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*Original Article*

# Overexpression of peptidyl-prolyl isomerase 1 (Pin1) and cyclin D1 in endometrial cancer

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**Abstract:** The aim of this study is to investigate the expression and correlation of peptidyl-prolyl isomerase 1 (Pin1) and cyclin D1 in endometrial cancer. This retrospective study included 52 newly diagnosed endometrial cancer patients in Tai'an Central Hospital from January 2011 to June 2015. Endometrial cancer samples were obtained from all 52 patients. Adjacent non-cancerous endometrium was also collected from 15 patients and used as control. The endometrium from 29 patients with uterine leiomyomata in the same period was also obtained as control. The expression of Pin1 and cyclin D1 in endometrium was detected by immunohistochemical staining (SABC method). The expression of Pin1 and cyclin D1 in different groups was compared by chi-square tests. The association between protein expression and clinical characteristics was also analyzed by chi-square tests. The relationship between Pin1 and cyclin D1 expression was analyzed by Pearson's correlation test. The expression of Pin1 and cyclin D1 in endometrial cancer was significantly higher compared with both normal endometrium and adjacent non-cancerous endometrium ($P < 0.05$). High expression of Pin1 and cyclin D1 was significantly associated with high clinical stage, pathological grade, lymphatic metastasis and the depth of myometrial invasion ($P < 0.05$). The expression of Pin1 was positively correlated with cyclin D1 ($P = 0.001$). In conclusion, Pin1 and cyclin D1 are highly expressed in endometrial cancer, and is associated with clinical stage, pathological grade, lymphatic metastasis and the depth of myometrial invasion, suggesting their regulatory role in the occurrence and development of endometrial cancer.

**Keywords:** Endometrial cancer, Pin1, Cyclin D1, immunohistochemical analyses

## Introduction

Endometrial cancer, the epithelial malignancy that arises from the endometrium, is the third most common cancer of the female reproductive system, behind ovarian cancer and cervical cancer [1]. Recently, the incidence rate of endometrial cancer has been gradually increased probably due to the increasing number of elderly people and obesity patients [2]. The occurrence and development of endometrial cancer is a complex, multi-step process involving a series of oncogenes and signal pathways [3].

Pin1, a prolyl cis/trans isomerase (PPIase) from the parvulin family, can specifically recognize the phosphorylated serine/threonine-proline sequences (pSer/Thr-Pro), leading to conformational and functional changes of proteins [4, 5]. Pin1 is also known as an important regulator of cell cycle [6-8]. It has been known that abnormity in Pin1 expression is involved in several physiological and pathological processes, including immune response, apoptosis and various types of cancers [9-11]. Cyclin D1, a member of the cyclin family, is a key regulator of cell cycle in eukaryotic cells. It binds to cyclin-dependent kinase CDK4 or CDK6, forming the cyclin D-CDK4/6 complex, which is required for cell cycle progression in G1 phase. Moreover, cyclin D1 can regulate cell functions through promoting the phosphorylation of tumor suppressor Rb and its family members [12, 13]. Numerous studies have reported cyclin D1 overexpression in a variety of cancers, including lung cancer, breast cancer, esophageal cancer, colorectal cancer, and pancreatic cancer [14-18]. It is believed that cyclin D1-induced cell cycle disorder is associated with the occurrence and progression of cancers [12, 19]. Overexpression of Pin1 can stimulate the expression of downstream genes including cyclin D1 through Ras, Wnt/β-catenin, and C-Jun/AP-1 pathways, leading to abnormal cell

metabolism and cell cycle, excessive proliferation, and even tumorigenesis [20, 21].

Although Pin1 inhibitors and Pin1-targeted gene therapy has received considerable attention, the expression of Pin1 and its association with cyclin D1 in endometrial cancer has not yet been clarified. In this study, we examined the expression of Pin1 and cyclin D1 in 52 cases of endometrial cancer, and further analyzed their association with clinical characteristics of the cancer. The current study should elucidation the role of both proteins in the pathogenesis of endometrial cancer, and might provide new insight into gene therapy of the malignant disease.

