cancer progression, we conducted a comprehensive *in silico*

evaluation of publicly available MSKCC Prostate Oncogenome Project data. *POU5F1* expression tended to be higher in more aggressive forms of prostate cancer (P = 0.006, Figure 2B). Furthermore, a risk score was calculated for each patient based on the Cox regression coefficient of *POU5F1* expression and was used to classify patients into low- and high-risk groups using an optimization algorithm for the minimum P value. *POU5F1* upregulation was strongly associated with a higher risk of prostate cancer recurrence (P = 0.014, Figure 2C). Moreover, analysis of publicly available datasets from two additional cohorts of patients with prostate cancer showed that high *POU5F1* levels additionally indicated poor BCR-free and overall survival ($P \leq$ 0.036, Figures 2D and E). These results provided a clinical rationale for using *POU5F1* as a prognostic marker in advanced prostate cancer.

Discussion

Biomarkers that allow predicting the individual clinical course of a disease are desirable. Genetic markers have certain advantages over clinicopathological indicators in that they can be utilized preoperatively, are easy to assess using blood samples, and allow objective interpretation without individual bias. We found that the genetic biomarker rs2394882 in the prostate CSC marker gene *POU5F1* was associated with disease recurrence. Additionally, elevated *POU5F1* gene expression correlated with aggressive cancers and poor clinical outcomes. If these findings are confirmed, *POU5F1*/rs2394882 should be considered as a biomarker for optimizing treatment modalities to improve the survival of patients with prostate cancer.

Table 2. Association of *PROM1* rs2078622 and *POU5F1* rs2394882 with BCR after RP

Gene SNP	n	BCR	Univariate analysis		Multivariate analysis*	
Genotype			HR (95% CI)	*P*	HR (95% CI)	*P*
PROM1 rs2078622						
CC	211	82	1.00		1.00	
CT	74	20	**0.61 (0.37–0.99)**	**0.044**	0.76 (0.44–1.30)	0.310
TT	5	2	0.93 (0.23–3.79)	0.920	0.36 (0.05–2.69)	0.319
CT/TT vs. CC			0.63 (0.39–1.00)	0.051	0.72 (0.42–1.22)	0.216
Trend			0.68 (0.44–1.05)	0.078	0.72 (0.44–1.15)	0.168
POU5F1 rs2394882						
CC	109	43	1.00		1.00	
CA	132	51	0.96 (0.64–1.44)	0.832	1.00 (0.62–1.61)	0.988
AA	49	9	**0.36 (0.18–0.75)**	**0.006**	**0.32 (0.14–0.73)**	**0.007**
CA/AA vs. CC			0.77 (0.52–1.14)	0.192	0.76 (0.48–1.20)	0.237
AA vs. CC/CA			**0.37 (0.19–0.74)**	**0.005**	**0.32 (0.14–0.71)**	**0.005**
Trend			**0.71 (0.54–0.93)**	**0.014**	**0.68 (0.49–0.93)**	**0.018**

Abbreviations: BCR, biochemical recurrence; RP, radical prostatectomy; SNP, single nucleotide polymorphism; HR, hazard ratio; CI, confidence interval; PSA, prostate-specific antigen.

*Adjusted by age, PSA at diagnosis, pathologic Gleason score, pathologic stage, surgical margin, and lymph node metastasis.

$P < 0.05$ are in boldface.

