PVT1 Assumes Signifying Capacity in Cervical Cancer Diagnosis.

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Research article

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Abstract

Background: Cervical cancer (CC) is the second most prevalent malignancy among women, which severely threatens patients' health. The study was conducted to determine the diagnostic role of plasmacytoma variant translocation 1 (*PVT1*) in CC to improve patients outcomes.

Methods: The qRT-PCR was used to determine the expression level of *PVT1* mRNA in CC samples and healthy controls. Chi-square test was used to determine the clinical effects of patients' features on *PVT1* expression. The receiver operating characteristics (ROC) curve with the area under the curve (AUC) was used as a tool for assessing the diagnostic role of *PVT1* expression in CC.

Results: The *PVT1* mRNA level was significantly higher in CC samples than healthy controls (P<0.0001). Large tumor size (P=0.006), positive uterus infiltration (P=0.031) and advanced FIGO stages (P=0.011) were contributed to the elevated expression of *PVT1* level. However, there was no close relationship between *PVT1* expression and other clinical parameters, including age (P=0.205), family history (P=0.073), positive HPV infection (P=0.155 and histological type (P=0.159). The ROC curve showed the optimal cutoff point for *PVT1* was 2.325, providing the sensitivity and specificity of 85.84% and 72.15%, respectively. Moreover, the AUC was 0.856, suggesting *PVT1* level could be regarded as a diagnostic biomarker in CC (P<0.0001, 95%CI= 0.803-0.909).

Conclusion: In summary, the level of *PVT1* mRNA was significantly increased in CC samples and the up-regulation of *PVT1* could distinguish CC patients from healthy controls.

Background

Cervical cancer (CC) is considered to be the second most frequent malignancy in women worldwide, especially in developing countries and low-income districts [1-3]. As a malignant type of tumor, CC is found to be accompanied with high mortality and morbidity, and approximately 500,000 newly confirmed CC cases and 270,000 dead CC cases are reported every year according to the World Health Organization (WHO) all around the world [4, 5]. At present, the commonly used methods for CC treatments, including surgical resection, chemotherapy and radiotherapy, have been improved in recent years [6, 7]. However, the prognosis of CC patients are still dismal, especially those with advances stages, among whom the overall survival rate might be less than 40% [8, 9]. Moreover, it is reported that CC is the only malignant tumor that could be preventable and curable if diagnosed early [10]. As a result, early diagnosis of CC is of great importance in the prevention and treatment of this disease. Therefore, biomarkers that could efficiently and timely diagnose CC are in urgent need.

Long non-coding RNAs (lncRNAs), longer than 200 nucleotides in length, are a series of RNAs that are lack of the protein-coding capacity [11, 12]. A growing number of studies have demonstrated that lncRNAs are involved in various biological processes, such as gene expression and transcription, cell proliferation and metastasis, apoptosis, and disease development [13-15]. *PVT1*, a member of lncRNA family, maps to human chromosome 8q24, and could encode about 12 exons and several transcripts [16,

17]. Besides, as an oncogene, *PVT1* has been demonstrated to stimulate cell proliferation, mobility and cell cycle and so on [18, 19]. Otherwise, emerging evidence has proved that *PVT1* is dysregulated in a variety of malignancies, including gastric cancer, breast cancer and colorectal cancer [20-22].

In the retrospective study, we wanted to investigate the expression of *PVT1* mRNA in CC samples and healthy controls, and then to explore the role of *PVT1* in the diagnosis of CC.

