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Long non-coding RNA *PCGEM1* as a biomarker for prostate cancer

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ABSTRACT: Prostate cancer (PC) is the second most common diagnosed malignant disease. Long non-coding RNAs (lncRNAs), which account for approximately 98% of the human genome, are becoming increasingly interesting with regard to various diseases and have great potential for diagnosis and prognostic monitoring of PC. To identify a new diagnosis marker for metastatic PC, we enrolled a total of 144 patients with PC, including 57 metastatic and 87 localized PC patients, and 148 healthy subjects. Patients were followed up routinely at 3-month intervals for 5 years. The expression of 10 selected lncRNAs in peripheral blood from participants was measured. Among the 10 selected lncRNAs, the expression of *PCGEM1* in the metastatic group was 2.57 times that in the localized group ($p < 0.001$) and 2.96 times that in the control group ($p < 0.001$). Patients with higher AJCC stage had significantly elevated *PCGEM1* expression (one-way ANOVA, $p < 0.001$). The relative expression of *PCGEM1* in patients with higher Gleason score was also higher than that in patients with lower Gleason score ($p = 0.003$). Moreover, patients with more than 5 years survival time had significantly lower *PCGEM1* expression than the rest ($p = 0.017$) and patients with elevated *PCGEM1* relative expression had significantly shorter survival time ($p < 0.001$). The present study suggested that *PCGEM1* expression in peripheral blood could act as a diagnostic marker of metastatic PC. It would also be a prognostic marker to predict the survival time.

KEYWORDS: peripheral blood, survival time, diagnostic, prognostic

INTRODUCTION

Prostate cancer (PC) is the second most common diagnosed malignant disease and the sixth leading cause for cancer related death among men worldwide. It was estimated that there were 899 000 new cases and 258 000 deaths in 2008 worldwide[1]. With the use of prostate-specific antigen in the serum, the proper rate of PC diagnosis increased over the past decades[2]; however, this procedure is limited by false-negative biopsies and overdiagnosis of clinically insignificant malignancies[3].

The ENCODE project has recently reported that more than 90% of the human genome can be transcribed and approximately 98% of these transcripts were of no protein-coding capacity, among which long non-coding RNAs (lncRNAs) are the most common type[4]. They are arbitrarily defined as being more than 200 nt in length and are involved in regulating a wide variety of important cellular functions, such as genome imprinting, gene expression, recruitment of chromatin modifying machinery, and regulation of X chromosome inactivation[5].

Notably, lncRNAs were found to be specifically regulated, suggesting the potential to serve as novel biomarkers and therapeutic targets[6]. Until now, many lncRNAs were detected in PC patients with specific expression pattern and were suggested to be biomarker of PC. For example, lncRNA *PCA3* was found to be strongly expressed in more than 95% of primary PC specimens and was not expressed in other normal human tissues[7]. The PROGENSA PCA3 test is the first urine-based molecular diagnostic test for PC which was approved by American FDA[8]. The lncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) was another marker of PC, the expression of which was correlated with poor prognosis in PC patients[9]. Crea et al found a total of 153 upregulated and 77 downregulated lncRNAs in metastatic versus nonmetastatic xenografts and focused on *PCAT18* for biomarker analysis[10]. Based on the expression profiles, we investigated the expression pattern of the 10 most dysregulated lncRNAs in our blood samples of PC patients and normal controls, and found *PCGEM1* could serve as a new diagnosis marker for metastatic

prostate cancer for AJCC for the following reasons: (1) the expression of *PCGEM1* was higher in metastatic group than that in localized and control groups ($p < 0.001$); (2) PC patients with higher AJCC stage had significantly elevated *PCGEM1* expression ($p < 0.001$); (3) PC patients with lower *PCGEM1* showed prolonged survival time.

