Annals of Diagnostic Pathology 34 (2018) 161–165

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

High expression of PXDN is associated with poor prognosis and promotes proliferation, invasion as well as migration in ovarian cancer

Ying-Ze Zheng, Lei Liang*

Department of Obstetrics, Daqing People's Hospital, Daqing 163000, China

ARTICLE INFO

Keywords:
Ovarian cancer
Peroxidasin
PI3K/Akt pathway
Proliferation

ABSTRACT

Background: Peroxidasin (PXDN) is an extracellular matrix protein with peroxidase activity. PXDN has been reported to participate in the processes of epithelial mesenchymal transition. However, the roles of PXDN in progression of cancers are still rare.

Methods: Expression profiles of PXDN in ovarian cancer (OC) tissues were obtained from GEO and TCGA database. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to measure the expression of PXDN in OC cells. Kaplan-Meier method was used to analyze the overall survival of OC patients. Furthermore, effects of PXDN knockdown on the proliferation, invasion as well as migration of HEY cells were examined by Cell Counting kit-8 (CCK-8), wound healing and transwell assay. Additionally, western blot assay was conducted to detect the levels of several key proteins in PI3K/Akt pathway.

Results: PXDN was highly expressed in OC tissues and cells. OC Patients with high PXDN expression showed poorer overall survival rate compared to the OC patients with low PXDN expression. The results of the present study demonstrated that knockdown of PXDN significantly suppressed the proliferation, invasion and migration of HEY cells. In addition, after silencing PXDN in HEY cells, the expression levels of the key protein phosphorylation in PI3K/Akt pathway were obviously decreased, including p-PI3K and p-Akt, that resulting in the inhibition of PI3K/Akt pathway activation.

Conclusion: PXDN might play a promoter role in the proliferation, invasion and migration of OC cells through regulating the activation of PI3K/Akt pathway. Therefore, PXDN might be regarded as a potential target for OC therapy.

1. Introduction

Ovarian cancer (OC) is one of the most aggressive and deadliest cancer-associated deaths among women worldwide [1,2]. It has been reported that nearly 200,000 new ovarian cancers occur and die about 130,000 each year in the world [3]. Over the past decade, the therapeutic effects with surgery and chemotherapy have made great progress, and the 5-year survival rate for most of patients with OC at early stages (I and II) can reach over 90%. Unfortunately, most OC patients who are diagnosed with advanced stages (III and IV) were reported to have a low 5-year survival rate [4]. Currently, the early diagnosis for OC patients is still a challenge, since an accurate non-invasion diagnostic method is not found. Therefore, new biomarkers and novel therapeutic targets for improving treatment strategies of OC patients are urgently needed.

Peroxidasin (PXDN) is a unique peroxidase that was fist found in *Drosophila melanogaster* [5]. It not only contains peroxidase domain, but also has domains feature of proteins of extracellular matrix. PXDN has been reported to be expressed in various cells of human and mouse, including endothelial cells, epithelial cells, and fibroblasts [6,7]. The aberrant expression of PXDN in hepatocellular carcinoma has been reported by Holmila et al. [8]. In addition, PXDN expression in cervical carcinoma was reported to be down-regulated [9]. However, McKeown et al. found that PXDN was highly expressed in metastatic melanoma tumors and could play a promoter role in melanoma cellular invasion [10]. Although PXDN has been studied in many literatures, very few attentions have been paid to the function roles of PXDN in the development of OC.

In this study, we examined the expression of PXDN in OC, and analyzed the prognostic significance. The function roles and underlying mechanisms of PXDN in OC cells were assessed by analyzing the effect of PXDN on proliferation, invasion as well as migration of OC cells. Our results suggest that PXDN is associated the poor prognosis of OC patients and may act as a promoter in the progression of OC.

* Corresponding author at: Department of Obstetrics, Daqing People's Hospital, No 213, Construction Road, Daqing 163000, Heilongjiang, China.
E-mail address: Lianglei-dq@163.com (L. Liang).

https://doi.org/10.1016/j.anndiagpath.2018.03.002

2. Materials and methods

2.1. Patients and tissue samples

In this work, the expression profile of PXDN in OC tissues was collected from GSE54388 dataset in Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) database. A total of 22 samples, including 16 tumor epithelial component samples and 6 normal ovarian surface epithelium samples, were selected to analyze the expression. Moreover, the expression profile dataset consisting of 586 OC patients and 8 normal healthy samples was obtained from Oncomine (https://www.oncomine.org/resource/login.html).

2.2. Cell culture

OC cell lines HEY and A2780, as well as a normal ovarian epithelial cell line IOSE80 were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy IOSE80of Sciences (Shanghai, China). Cells were cultured in 90% Roswell Park Memorial Institute (RPMI)-1640 (Gibco) that contains 10% fetal bovine serum (FBS) (Gibco), 100 μg/ml streptomycin (Sigma-Aldrich) and 100 U/ml penicillin (Sigma-Aldrich) in a humidified chamber with 5% CO_2 at 37 °C.

2.3. RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis

According to the manufacture's instructions of TRIZOL reagent (Invitrogen), total RNA of OC tissues and cells was isolated and reversely transcribed to complementary DNA (cDNA) with a reverse transcription kit (Takara, Dalian, China). For qRT-PCR analysis, the relative quantification of PXDN expression was computed by comparative C_T method ($\Delta\Delta C_T$). The gene-specific primers were as follows: PXDN: Forward: 5′- CAGGGACCTCGTTAATGGCT -3′, Reverse: 5′-CCGTT- CAGACAGCTGACGTT -3′, Tubulin: Forward: 5'-GGAGCGAGA TCCCTC- CAAAAT -3′: Reverse: 5′–GGCTGTTGTCATACTTCTCATGG-3′. The primer of GAPDH gene was used to verify the completeness of RNA by conducting PCR amplification.