Materials And Methods

Patients and specimens

Sampling of this study consisted of 192 women, including 113 CC patients who were pathologically confirmed in Harrison International Peace Hospital, and 79 blood donors in the same hospital. All the patients were graded according to the protocols of the International Federation of Gynecology and Obstetrics (FIGO). They received no radio- or chemo- therapy before investigation. And those patients with cancers other than CC were excluded from our study. Blood samples were obtained from both CC patients and healthy controls and stored at -80℃ until use. This study was supported by the Ethics Committee of Harrison International Peace Hospital. And all participants had provided the informed consents in advance.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from CC samples and healthy controls with the RNeasy Mini Kit (Qiagen, Germany) following the instructions. Then the reverse transcription was conducted to synthesize cDNA with 5μl of total RNA using SuperScript II RNses H-Rverse transcriptase (Invitrogen). Then real-time PCR was performed with cDNA using the Universal PCR Master Mix (Applied Biosystems) under optimal conditions. *GAPDH* was taken as an internal reference to normalize the *PVT1* mRNA expression. The $2^{-\Delta\Delta Ct}$ method was applied to calculate the expression of *PVT1.*

Statistical analysis

All data were analyzed by Sigmaplot 12.5 and GraphPad Prism 5.0 softwares. Student's t-test was adopted to compare the difference of *PVT1* expression between groups. Chi-square test was carried out to explore the association between *PVT1* expression and clinical characteristics of patients. The diagnostic performance of *PVT1* in CC was estimated by the receiver operating characteristic (ROC) curve with the area under the curve (AUC). The difference was statistical significant with the *P* value of less than 0.05.

Results

Up-regulation of *PVT1* mRNA in CC samples

The expression of *PVT1* mRNA in CC samples and normal controls was examined via qRT-PCR. The level of *PVT1* mRNA in CC patients was 3.04±0.71 (mean±SD), which was obviously higher than that in healthy controls (2.00±0.65), and the difference was statistical significant (*P*<0.0001).(**Figure 1)**

Association of *PVT1* expression and clinical parameters

To determine the effects of clinical parameters on *PVT1* expression, Chi-square test was conducted. Patients were first classified by the median expression of *PVT1* (3.02) into high *PVT1* group (n=57) and low *PVT1* group (n=56). According to the results in **Table 1**, *PVT1* expression was significantly affected by large tumor size (*P*=0.006), uterus infiltration (*P*=0.031) and high FIGO stages (*P*=0.011). However, there was no link between *PVT1* expression and other clinical characteristics, such as age (*P*=0.205), family history (*P*=0.073, positive HPV infection (*P*=0.155) and histological type (*P*=0.159).

Diagnostic performance of *PVT1* in CC

To calculate the diagnostic significance of lncRNA *PVT1* in CC, the ROC curve was performed. As shown in **Figure 2**, the optimal cutoff value of *PVT1* in CC was 2.325, giving the sensitivity of 85.84% and the specificity of 72.15%. Furthermore, the AUC was 0.856, indicating *PVT1* could discriminate CC patients from healthy individuals (*P*<0.0001, 95%CI=0.803-0.909).

Discussion

CC is one of the most frequent gynecologic malignancies in female patients globally, and the incidence rate exists obvious differences between regions. In recent years, with the prevalence of sexually transmitted diseases and their induction factors, such as human papillomavirus (HPV), CC becomes a heavy burden for female human beings. Besides, the onset ages of CC patients become younger and younger. What's more, CC is reported to be the only malignancy that could be preventable and curative if it was found at early stages. Therefore, early diagnosis and timely treatment are of great importance to improve the survival rate of CC patients. Recently, tumor biomarkers, such as chain antigen 19-9 (CA19-9), carcino-embryonic antigen (CEA) and cancer antigen 125 (CA125) are more and more used in the diagnosis and prognosis of various diseases, including CC [23, 24]. However, the biomarkers are lack specificity for CC. So, we attempted to find some specific and effective candidates for CC.