## Materials and methods

### *Subjects and sample collection*

This study selected 52 newly diagnosed endometrial cancer patients in Tai'an Central Hospital from January 2011 to June 2015. The inclusion criteria were as follows: patients without any prior cancer treatment including radiotherapy, chemotherapy, surgery, etc.; the diagnosis was confirmed by post-operative pathological examination; without other concurrent primary malignancies; without any hormone-related diseases; and paraffin specimens were well preserved. Endometrial cancer samples were obtained from all 52 patients. Adjacent non-cancerous endometrium was also collected from 15 patients and used as control. In addition, the endometrium from 29 patients with uterine leiomyomata in the same period was also obtained as control. The tissues were fixed with 4% paraformaldehyde for 72 h, embedded in paraffin and cut into 4-μm sections. This study was approved by the Research Ethics Committee at the Tai'an Central Hospital. All patients were required to sign the informed consent for the research use of tissues.

### *Main reagents*

Rabbit anti-human Pin1 polyclonal antibody and rabbit anti-human cyclin D1 monoclonal antibody was purchased from Abcam (Cambridge, MA, USA). SABC immunohistochemistry kit and DAB chromogenic reagent kit were purchased from Boster Biotech. (Wuhan, China). Neutral gum was purchased from Specimen and Model Factory (Shanghai, China). All other reagents were purchased from (ZSGB Biotech, Beijing, China) unless specified otherwise.

### *Immunohistochemical staining*

The expression of Pin1 and cyclin D1 in endometrium was detected by immunohistochemical staining (SABC). Briefly, paraffin sections were deparaffinized in xylene, rehydrated with serial ethanol (100, 90, and 80% ethanol), and rinsed with running water. The sections were boiled in antigen retrieval solution for 5 min, and incubated in 3% hydrogen peroxide for 10 min to block the endogenous peroxidases. The sections were then blocked with 5% Bovine serum albumin (BSA) for 20 min, and incubated with appropriate primary antibody overnight at 4°C. The sections were rinsed and incubated with biotinylated mouse anti-rabbit secondary antibody at 37°C for 20 min. The sections were rinsed and incubated with SABC at 37°C for 20 min, followed by DAB color development for 5 min. The sections were further counterstained with hematoxylin, dehydrated through serial alcohols, cleared in xylene and covered with neutral gum. The endometrium from 29 uterine leiomyomata patients and adjacent non-cancerous endometrium from 15 endometrial cancer patients were used as control. PBS was used as negative control, and breast cancer tissue was used as positive control.

### *Determination of expression level*

The sections were independently examined for the extent and intensity of staining using a Zeiss axiovert 40 c inverted microscope by two experienced pathologists in a blinded manner. Yellow or brown-stained granules in cytoplasm and nuclei were identified as positive expression. The staining intensity and positive staining rate of five randomly selected visual fields was quantified using the Image-Pro Plus 5.0 software. Pin1 was primarily localized in cell cytoplasm and/or nuclei (**Figure 1**). (-) was defined as negative staining or light brown staining, (+) as moderate-brown, (++) as brown, and (+++) as extensive dark-brown particles in both cytoplasm and nuclei. Positive staining rates of 0%-9%, 10%-33%, 34%-66%, and 67% or higher were scored as 0, 1, 2 and 3, respec-