MATERIALS AND METHODS

Study population

A total of 292 participants, which included 144 consecutive patients who had a histopathologic diagnosis of PC and 148 geographically and sexually matched healthy controls, were recruited from outpatient Department of the Second Hospital of Sichuan. Detailed clinical and pathology data were obtained from physician records and hospital notes. Among the 144 patients, cancer metastasis was diagnosed in 57 patients (40%); the metastatic sites included lymph nodes, bones, and other distant organs. All patients were followed-up at 3-month intervals for 5 years, routinely. At each visit, a comprehensive examination was carried out. Survival time was measured from the date of histopathology diagnosis until the date of death or last follow-up. Two or more pathologists classified the stages of PC patients according to the American Joint Committee on cancer (AJCC), 7th edition[11]. Each subject signed written informed consent to participate in the study. This study was approved by the Institutional Review Board of the Second Hospital of Sichuan (project no. B-133).

RNA samples

At the first visit, 5 ml venous blood samples of each subject were collected into a blood collection tube. Total RNA was extracted from lymphocytes using PAXgene RNA collection tubes (QIAGEN, USA). The concentration of RNA samples was quantified using a NanoDrop ND-1000 spectrophotometer; the quality of RNA was assessed with denaturing agarose gel electrophoresis.

Real-time PCR

First, total RNA was reversely transcribed to cDNA with PrimeScript RT reagent Kit equipped with gDNA Eraser (TaKaRa, China) strictly according to the manufacturer's instructions. Then, real-time PCR was performed using SsoFast EvaGreen Supermix (Bio-Rad, USA) on a CFX96 Real-Time PCR Detection System (Bio-Rad, USA).

The PCR procedures included an initial step at 95 °C for 30 s, followed by 40 cycles of amplification and quantification (95 °C for 5 s, 60 °C for 5 s). Each cDNA sample was performed in triplicate in a final volume of 25 μl containing 1 μl of cDNA and 400 nmol of forward and reverse gene-specific primers.

Relative gene expression level was quantified based on the cycle threshold (Ct) values, where *GAPDH* was used as an internal control whose expression was stable in primary and metastatic PC[10]. For quantitative results, expression of each gene was represented as a fold change using the following mathematical model:

$$\text{fold change} = \frac{(E_{\text{target}})^{\Delta \text{Ct}_{\text{target}}}}{(E_{\text{ref}})^{\Delta \text{Ct}_{\text{ref}}}}$$

where E_{target} and E_{ref} are the PCR efficiency of target gene transcript and reference gene transcript, respectively; $\Delta\text{Ct}_{\text{target}}$ and $\Delta\text{Ct}_{\text{ref}}$ are the Ct deviation of control minus sample of the target gene transcript and the reference gene transcript, respectively[12]. All primer pairs are available upon request.

Statistical analysis

The programs GRAPHPAD PRISM 5, SPSS 17.0, and MICROSOFT OFFICE EXCEL 2007, were used for data analysis. The data are presented as mean ± SD for continuous variables or percentages for categorical variables. Specific analysis methods were used with detailed description. A value of $p < 0.05$ indicated a statistically significant result.

RESULTS

Cohort characteristics

A total of 292 subjects, including 144 BC patients (aged between 38 and 81, median 72 years) and 148 age- and gender-matched healthy controls (aged between 42 and 77, median 67 years) were enrolled in the present study. The clinical characteristics of all participants are summarized in Table 1. The distribution of race and marital status were compatible in both groups.

Real-time PCR validation of 10 lncRNAs candidates

Through RNA sequencing, Crea et al identified 153 upregulated and 77 downregulated lncRNAs in metastatic xenografts, compared to nonmetastatic xenografts[10]. We selected 10 lncRNAs with most drastic expression fluctuation for validation

Table 1 Characteristics of the study participants.

