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ORIGINAL RESEARCH

The Clinical Significance and Biological Function of *PCDH7* in Cervical Cancer

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Purpose: Cervical cancer is a common cancerous tumor in women that is prone to recurrence and metastasis. Recently, many people have explored the role of protocadherin 7 (*PCDH7*) in cancer and found that *PCDH7* is abnormally expressed in many cancers. The purpose of this study is to explore the expression and mechanism of *PCDH7* in cervical cancer and evaluate its clinical prognostic significance.

Materials and Methods: The expression of *PCDH7* in cervical cancer and cells was measured by qRT-PCR. The relationship between *PCDH7* expression and the clinical prognosis was calculated using the Kaplan–Meier method and Cox regression analyses. Effects of *PCDH7* on cancer cell proliferation, migration, and invasion were studied by MTT assay and transwell assays.

Results: The expression of *PCDH7* in cervical cancer tissues and cell lines was notably downregulated compared with the corresponding control. Low *PCDH7* expression was associated with a low survival rate. *PCDH7* expression was correlated with lymph node metastasis, cell differentiation, and FIGO staging. *PCDH7* can be used as an independent prognostic factor for cervical cancer. Up-regulation of *PCDH7* significantly inhibited the proliferation ability, migration potential, and invasion capacity of cancer cells.

Conclusion: *PCDH7* may be used as a prognostic biomarker for cervical cancer patients.

Keywords: prognosis, proliferation, migration, invasion

Introduction

Cervical cancer is one of the most common malignant tumors in women and ranks second in developing countries.[1,2] With the rapid progression of cervical cancer screening and the widespread application of human papillomavirus (HPV) vaccines, great progress has been made in reducing mortality, especially in developed countries.[3,4] Although great progress has been made in early prevention, the rate of cervical cancer metastasis and recurrence is still high, the prognosis is poor, and the patient population becomes younger and younger.[5,6] At present, there are few accurate and specific markers in the prognosis of cervical cancer.[7,8] Thereby, in order to assess the prognosis of cervical cancer, it is necessary to explore new prognostic biomarkers to predict the risk or prognosis of cervical cancer.[9,10]

Protocadherin (*PCDH*) is the largest subfamily of the cadherin family, which can strengthen nerve synapses and play a certain role in signal transduction.[11,12] The *PCDH* family is divided into clustered *PCDH* and non-clustered *PCDH* according to its gene structure.[13] The protein encoded by non-clustered *PCDH7* has an extracellular domain containing 7 cadherin repeats, playing a crucial role in cell recognition and adhesion, and is concentrated in the brain and heart.[14,15]

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Previous studies have found that a variety of genes are abnormally expressed in cervical cancer.[16,17] There have been many studies showings that *PCDH* is abnormally expressed in various cancers and has a carcinogenic or anti-tumor effect.[18–20] However, few studies are focusing on the expression and role of individual *PCDHs* in cancer. Previous studies have found that the expression of *PCDH7* was significantly up-regulated in human non-small cell lung cancer (NSCLC).[21] *PCDH7* was significantly down-regulated in non-muscle invasive bladder cancer (NMIBC) and Cox analysis found that *PCDH7* can be used as an independent predictor of NMIBC.[22] The above investigations demonstrate that *PCDH7* plays a role in a variety of cancers, but the role and mechanism of *PCDH7* in cervical cancer remain unclear.

In the present study, we first determined the abnormal expression of *PCDH7* in cervical cancer tissues and cells. Then we assessed the relationship between *PCDH7* expression and clinical characteristics and survival status to understand its role in prognosis. In addition, we explored the role of *PCDH7* expression in cell proliferation capacity, migration, and invasion abilities using cervical cancer cells to understand its mechanism of action in cervical cancer. Through the entire study, we will explore whether *PCDH7* could act as a prognostic biomarker for patients with cervical cancer.