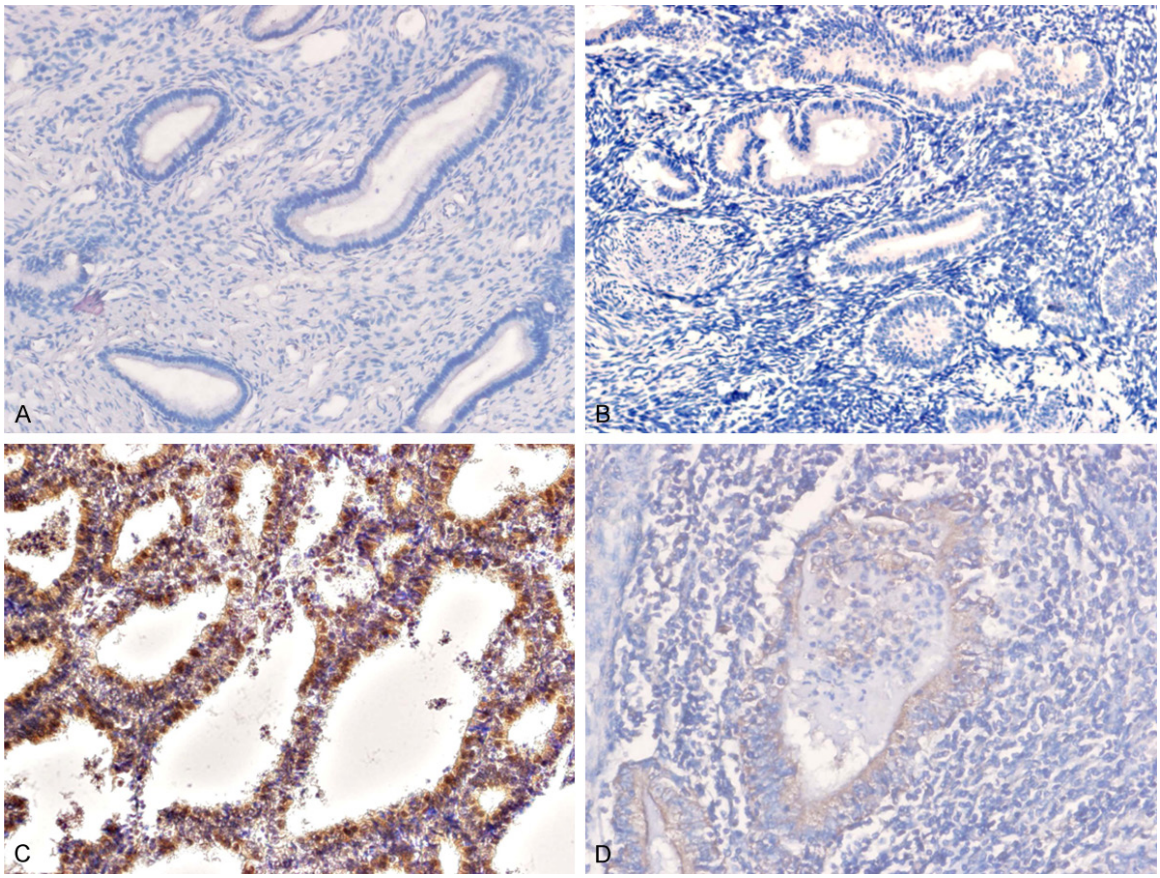


**Figure 1.** Representative immunohistochemical images (SABC method) comparing the expression of Pin1 in different groups. A. High Pin1 expression in normal endometrium (200×); B. Low Pin1 expression in normal endometrium (200×); C. High Pin1 expression in endometrial cancer (400×); D. Low Pin1 expression in endometrial cancer (400×).

tively. If the sum of the average intensity score and average stinging rate score was above 3, the sample was considered high Pin1 expression. Otherwise, the sample was considered low expression. Cyclin D1 was also mainly distributed in cell nuclei (**Figure 2**). Colorless, light brown, brown and dark brown were scored as (-), (+), (++) and (+++), respectively. Positive staining rates of 0-4%, 5-25%, 26-50%, 51-75% and 76-100% were scored as 0, 1, 2, 3 and 4, respectively. If the sum of the two scores was above 1, the sample was considered cyclin D1-positive. A sample with a score of 1 or lower was considered negative expression.

*Statistical analyses*

Data was analyzed using SPSS19.0 statistical software (IBM SPSS., Chicago, IL, USA). The expression of Pin1 and cyclin D1 in different groups was compared by chi-square tests. The relationship between Pin1 and cyclin D1 expression was analyzed by Pearson's correlation test. *P* values smaller than 0.05 were considered statistically significant.