Characteristics	Bladder cancer ($n = 144$)	Healthy population ($n = 148$)	p value
Age (median, range) (years)	72 (38–81)	67 (42–77)	0.271
Race:			
Han (n, %)	132 (92%)	139 (94%)	0.456
Non-Han (n, %)	12 (8%)	9 (6%)	
Marital status:			
Married (n, %)	119 (83%)	124 (77%)	0.794
Not married (n, %)	25 (17%)	24 (23%)	
Smoker (n, %)	96 (67%)	43 (34%)	< 0.001

in our cohorts consisting of 20 metastatic samples and 20 nonmetastatic samples. The 10 lncRNAs included 7 upregulated lncRNAs, namely *GTF2IRD2P1*, *C3orf51*, *LOC339535*, *LOC285692*, *PCGEM1*, *SH3GL1P1*, and *TYRO3P*; and 3 downregulated lncRNAs, namely *LOC100132215*, *FLJ37307*, and *AMZ2P1*. A general consistent result of each lncRNA between our cohort and the cohort used by Crea was confirmed in 7 lncRNAs in terms of regulation direction (upregulation or downregulation) and significance except *LOC285692*, *LOC100132215* and *AMZ2P1* (Fig. 1). Among the 7 lncRNAs, *PCGEM1* expression in metastatic group was 2.57 times compared to the expression in nonmetastatic group. We therefore focused on this lncRNA in further analysis.

PCGEM1 expression was positively correlated with PC progression

We further investigated the expression of *PCGEM1* in another large cohort consisting of 148 healthy controls, 59 metastatic PC patients and 85 localized PC patients (Table 2). The results showed that the expression of *PCGEM1* was higher in metastatic group than that in localized and control groups ($p < 0.001$, Fig. 2).

Table 2 Relative expression of *PCGEM1* in PC patients of different AJCC stages and Gleason score.

	Patients (n, %)	Relative expression of *PCGEM1*	p value
AJCC stage:			
I/II	31 (22%)	1.00 ± 0.37	
III	29 (20%)	1.49 ± 0.88	< 0.001
IV	84 (58%)	3.9 ± 1.3	
Gleason score:			
⩽ 5	9 (6%)	1.00 ± 0.29	
6	16 (11%)	1.7 ± 1.0	0.003
7	58 (40%)	1.92 ± 0.92	
⩾ 8	61 (42%)	4.2 ± 1.9	
Tumour metastasis:			
Metastasis	57 (40%)	1.00 ± 0.49	< 0.001
Localized	87 (60%)	3.5 ± 1.7	

According to AJCC, we divided all the PC patients into 4 stages, namely I, II, III, and IV stage. The number of patients and the relative expression of *PCGEM1* are described in Table 2. A total of 31 patients (22%) were grouped into stage I and II, 29 (20%) in group III, and 84 (58%) in group IV. Patients with higher AJCC stages had significantly elevated *PCGEM1* expression (one-way ANOVA, $p < 0.001$). The relative expression in patients with different Gleason scores (⩽ 5, 6, 7, and ⩾ 8) were also investigated, of which the result was consistent to the result reflected by AJCC stage (one-way ANOVA, $p = 0.003$).

Besides, the cases with tumour metastasis had significantly higher *PCGEM1* expression compared with those with localized tumours (non-paired

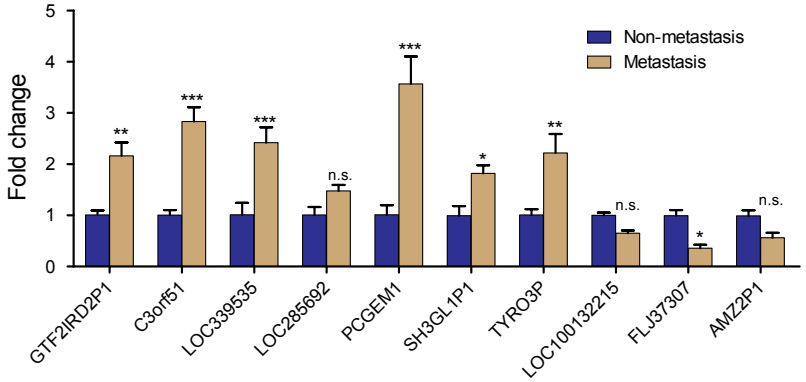

Fig. 1 Real-time PCR validation of 10 lncRNAs candidates. Fold changes were calculated by the $2^{-\Delta\Delta Ct}$ method. The data were analysed using two-way ANOVA. The bars represent the means ± SEM ($n = 20$). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, which were determined using Bonferroni's *post-hoc* test, represented significant differences; n.s., not significant.