LncRNAs, which are becoming more and more prevalent in the life science research in recent years, have been demonstrated to be potential targets in detection and treatment of cancers [25]. It has been confirmed that there are thousands of lncRNAs in mammalian genomes [26]. And plenty of lncRNAs have been investigated in various malignant diseases. For example, Ji et al. have reported that lncRNA *TUG1* was involved in the cell transference and invasion in gastric cancer through regulating miR-144/c-Met axis [27]. Yang et al. claimed that lncRNA *GAPLINC* enhanced invasion of colorectal cancer via binding to *PSF* and *NONO* [28]. These studies indicated that lncRNAs might be related with the development and progression of malignancies. *PVT1*, a lncRNA, as mentioned previously, was observed to be elevated in

various cancers. And Iden et al. also found that *PVT1* was increased in CC tissues compared with healthy controls [25]. Thereby, we were engaged in exploring the role of *PVT1* in CC diagnosis in this study.

We first measured the expression of *PVT1* mRNA in CC samples and healthy controls using the qRT-PCR method. And we observed that the *PTV1* mRNA expression was significantly high in CC samples compared with healthy controls, which agreed with and confirmed the previous results. The following Chi-square test showed that elevated expression of *PVT1* was significantly contributed to large tumor size, positive uterus infiltration and advanced FIGO stages. All the above results suggested that *PVT1* expression might be related with the occurrence and development of CC. So we further detected the link between *PVT1* expression and CC diagnosis. The final ROC analysis illustrated that high expression of *PVT1* could be regarded as a potential diagnostic marker for CC with high sensitivity and specificity.

Compared with miRNAs, the structure of lncRNAs is more complex, which leads to the diverse action modes of lncRNAs on tumors and genes, and more subtle regulation of the life processes. So far, the precise mechanism of *PVT1* on CC tumorigenesis is still not well understood. In fact, multiple studies have provided the mechanistic data of *PVT1* and suggested that it exerts its effect in a cell-type or disease-specific manner. For example, *PVT1* is reported to locate at about 55 kb downstream of the well known oncogene *MYC*, and is found to share close relationships with *MYC* in various cancers [29, 30]. Thus , we sought to be better define the role of *PVT1* in CC. This finding could provide a novel research area for our further studies.

Conclusions

In conclusion, *PVT1* mRNA was highly expressed in CC samples compared with healthy controls. And the we could distinguish the CC patients from healthy individuals with the up-regulated expression of *PVT1*.

Abbreviations

Cervical cancer (CC)

plasmacytoma variant translocation 1 (*PVT1*)

receiver operating characteristics (ROC)

area under the curve (AUC)

World Health Organization (WHO)

Long non-coding RNAs (lncRNAs)

Federation of Gynecology and Obstetrics (FIGO).

Quantitative real-time PCR (qRT-PCR)

human papillomavirus (HPV)

chain antigen 19-9 (CA19-9)

carcino-embryonic antigen (CEA)

cancer antigen 125 (CA125)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Harrison International Peace Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

S.C. design of the work; S.C. the acquisition, analysis, L.Z. interpretation of data; L.Z. the creation of new software used in the work; S.C. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Tables

Table 1. Relationship of *PVT1* expression and clinical characteristics

Clinical features	Case	Expression		c^2	*P* value
	NO.	High	Low		
Age (years)				1.610	0.205
≦50	68	31	37		
>50	45	26	19		
Family history				3.207	0.073
No	49	20	29		
Yes	64	37	27		
HPV infection				2.025	0.155
Never	61	27	34		
Ever	52	30	22		
Histological type				1.983	0.159
SCC	53	23	30		
AC	60	34	26		
Tumor size (cm)				7.439	0.006
≦4	56	21	35		
>4	57	36	21		
Uterus infiltration				4.674	0.031
Absent	55	22	33		
Present	58	35	23		
FIGO stage				6.486	0.011
I,II	59	23	36		
III,IV	54	34	20		

SCC: squamous cell carcinoma; AC: adenocarcinoma.