Materials and Methods

Patients and Tissue Samples Collection

We selected 106 patients with cervical cancer who underwent surgery from July 2013 to June 2015 in Ningbo Women and Children's Hospital and ensured that they have not received other treatment before surgery. All patients were diagnosed as primary cervical cancer confirmed by pathologists according to the 7th International Federation of Gynecology and Obstetrics (FIGO) staging system.[23] The patient's tumor tissues and the corresponding surrounding non-tumor tissues were obtained during surgery or biopsy and then quickly frozen in liquid nitrogen. Each patient signed an informed consent form before the operation and agreed to use the tissues for this research. According to the pathological type, there are 85 patients with cervical squamous cell carcinoma, 19 patients with cervical adenocarcinoma, and 2 patients with cervical adenosquamous carcinoma. The clinical case characteristics of each patient were recorded in Table 1, and each patient was followed up by telephone for five years to understand his survival status. This present study has been approved by the Ningbo Women and Children's Hospital ethics committee and in accordance with the Declaration of Helsinki.

Cell Lines and Transfection

Human cervical cancer cell lines HeLa, SiHa, C33A, and CaSki, and normal human cervical cell lines Ect1/E6E7 were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). All these cells were incubated in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, Invitrogen, USA), and stored in a humidified incubator at 37°C with 5% CO_2.

Effectene transfection reagent (QIAGEN Companies) was used for cell transfection of pcDNA3.1- *PCDH7* (Invitrogen; Carlsbad, USA) according to the manufacturer's instructions. Cells transfected with pcDNA3.1-control and cells without any transfection were used as controls. Each experiment was repeated at least three times.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Following the manufacturer's instructions, TRIzol reagent (Invitrogen, Carlsbad, California, USA) was used to isolate total RNA from tissues and cell lines. Reverse transcription was performed by transcriptor first-strand cDNA synthesis kit (Roche, Vilvoorde, Brussels, Belgium) to synthesize complementary DNA (cDNA). SYBR Green I Master Mix kit (Invitrogen) was used for qRT-PCR and then run on 7300 real-time PCR system (Applied Biosystems, USA) to study the expression of *PCDH7*. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of *PCDH7*.

Cell Proliferation Assay

Colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to explore the effect of *PCDH7* on the proliferation of cervical cancer cells (HeLa and SiHa cells). First, the cells were incubated in a 96-well plate (4×10^3/well). We added MTT reagent (Sigma-Aldrich, USA, 5mg/mL) at 0, 1, 2, 3, and 4 days, and then incubated at 37°C for 4 hours. After that, we added 100 μL DMSO (Sigma-Aldrich, USA) into the plate and used a microplate reader (Bio-Rad, Inc., Hercules, CA, USA) to measure its absorbance value at

Table 1 Association of *PCDH7* Expression with Clinical Features of Cervical Cancer Patients

Features	Total No. N=106	*PCDH7* Expression		*P* values
		High (n=48)	Low (n=58)	
Age (Years)				0.595
≤50	50	24	26	
>50	56	24	32	
Tumor size (cm)				0.947
≤4	46	21	25	
>4	60	27	33	
HPV status				0.887
Negative	28	13	15	
Positive	78	35	43	
Lymph node metastasis				0.007
Negative	67	37	30	
Positive	39	11	28	
Differentiation				0.039
Well	48	27	21	
Moderately/Poorly	58	21	37	
FIGO stage				0.001
I–IIA	56	33	23	
IIB-IV	50	15	35	
Pathological type				0.027
Squamous	85	43	42	
Adeno/adenosquamous	21	5	16	

a wavelength of 490 nm. The assay was repeated at least three times for each sample.

Cell Migration and Invasion Assays

Effects of *PCDH7* on cell migration capacity and invasion potential were analyzed by transwell (24-well; Corning Life Sciences, New York, USA) assays using HeLa and SiHa cells. The invasion assay required Matrigel (Bedford, Massachusetts, USA) to be pre-coated on the bottom membrane of the upper chamber, while the migration assay did not. The HeLa and Siha cells were suspended in serum-free RPMI-1640 medium, and then the cell suspension was added to the upper chamber of the transwell (2×10^4/well), while 600 μL of RPMI-1640 medium containing 10% FBS was added to the lower chamber as a chemokine. After removing the cells that have not migrated or invaded the upper layer of the bottom membrane, the bottom membrane was fixed in 4% paraformaldehyde for 30 minutes and stained with 0.1% crystal violet for 20 minutes. Cells were counted using an optical microscope.