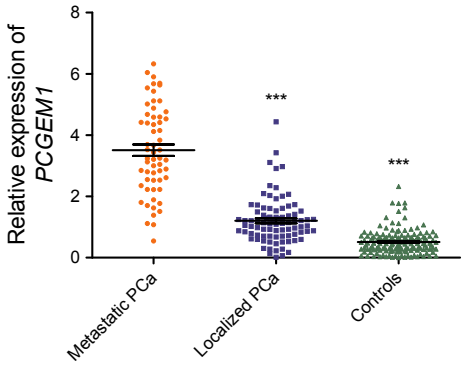

Fig. 2 Relative expression of *PCGEM1* in peripheral blood in metastatic PC, localized PC, and control groups. Fold changes were calculated by the $2^{-\Delta\Delta Ct}$ method. Error bars represent means ± SEM. The data were analysed using one-way ANOVA. $***p < 0.001$, which were determined using Turkey multiple comparison test, represent significant differences.

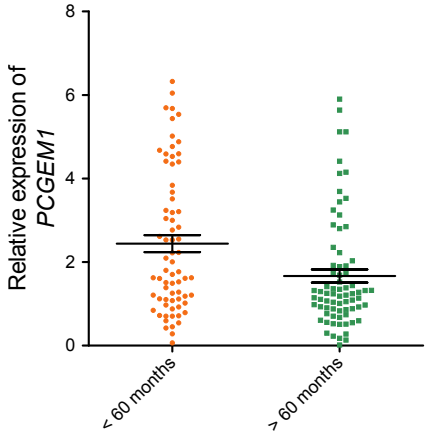

Fig. 3 PC patients surviving over 5 years had lower *PCGEM1* relative expression. Comparison between two groups was performed by Mann-Whitney test. Survival time: the time from the date of histopathology diagnosis to the date of death.

t-test, $p < 0.001$).

Patients with lower *PCGEM1* expression showed prolonged survival time

To analyse the association of *PCGEM1* expression with patients' survival time, we first compared the relative *PCGEM1* expression in those with survival time more than 5 years and those died in recent 5 years. The result showed that patients with more than 5 years survival time had significantly lower *PCGEM1* expression than the rest ($p = 0.017$, Fig. 3).

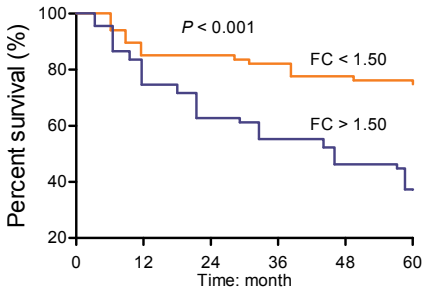

Fig. 4 PC patients with lower *PCGEM1* relative expression had longer survival time.

We further divided all the cases into two groups according to the relative *PCGEM1* expression to controls, one with elevated *PCGEM1* relative expression (> 1.5, $n = 76$) and the other with lower *PCGEM1* relative expression (< 1.5, $n = 68$). Survival curve analysis revealed that patients with elevated *PCGEM1* relative expression had significantly shorter survival time ($p < 0.001$) and were at 2.54 times higher risk of death compared with those with lower *PCGEM1* relative expression ($p < 0.001$, Fig. 4).

DISCUSSION

In recent years, the functions of lncRNAs in different diseases have been widely investigated and reported. For example, spinocerebellar ataxia type 8 (SCA8) patients have a trinucleotide expansion in ataxin 8 opposite strand (ATXN8OS), a lncRNA that is antisense to the *KLHL1* gene [13]. High expression levels of lncRNA *HOTAIR* has been shown to be an independent prognostic factor of hepatocellular carcinoma after liver transplantation [14]. In PC students, dysregulation of lncRNAs was noticed in several studies. However, we found that most of these studies focused on tumour tissues, but not peripheral blood, for lncRNAs analysis, which conferred big trouble for diagnosis and prognostic monitoring of PC. If dysregulated expression of certain lncRNAs can be detected in peripheral blood, it may bring great convenience for PC diagnosis.