## Results

*Clinical characteristics*

Of the 52 patients in this study, 21 patients were aged below 50 and 31 were aged 50 or older. There were 38 cases of adenocarcinoma, 10 adenocarcinoma with squamous differentiation and 4 serous adenocarcinoma. Based on the International Federation of Gynecologists and Obstetricians (FIGO) classification 2009, 23 patients were in stage I, 18 in stage II, and 11 in stage III/IV patients. There were 20 cases of in G1 grade, 22 in G2 and 10 in G3. Only 5 out of the 52 patients had lymphatic metastasis. Nineteen patients had the depth of myometrial invasion less than 1/2 and the remaining

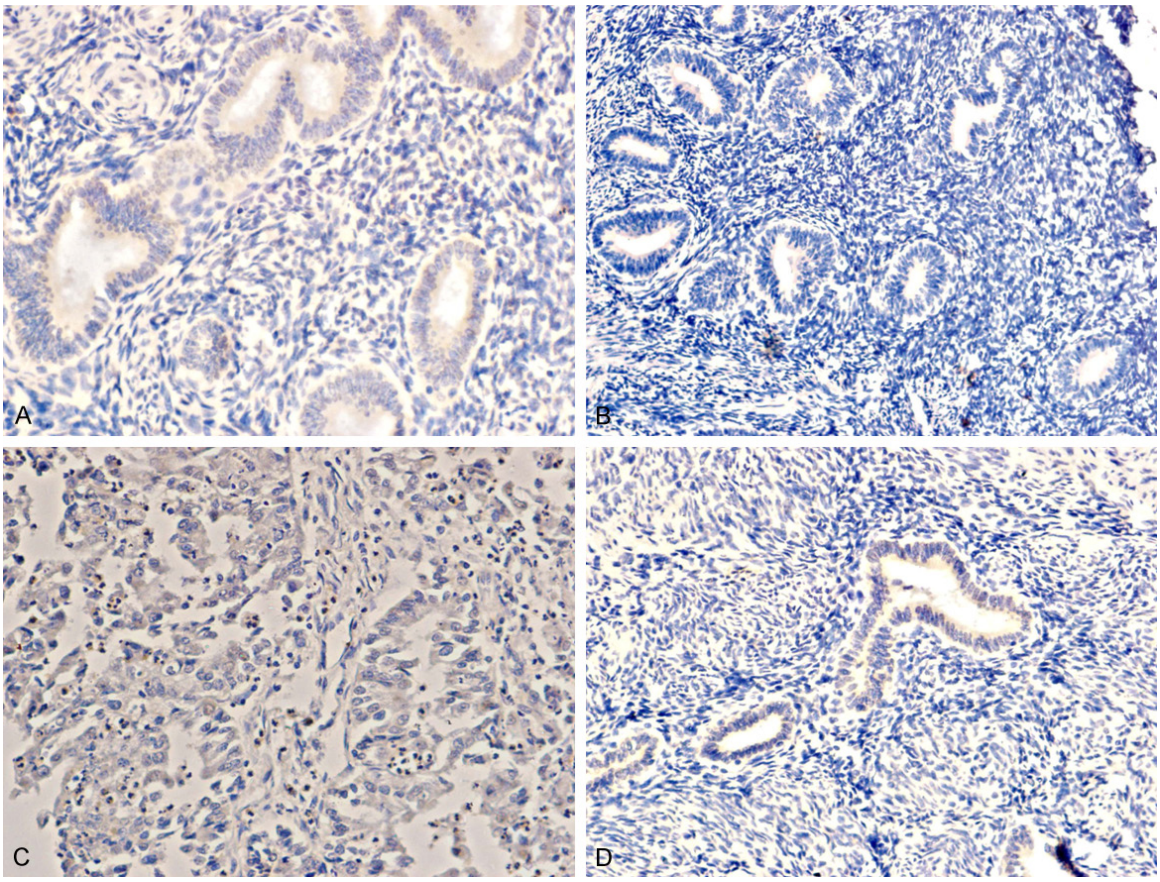


**Figure 2.** Representative immunohistochemical images (SABC method) comparing the expression of cyclin D1 in different groups. A. Positive cyclin D1 expression in normal endometrium (200×); B. Negative cyclin D1 expression in normal endometrium (200×); C. Positive cyclin D1 expression in endometrial cancer (200×); D. Negative cyclin D1 expression in endometrial cancer (200×).