Statistical Analysis

The statistical analysis of the data was performed by SPSS 23.0 (SPSS Inc., Chicago, IL) and GraphPad 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Differences between the groups were analyzed by Student's *t*-test or one-way ANOVA. The Kaplan–Meier method and multivariate Cox regression analyses were used to evaluate the relationship between *PCDH7* and the clinicopathological characteristics and prognosis of patients. All data are expressed as mean ± SD, and the data is considered statistically significant when $P < 0.05$.

Results

PCDH7 Expression in Tissue Specimens and Cells

The expression of *PCDH7* in cervical cancer tissues and cells was studied by qRT-PCR. It can be seen from the results that the expression of *PCDH7* in cancer tissues was significantly down-regulated compared with that in non-tumor tissues ($P < 0.001$, Figure 1A). Then we chose to verify this result in cervical cancer cell lines (HeLa, SiHa,

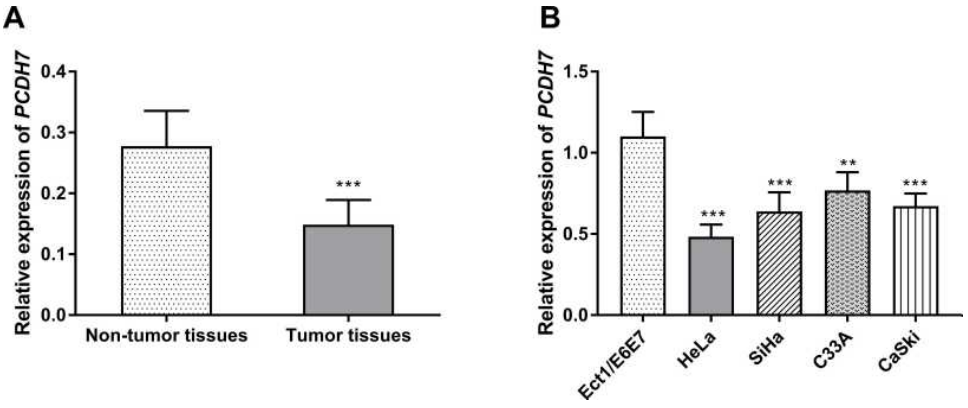

Figure 1 The relative expression level of *PCDH7* in cervical cancer tissues and cells. (**A**) Compared with surrounding non-tumor tissues, *PCDH7* expression in cervical cancer tissues was downregulated (***$P < 0.001$). (**B**) The expression of *PCDH7* in cervical cancer cell lines is lower than normal human cervical cells (**$P < 0.01$, ***$P < 0.001$).

C33A, and CaSki). It can be seen from the results that *PCDH7* expression in cervical cancer cells was remarkably lower than in normal human cervical cells (all $P < 0.01$, Figure 1B). At the same time, HeLa and SiHa cells had relatively lower expression among four cell lines,; thus, HeLa and SiHa cells were selected for subsequent assays.

The Association Between the Expression of PCDH7 and the Characteristics of Cervical Cancer Clinical Cases

Table 1 shows the relationship between *PCDH7* expression and clinical features in patients with cervical cancer. Using the average expression level of *PCDH7* expression (0.1486) in cancer tissues as a critical point, patients were divided into high expression groups (n = 48) and low expression groups (n = 58). As can be seen from Table 1, the expression of *PCDH7* was significantly associated with lymph node metastasis ($P = 0.025$), cell differentiation ($P = 0.039$), FIGO staging ($P = 0.001$), and pathological type ($P = 0.027$). Nevertheless, there was no correlation between the expression of *PCDH7* and age, tumor size, and HPV status (all $P > 0.05$).