In the present study, we found that the expression of *PCGEM1* in the metastatic group was significantly higher than that in the localized and control groups. Besides, patients with higher AJCC stages had significantly elevated *PCGEM1* expression, compared to those with lower AJCC stages. Moreover, patients with more than 5 years survival time had significantly lower *PCGEM1* expression than the rest; and vice versa, patients with elevated *PCGEM1*

relative expression had significantly shorter survival time. All the abovementioned evidence suggested that *PCGEM1* could act as a potential biomarker for PC diagnosis and prognostic monitoring. Besides, *PCGEM1* could potentially become a part of prognosticating tool along with GPS and Polaris biopsy PCR kits to determine suitability of men on active surveillance.

In previous studies, *PCGEM1* was identified as a prostate cancer specific lncRNA that is capable of promoting proliferation and inhibiting apoptosis. *PCGEM1* overexpression in LNCaP cells results in delayed induction of p53 and p21 induced by doxorubicin, which could therefore inhibit cell apoptosis[15]. Besides, *PCGEM1* is an in vivo androgen-regulated transcript with potential nuclear and/or cytoplasmic function[16]. Although the involvement of *PCGEM1* in PC was widely reported, the role of *PCGEM1* in PC is still controversial. For example, androgen deprivation is able to induce *PCGEM1* and causes its accumulation in nuclear speckles[17], while this was not the case in another study which revealed that *PCGEM1* was stimulated by androgen and downregulated by castration in xenograft models[16]. Similarly, *PCGEM1* along with *PRNCR1* can impact AR signalling through interaction with AR to promote castration resistance[18], however, this is not supported by a comprehensive analysis of RNA-sequencing data[19]. Genetic analysis also supported the involvement of *PCGEM1* in PC risk. *PCGEM1* polymorphisms might contribute to PC risk in Chinese men and SNP rs6434568 C carriers or rs16834898 C carriers had significantly decreased risk of PC[20].

For real-time PCR validation, we only selected 10 lncRNA candidates. In fact, many other lncRNAs surpassing the lncRNA list in our study could be potential biomarker for PC, since as many as 153 upregulated and 77 downregulated lncRNAs have been identified[10]. It is quite meaningful to investigate the potential of other lncRNAs to be biomarker of PC.

Another issue to be considered is the accuracy of a single molecule to diagnose diseases. In fact, many studies showed that combinational usage of several molecules could be more efficient for diagnosis and prognostic monitoring of diseases. Meng et al[21] showed that a four-lncRNA (U79277, AK024118, BC040204, AK000974) expression signature could be used to predict breast cancer survival[21]. Using a risk score based on the expression signature of these lncRNAs, the authors separated the patients into low-risk and high-risk groups with significantly different survival times, which was validated in three other cohorts[21]. Li et al[22] identified a three-lncRNA signature (including the lncRNAs ENST00000435885.1, XLOC_013014, and ENST00000547963.1) that was able to classify the oesophageal squamous cell carcinoma patients into two groups with significantly different overall survival[22]. In a future study, we could investigate using lncRNA combination(s) to diagnose PC.