**Table 1.** Comparison of Pin1 and cyclin D1 expression in endometrial cancer and normal endometrium

| Expression | | Endometrial cancer | | Normal endometrium | | $\chi^2$ (P) |
|---|---|---|---|---|---|---|
| | | n | Ratio | n | Ratio | |
| Pin1 | HE | 28 | 53.9% | 4 | 13.8% | 12.497 (0.000) |
| | LE | 24 | 46.1% | 25 | 86.2% | |
| Cyclin D1 | Positive | 22 | 42.3% | 3 | 10.3% | 8.914 (0.020) |
| | Negative | 30 | 57.7% | 26 | 89.7% | |

HE: High expression; LE: Low expression.

had the depth of myometrial invasion more than 1/2.

*Increased expression of Pin1 and cyclin D1 in endometrial cancer compared with normal endometrium*

While Pin1 was mainly expressed in the nucleus and cytoplasm of endometrial cancer cells, cyclin D1 was primarily expressed in the nucleus. Yellow or brown-stained granules in cytoplasm and nuclei were identified as positive expression (**Figures 1** and **2**). As shown in **Table 1**, 4 out of 29 normal endometrium (13.8%) had high Pin1 expression, whereas 53.9% of endometrial cancer exhibited high Pin1 expression ($\chi^2$ = 12.497, P = 0.000). The ratio of high Pin1 expression in endometrial cancer (53.3%) was also significantly higher compared with adjacent non-cancerous endometrium (6.7%, $\chi^2$ = 4.658, P = 0.040, **Table 2**). Similarly, the ratio of positive cyclin D1 expression in endometrial cancer was significantly high than both normal endometrium (10.3%, $\chi^2$ = 8.914, P = 0.020, **Table 1**) and adjacent non-cancerous endometrium (0.00%, $\chi^2$ = 6.000, P = 0.021, **Table 2**).

**Table 2.** Comparison of Pin1 and cyclin D1 expression in endometrial cancer and adjacent non-cancerous endometrium

| Expression | | Endometrial cancer | | Adjacent non-cancerous endometrium | | $\chi^2$ (P) |
|---|---|---|---|---|---|---|
| | | n | Ratio | n | Ratio | |
| Pin1 | HE | 8 | 53.3% | 1 | 6.7% | 4.658 (0.040) |
| | LE | 7 | 46.7% | 14 | 93.3% | |
| Cyclin D1 | Positive | 5 | 33.3% | 0 | 0.00% | 6.000 (0.021) |
| | Negative | 10 | 66.7% | 15 | 100.0% | |

HE: High expression; LE: Low expression.

These results suggested an abnormal increase in Pin1 and cyclin D1 expression in endometrial cancer.

*Association between Pin expression and clinical characteristics*

As shown in **Table 3**, the ratio of high Pin1 expression in stage III/IV patients (90.9%) was significantly higher compared with stage I and II patients (30.4% and 61.1%, respectively, $\chi^2$ = 11.658, P = 0.030). The ratio of high Pin1 expression in G3 grade (100%) was significantly higher than that in G1 and 2 grade (35.0% and 50.0%, respectively, $\chi^2$ = 12.59, P = 0.020). The ratio of high Pin1 expression in patients with lymphatic metastasis (100%) was significantly higher compared with other patients without metastasis (0%, $\chi^2$ = 4.742, P = 0.038). The ratio of high Pin1 expression in patients with depth of myometrial invasion ≥ 1/2 (75.8%) was significantly higher than that in other patients with depth of myometrial invasion < 1/2 (15.8%, $\chi^2$ = 17.448, P = 0.000). These results suggested that strong Pin1 expression was correlated with higher clinical stage, pathological grade, lymphatic metastasis, and depth of myometrial invasion. Moreover, no association was detected between Pin1 expression and age, histological type or menopause ($\chi^2$ = 0.154, P = 0.457, $\chi^2$ = 2.715, P = 0.283, and $\chi^2$ = 0.30, P = 0.543, respectively, **Table 3**).