HPV: human papillomavirus

Figures

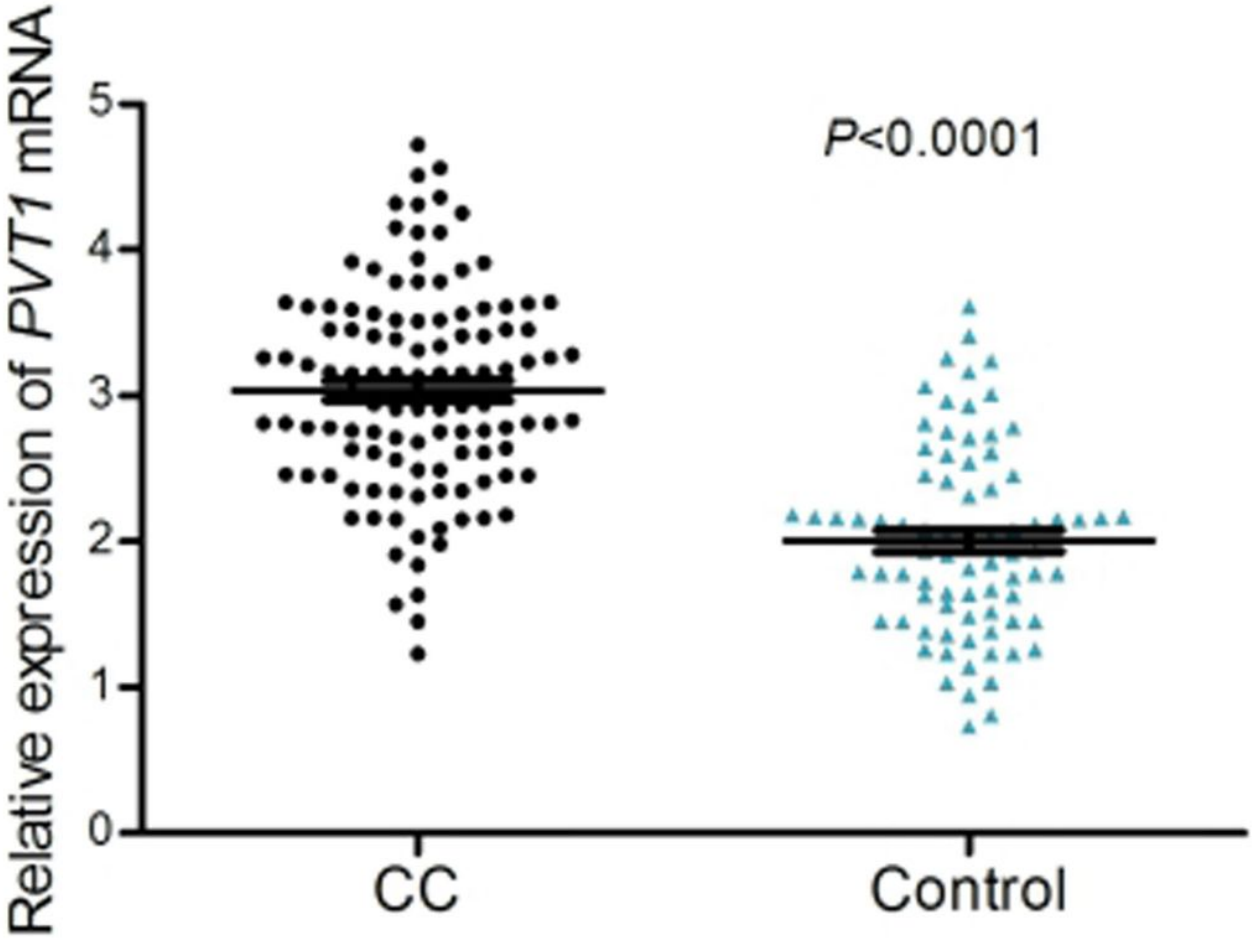

Figure 1

The expression levels of PVT1 mRNA in CC samples and healthy controls were examined using qRT-PCR. The result showed that PVT1 mRNA was obviously elevated in CC patients (P<0.0001).