Significance of PCDH7 Expression in the Prognosis of Cervical Cancer

Through the Kaplan–Meier method and Cox regression model, the relationship between the survival information of cervical cancer patients and the expression of *PCDH7* was analyzed to determine the potential prognostic value of *PCDH7* expression. It can be seen from the figure that the five-year survival rate of patients with high PCDH7 expression is better than patients with low expression (log-rank $P = 0.011$, Figure 2). Table 2 showed the correlation analysis between patient clinical information and *PCDH7* expression. It can be seen from the table that *PCDH7* expression (HR = 4.115, 95% CI = 1.169–14.489 and $P = 0.028$), lymph node metastasis (HR = 2.596, 95% CI = 1.085–6.210 and $P = 0.032$), FIGO staging (HR = 3.830, 95% CI = 1.098–13.364 and $P = 0.035$), and pathological type (HR = 2.503, 95% CI = 1.007–6.217 and $P = 0.048$) are independent prognostic factors for cervical cancer patients.

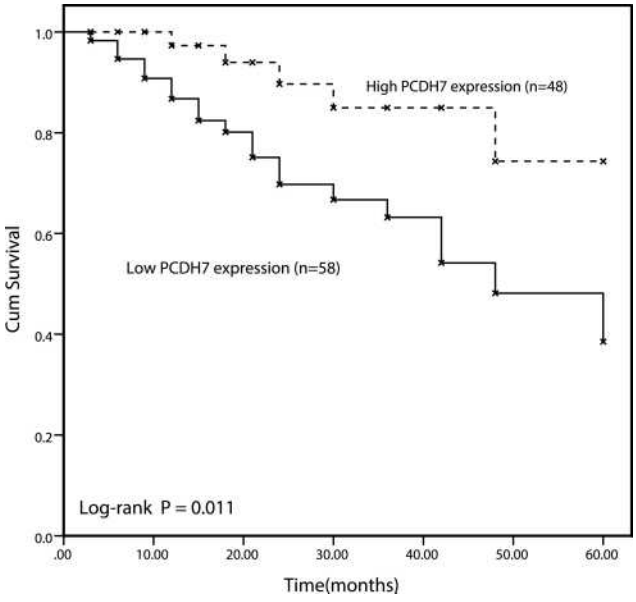

Figure 2 The survival curve of cervical cancer patients based on *PCDH7* expression. The five-year survival rate of patients with low *PCDH7* expression is lower ($P = 0.011$).

Table 2 Multivariate Cox Regression Analysis for Risk Prognostic Factors to the Overall Survival of Patients

Parameters	Multivariate Analysis		
	HR	95% CI	P
PCDH7 expression	4.115	1.169–14.489	0.028
Age	2.576	0.859–7.731	0.091
Tumor size	2.392	0.739–7.740	0.146
HPV status	2.912	0.745–11.385	0.124
Lymph node metastasis	2.596	1.085–6.210	0.032
Differentiation	3.244	0.901–11.676	0.072
FIGO stage	3.830	1.098–13.364	0.035
Pathological type	2.503	1.007–6.217	0.048

The Upregulation of PCDH7 Inhibited the Proliferation, Migration, and Invasion of Cervical Cancer Cells

In order to further determine the biological role of *PCDH7* in cervical cancer, we conducted proliferation, migration, and invasion assays on cervical cancer cells. Firstly, it was found that the expression of *PCDH7* in cells was increased after the transfection of pcDNA3.1-*PCDH7* by qRT-PCR ($P < 0.001$, Figure 3A). The effect of *PCDH7* on cell proliferation was studied by MTT assay. It can be seen from the figure that the high expression of *PCDH7* significantly inhibited the proliferation of HeLa and SiHa cells ($P < 0.01$, Figure 3B and C). After that, we evaluated the effect of *PCDH7* on cell migration and invasion through transwell assays. It can be seen from the figure that increased expression of *PCDH7* noteworthy reduced the migratory ability of HeLa and SiHa cells and suppressed the invasion of both cells ($P < 0.001$, Figure 4A and B).