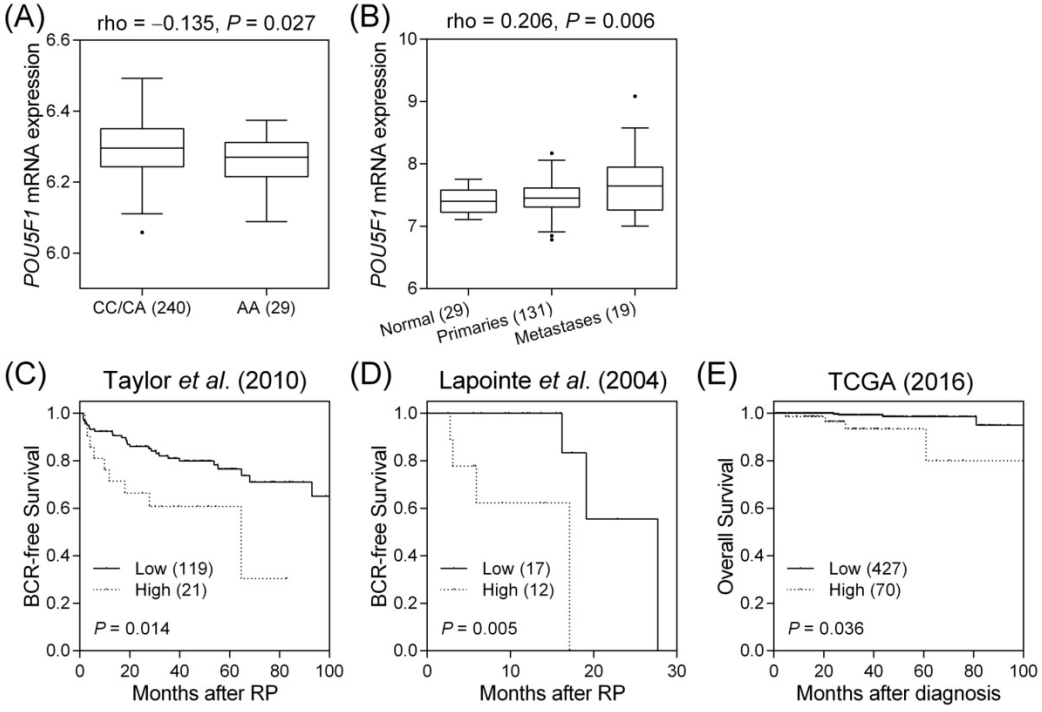

Figure 2. Functional analyses of *POU5F1* rs2394882. (A) Correlation of rs2394882 genotypes with *POU5F1* expression: *POU5F1* mRNA expression tended to be lower in rs2394882 AA carriers. (B) More advanced prostate cancers tended to show higher *POU5F1* expression. Expression of *POU5F1* mRNA correlates with (C) BCR-free survival in the dataset from Taylor *et al.* (2010), (D) BCR-free survival in the dataset from Lapointe *et al.* (2004), and (E) overall survival in the TCGA dataset. Increased *POU5F1* expression was significantly associated with poor prostate cancer prognosis. Numbers in parentheses indicate numbers of patients.

The SNP rs2394882 is located within intron 3 of *POU5F1* and is predicted to affect mRNA splicing by altering exonic splicing enhancer binding (Table 1). POU5F1 (also known as OCT4) is a member of the POU family of transcription factors, whose main function is to bind the octameric sequence motif (ATGCAAAT), thus activating target gene expression [25]. The *POU5F1* gene undergoes alternative splicing to generate three mRNA isoforms: OCT4A, OCT4B, and OCT4B1 [26]. OCT4A, the main isoform, acts as a transcription factor to regulate stem cell pluripotency and self-renewal [27]. On the other hand, as OCT4B1 levels have been observed to increase following heat stress, OCT4B and OCT4B1 are thought to be related with the stress response and anti-apoptotic properties rather than with stemness maintenance [28]. Further, Roadmap Epigenomics data indicate that rs2394882 and several linked SNPs are situated at a locus with enhancer-related histone modification patterns in the human induced pluripotent stem cell line iPS DF 19.11 and in other cell types (Table S2). Additionally, rs2394882 is predicted to alter multiple transcription factor-binding sites; the present expression quantitative trait locus analysis supported that the risk allele C at rs2394882 is associated with increased *POU5F1* expression (Figure 2A). *POU5F1* is overexpressed in a wide variety of human cancers, including bladder, brain, lung, ovarian, pancreatic, prostate, renal, seminoma, and testicular cancers [29]. *POU5F1* overexpression has been also observed in the tissues of patients with recurrent prostate cancer [30, 31]. Moreover, *POU5F1* reportedly is upregulated in both docetaxel- and mitoxantrone-resistant human prostate cancer cells [32]. Taken together, these data are consistent with our present finding that patients carrying the rs2394882 risk allele C have higher *POU5F1* expression, which in turn is correlated with more aggressive forms of prostate cancer and poor patient prognosis (Figure 2). Validation of the functional roles of *POU5F1* during prostate cancer progression should provide novel insights into the involvement of CSCs in carcinogenesis, as well as into the potential of *POU5F1* as a therapeutic target.

In conclusion, our study provided evidence that genetic variants of CSC-related genes influence patient outcome, and revealed *POU5F1* as a potential therapeutic target in prostate cancer. However, our findings in the homogeneous Taiwanese cohort used in this study may not be generalizable to other ethnic groups. A selection bias may be present due to the retrospective, hospital-based nature of this study. Furthermore, the relatively small sample size and limited outcome events in some strata may have increased the role of chance in the present findings. Additional and larger studies are warranted to validate our findings and enable the development of more effective personalized treatment for patients with prostate cancer.

Supplementary Material

Supplementary tables.
http://www.medsci.org/v14p1301s1.pdf

Abbreviations

RP: radical prostatectomy; CSC: cancer stem cell; SNP: single nucleotide polymorphism; BCR: biochemical recurrence; PSA: prostate-specific antigen.

Acknowledgments

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Competing Interests

The authors have declared that no competing interest exists.

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