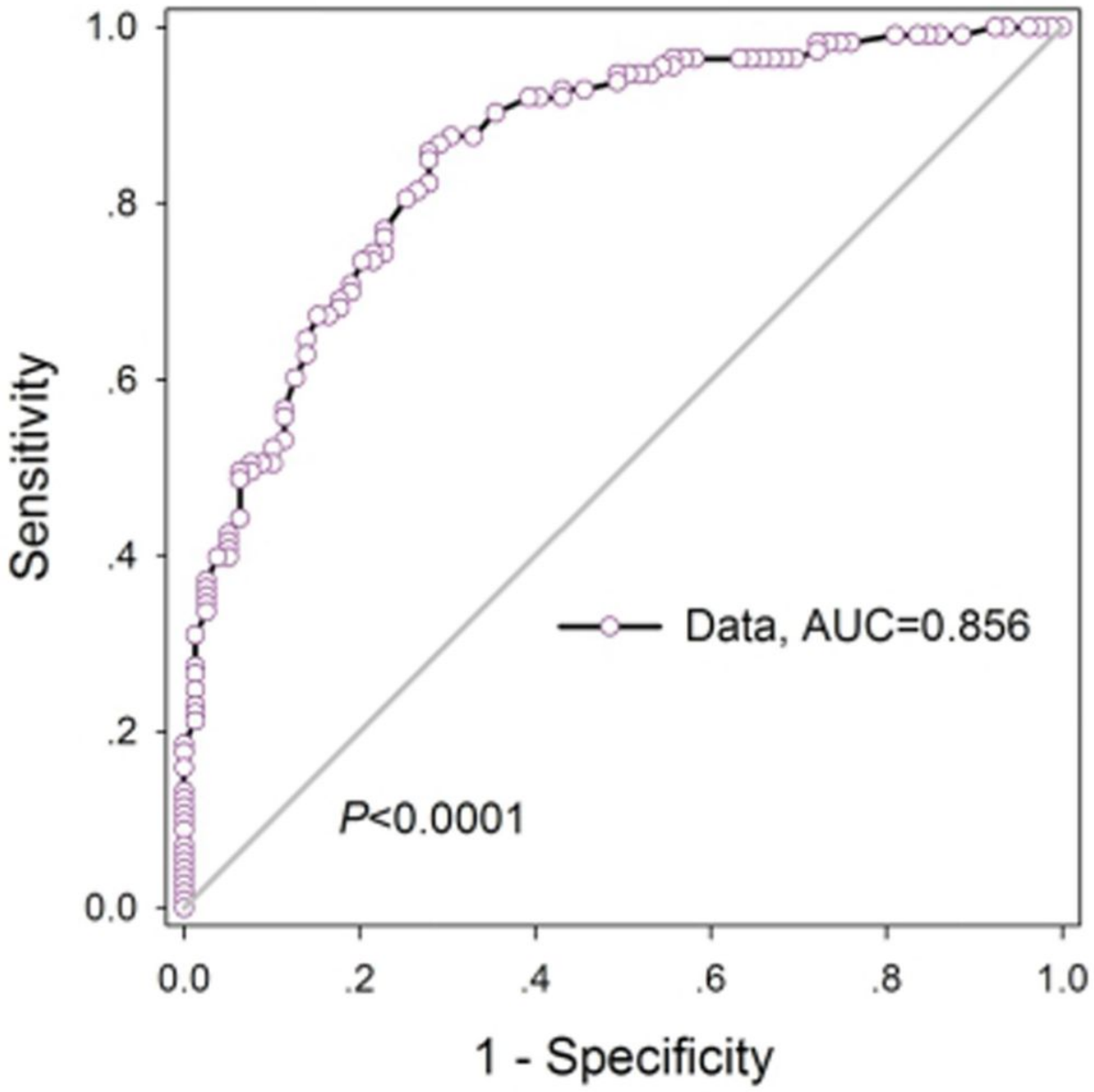

Figure 2

ROC analysis was performed to assess the diagnostic value of PVT1 level in CC. It showed that PVT1 was of great diagnostic significance for CC with the AUC of 0.856 (P<0.0001, 95%CI=0.803-0.909).