2.4. Cell transfection

According to the manufacture's instructions of Lipofectamine 2000 (Invitrogen), the siTNA targeting PXDN (si-PXDN) or a scrambled negative control (si-NC) (GenePharma, China) was transfected into HEY cells. The sequence used for PXDN siRNA was 5′-AACAGGGCAGAAA GUUGUA -3 and 5′- AUGAAAUGGGAUCAAGUGG -3. After transfection, cells were cultured for 48 h and measured with qRT-PCR.

2.5. Cell proliferation

The proliferation of OC HEY cells was measured with Cell Counting Kit-8 (CCK8, Dojindo). Cells after 48 h transfection were digested and counted for preparing cell suspensions. Subsequently, cells were placed into a 96-well plate at a density of 1×10^5 cells/well and cultured in an incubator containing CO_2. After incubation for 0 h, 24 h, 48 h, 72 h and 96 h, respectively, the viability of HEY cells was detected by the use of CCK8 reagent. The cell numbers were counted by examining the absorbance of cells at 450 nm. All the data were conducted in triplicate.

2.6. Western blot analysis

Western blot analysis was conducted by the following primary antibodies: PI3K (cell signaling; 1:1000), p-PI3K (cell signaling; 1:1000), Akt (cell signaling; 1:1000), p-Akt (cell signaling; 1:1000), and Tubulin (Millipore; 1:1500). Briefly, RIPA lysis buffer (Beyotime, Nantong, China) was used to lyse the simulated proteins and compute the concentration of proteins with a BCA Protein Assay Kit (cwbiotech, Beijing, China). Then, protein lysates was isolated using 12% SDS-PAGE gels and transferred to PVDF membranes (Millipore, Billerica, USA). The membranes were cultured with primary antibodies and secondary antibodies overnight at 4 °C. Eventually, the bound antibodies were measured with an ECL substrate (Advansta, Menlo Park, CA, USA).

2.7. Cell invasion and migration assays

The invasion and migration analyses were conducted in a 24-well transwell chamber. For invasion assay, stably transfected OC cells were seed into serum-free medium and added to the upper chamber of transwell chamber. Notably, fresh matrigel (Corning, Acton, MA) was precoated on the upper chamber. Furthermore, 300 ml supplemented with 20% FBS was added to the lower chamber. Cells attached in the upper chamber were removed using cotton-tripped swabs after 24 h incubation. Then, the cells were fixed in the lower chamber by the use of 4% paraformaldehyde. After fixation for 15 min, cells were stained using crystal violet. Finally, five fields were randomly selected under a microscope to count the number of cells. In the process of migration assay, the same procedures were performed, except that matrigel was coated to the transwell chamber for forming a matrix barrier.

Additionally, wound healing assay was performed to evaluate the migrated ability of cells. Briefly, HEY cells were seeded into a 12-well plate at a density of 1×10^5 cells/well and cultured with RPMI 1640 containing 3% FBS overnight. Then, cells attached on the well were scraped with a spatula. Notably, the attached cells were transfected with si-PXDN or si-NC after removing the detached cells and cultured in a humidified incubator containing 5% CO_2 at 37 °C. Eventually, after scratch for 24 h, wound healing between the edges of the scratch cells in cells transfected with si-PXDN was observed by comparing with those of cells treated with si-NC.

2.8. Statistical analysis

The PXDN expression level was presented as mean ± standard deviation (SD). The survival rate of OC patients was estimated using Kaplan-Meier method, and survival differences between groups were assessed by log-rank test. Additionally, the univariate and multivariate survival analyses were conducted by cox proportional hazards model to analyze the independent prognostic factors related to overall survival. GraphPad Prism 5 (San Diego, CA, USA) and SPSS 22.0 (Chicago, IL, USA) was employed to perform the statistical analysis of all data, and P-value < 0.05 was considered statistically significant.

3. Results

3.1. Up-regulation of PXDN in OC tissues and cells

To confirm the expression of PXDN in OC tissues, expression profiles of a total of 16 patients with OC and 6 normal healthy samples were collected and evaluated by qRT-PCR. The results showed that PXDN expression was obviously up-regulated in OC tissues compared to the normal groups (Fig. 1A, $P = 0.0045$). Furthermore, as shown in Fig. 1B, compared to the expression in normal tissues, PXDN was also demonstrated to be highly expressed in OC sample tissues, based on the expression data from TCGA. Moreover, qRT-PCR was performed to measure the expression level of PXDN in OC cells. Compared to the normal cells (IOSE80), PXDN expression in OC HEY and A2780 cells was observed to be up-regulated (Fig.1C). Notably, the expression level of PXDN in HEY cells is higher than that of A2780 cells. Thus, HEY cells were selected as the OC cell model. In addition, the survival curves of data obtained from TCGA database were plotted by Kaplan–Meier method and showed in Fig.1D. Consequently, we found that survival rate of OC patients with high PXDN expression was lower than that of low PXDN expression ($P = 0.0162$), indicating that high expression of PXDN was associated with poor survival in OC patients.