REFERENCES

1. Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, Bray F (2012) International variation in prostate cancer incidence and mortality rates. *Eur Urol* **61**, 1079–92.
2. Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJJ, Petros JA, Andriole GL (1991) Measurement of prostate-specific antigen in serum as a screening-test for prostate-cancer. *New Engl J Med* **324**, 1156–61.
3. Schröder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, Kwiatkowski M, Lujan M, et al (2009) Screening and prostate-cancer mortality in a randomized European study. *New Engl J Med* **360**, 1320–8.
4. Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, Margulies EH, Weng Z, Snyder M, et al (2007) Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* **447**, 799–816.
5. Hung T, Chang HY (2010) Long noncoding RNA in genome regulation: prospects and mechanisms. *RNA Biol* **7**, 582–5.
6. Gibb EA, Brown CJ, Lam WL (2011) The functional role of long non-coding RNA in human carcinomas. *Mol Canc* **10**, 38.
7. Hessels D, Klein Gunnewiek JMT, van Oort I, Karthaus HFM, van Leenders GJL, van Balken B, Kiemeney LA, Witjes JA, et al (2003) $DD3^{PCA3}$-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol* **44**, 8–15.
8. Hessels D, Schalken JA (2009) The use of *PCA3* in the diagnosis of prostate cancer. *Nat Rev Urol* **6**, 255–61.
9. Ren SC, Liu YW, Xu WD, Sun Y, Lu J, Wang F, Wei M, Shen J, et al (2013) Long noncoding RNA *MALAT-1* is a new potential therapeutic target for castration resistant prostate cancer. *J Urol* **190**, 2278–87.
10. Crea F, Watahiki A, Quagliata L, Xue H, Pikor L, Parolia A, Wang Y, Lin D, et al (2014) Identification of a long non-coding RNA as a novel biomarker and potential therapeutic target for metastatic prostate cancer. *Oncotarget* **5**, 764–74.
11. Edge SB, Compton CC (2010) The American Joint Committee on Cancer: the 7th edition of the *AJCC Cancer Staging Manual* and the future of TNM. *Ann Surg Oncol* **17**, 1471–4.

12. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**, e45.
13. Moseley ML, Zu T, Ikeda Y, Gao W, Mosemiller AK, Daughters RS, Chen G, Weatherspoon MR, et al (2006) Bidirectional expression of CUG and CAG expansion transcripts and intranuclear polyglutamine inclusions in spinocerebellar ataxia type 8. *Nat Genet* **38**, 758–69.
14. Yang Z, Zhou L, Wu L, Lai M, Xie H, Zhang F, Zheng S (2011) Overexpression of long non-coding RNA *HOTAIR* predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. *Ann Surg Oncol* **18**, 1243–50.
15. Fu X, Ravindranath L, Tran N, Petrovics G, Srivastava S (2006) Regulation of apoptosis by a prostate-specific and prostate cancer-associated noncoding gene, *PCGEM1*. *DNA Cell Biol* **25**, 135–41.
16. Parolia A, Crea F, Xue H, Wang Y, Mo F, Ramnarine VR, Liu HH, Lin D, et al (2015) The long non-coding RNA *PCGEM1* is regulated by androgen receptor activity in vivo. *Mol Canc* **14**, 46.
17. Zhang Z, Zhou N, Huang J, Ho TT, Zhu Z, Qiu Z, Zhou X, Bai C, et al (2016) Regulation of androgen receptor splice variant AR3 by *PCGEM1*. *Oncotarget* **7**, 15481–91.
18. Yang L, Lin C, Jin C, Yang J, Tanasa B, Li W, Merkurjev D, Ohgi KA, et al (2013) lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. *Nature* **500**, 598–602.
19. Prensner JR, Sahu A, Iyer MK, Malik R, Chandler B, Asangani IA, Poliakov A, Vergara IA, et al (2014) The lncRNAs *PCGEM1* and *PRNCR1* are not implicated in castration resistant prostate cancer. *Oncotarget* **5**, 1434–8.
20. Xue Y, Wang M, Kang M, Wang Q, Wu B, Chu H, Zhong D, Qin C, et al (2013) Association between lncRNA *PCGEM1* polymorphisms and prostate cancer risk. *Prostate Canc Prostatic Dis* **16**, 139–44.
21. Meng J, Li P, Zhang Q, Yang Z, Fu S (2014) A four-long non-coding RNA signature in predicting breast cancer survival. *J Exp Clin Canc Res* **33**, 84.
22. Li J, Chen Z, Tian L, Zhou C, He M, Gao Y, Wang S, Zhou F, et al (2014) LncRNA profile study reveals a three-lncRNA signature associated with the survival of patients with oesophageal squamous cell carcinoma. *Gut* **63**, 1700–10.