Discussion

Cervical cancer is cancer with a high incidence rate in women.[24] It has a high rate of metastasis and recurrence after the operation, and it is difficult to cure and control.[25] It is necessary to explore more sensitive biomarkers to monitor its prognosis for suppressing its metastasis and recurrence at the earliest possible time. *PCDH* is a cadherin that is aberrantly expressed in cancer and can affect its mechanism of action in recent studies, and *PCDH7* is a subfamily among them.[13,26,27] Previous studies have found that it is abnormally expressed in several

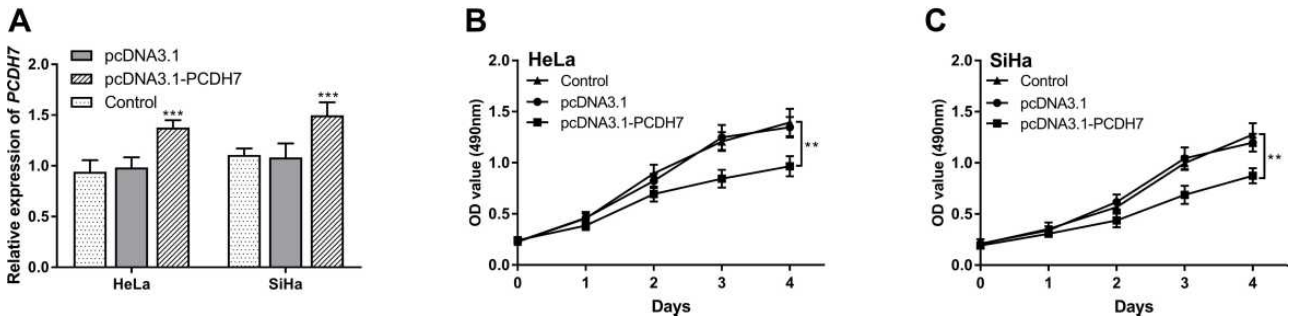

Figure 3 The upregulation of *PCDH7* affects cell proliferation. (**A**) The expression of *PCDH7* in cancer cells transfected with pcDNA3.1-*PCDH7* was significantly up-regulated, achieving the expected effect (***$P < 0.001$). (**B**) Increased expression of *PCDH7* inhibited the proliferation of HeLa cells (**$P < 0.01$). (**C**) Up-regulation of *PCDH7* inhibited the proliferation of SiHa cells (**$P < 0.01$).

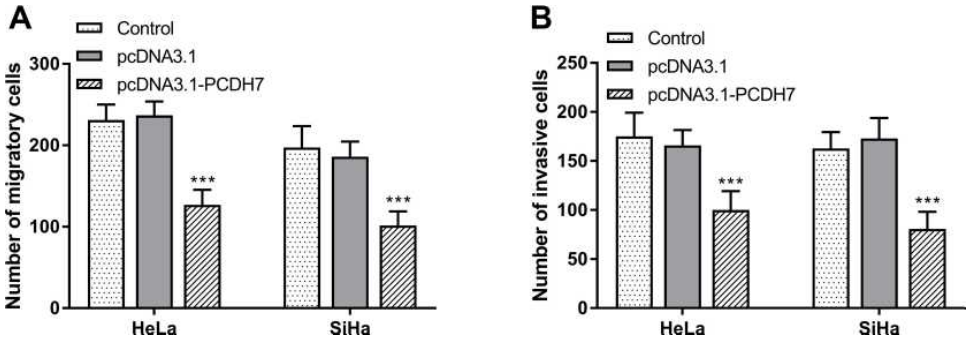

Figure 4 The up-regulation of *PCDH7* affects cell migration and invasion. (**A**) Up-regulation of *PCDH7* significantly inhibited the migration abilities of HeLa cells and SiHa cells (***$P < 0.001$). (**B**) Overexpression of *PCDH7* significantly suppressed the invasive ability of HeLa cells and SiHa cells (***$P < 0.001$).

cancers such as NSCLC, NMIBC, and gastric cancer.[21,22,28] So, we studied the abnormal expression and mechanism of *PCDH7* in cervical cancer.

First, we investigated the expression differences of *PCDH7* in cervical cancer tissues and cells. It can be seen from results that the expression of *PCDH7* in cervical cancer tissues is significantly lower than that in surrounding non-tumor tissues. Compared with normal human cervical cells, the expression of *PCDH7* was significantly down-regulated in cervical cancer cell lines. Previous studies have obtained similar results to this study. For example, *PCDH7* expression was significantly down-regulated in colorectal cancer tissues.[29] However, *PCDH7* was upregulated in castration-resistant prostate cancer (CRPC) cells and tissues, and *PCDH7* was overexpressed in NSCLC tumors.[21,30] The different results may be due to the different roles of *PCDH7* in different tumor tissues, but the abnormal expression of *PCDH7* in cancer tissues can be obtained. Other genes also have this phenomenon. For example, *FOXO1* expression in cervical cancer tissues was downregulated, and in epithelial ovarian cancer (EOC) tissues was significantly highly expressed.[31,32]

Then we verified the relationship between *PCDH7* expression and prognosis through the Kaplan–Meier survival curve and the Cox regression model. It can be observed that the five-year survival rate of patients who had low *PCDH7* expression is significantly shorter than those with high expression. At the same time, it was found that the low expression of *PCDH7* was significantly related to the characteristics of clinical cases such as positive lymph node metastasis, moderate/poor differentiation, advanced FIGO staging, and pathological type (adenocarcinoma and adenosquamous carcinoma). These data suggest that *PCDH7* expression may be used as an independent clinical prognostic factor for cervical cancer. Lin eet al. have proved that decreased expression of *PCDH7* was associated with a lower survival rate and can be used as an independent predictor of NMIBC.[22] Chen eet al. have proved that low *PCDH7* expression was remarkably associated with poor prognosis of gastric cancer.[28] Combined with the above results, it is indicated that the low expression of *PCDH7* may be used as a potential independent predictor for the prognosis of cervical cancer.

We have further studied the effects of *PCDH7* on the proliferation of potential, migration, and invasion abilities of cervical cancer cells. First, we transfected pcDNA3.1-*PCDH7* into cervical cancer cells to up-regulate the expression of *PCDH7*. Through the MTT assay, it was found that the up-regulated *PCDH7* significantly inhibited the proliferation abilities of cancer cells. Through the transwell assays, the increased expression of *PCDH7* reduced the migration and invasion ability of cancer cells. These assays show that the high expression of *PCDH7* can suppress cell proliferation abilities, migration, and invasion potential. Chen eet al. have confirmed that down-regulation of *PCDH7* inhibited the migration capacity and invasion abilities of gastric cancer cells by inhibiting E-cadherin.[28] Li eet al. have found that overexpression of *PCDH7* promoted the proliferation and invasion of breast cancer cells in vitro.[33] Therefore, the expression of *PCDH7* can affect the biological behaviors of cells to affect the development of cancer.

Although in vitro cell assays have been carried out in this research, the exploration of its mechanism is not deep enough. The next step will be to deepen the research on the functional mechanism of PCDH7 in cervical cancer.

Conclusion

In conclusion, *PCDH7* is down-regulated in cervical cancer tissues and cell lines. The downregulation of *PCDH7* was related to the shorter overall survival of patients. At the same time, the down-regulation of *PCDH7* promoted the proliferation capacity, migration potential, and invasion abilities of cervical cancer cells. All in all, *PCDH7* may be acted as a prognostic biomarker for cervical cancer.

Abbreviations

cDNA, complementary DNA; CRPC, castration-resistant prostate cancer; EOC, epithelial ovarian cancer; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMIBC, non-muscle invasive bladder cancer; NSCLC, non-small cell lung cancer; PCDH, protocadherin; qRT-PCR, quantitative real-time polymerase chain reaction; SPSS, Statistical Product and Service Solutions.

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Disclosure

The authors report no conflicts of interest in this work.

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