*Association between cyclin D1 expression and clinical characteristics*

As shown in **Table 4**, the ratio of positive cyclin D1 expression in stage III/IV patients (81.8%) was significantly higher compared with stage I and II patients (13.0% and 55.6%, respectively, $\chi^2$ = 16.789, P = 0.000). The ratio of positive cyclin D1 expression in G3 grade (90.0%) was significantly higher than that in G1 and 2 grade (25.0% and 36.4%, respectively, $\chi^2$ = 11.916, P = 0.020). The ratio of positive cyclin D1 expression in patients with lymphatic metastasis (100%) was significantly higher compared with other patients without metastasis (36.3%, $\chi^2$ = 7.544, P = 0.010). The ratio of high cyclin D1 expression in patients with depth of myometrial invasion ≥ 1/2 (60.6%) was significantly higher than that in other patients with depth of myometrial invasion < 1/2 (10.5%, $\chi^2$ = 12.389, P = 0.000), suggesting a significant correlation between positive cyclin D1 expression and higher clinical stage, pathological grade, lymphatic metastasis, and depth of myometrial invasion. In addition, there was no association between cyclin D1 expression and age, histological type or menopause ($\chi^2$ = 0.105, P = 0.483, $\chi^2$ = 1.841, P = 0.502, and $\chi^2$ = 0.256, P = 0.414, respectively, **Table 4**).

*Positive correlation between Pin1 and cyclin D1 expression of in endometrial cancer*

Among 28 cases of endometrial cancer with high Pin1 expression, 18 (64.3%) were cyclin D1-positive, whereas only 4 out of 24 (16.7%) endometrial cancer with low Pin1 expression exhibited positive cyclin D1 expression, suggesting a higher positive cyclin D1 expression rate in endometrial cancer patients with high Pin1 expression compared with those with low Pin1 expression ($\chi^2$ = 12.006, P = 0.001). Further Pearson's correlation test revealed that Pin1 expression was positively correlated with cyclin D1 expression in endometrial cancer (r = 0.480, P = 0.001, **Table 5**).

## Discussion

The occurrence and progression of endometrial cancer involves the regulation of several oncogenes and signal pathways. Pin1 plays an important role in the G1/S and G2/M transition [22], and Pin1 overexpression is associated with several types of cancer, such as lung cancer, breast cancer, cervical cancer, ovarian cancer, esophageal squamous cell cancer and prostate cancer [23, 24]. Consistently, we compared the expression of Pin1 in endometrial

**Table 3.** Comparison of Pin1 expression in endometrial cancer with difference clinical characteristics

| Clinical characteristics | n | Pin1 High expression | Ratio | Pin1 Low expression | Ratio | $\chi^2$ (P) |
|---|---|---|---|---|---|---|
| Age (years) | | | | | | |
| < 50 | 21 | 12 | 57.1% | 9 | 42.9% | 0.154 (0.457) |
| ≥ 50 | 31 | 16 | 51.6% | 15 | 48.4% | |
| Histological type | | | | | | |
| Adenocarcinoma | 38 | 23 | 60.5% | 15 | 39.5% | 2.715 (0.283) |
| Adenocarcinoma with squamous differentiation | 10 | 4 | 40.0% | 6 | 60.0% | |
| Serous adenocarcinoma | 4 | 1 | 25.0% | 3 | 75.0% | |
| Clinical stage | | | | | | |
| I | 23 | 7 | 30.4% | 16 | 69.6% | 11.658 (0.030) |
| II | 18 | 11 | 61.1% | 7 | 38.9% | |
| III/IV | 11 | 10 | 90.9% | 1 | 9.1% | |
| Pathological grade | | | | | | |
| G1 | 20 | 7 | 35.0% | 13 | 65.0% | 12.59 (0.020) |
| G2 | 22 | 11 | 50.0% | 11 | 50.0% | |
| G3 | 10 | 10 | 100.0% | 0 | 0.0% | |
| Lymphatic metastasis | | | | | | |
| Yes | 5 | 5 | 100.0% | 0 | 0.0% | 4.742 (0.038) |
| No | 47 | 23 | 48.9% | 24 | 51.1% | |
| Depth of myometrial invasion | | | | | | |
| < 1/2 | 19 | 3 | 15.8% | 16 | 84.2% | 17.448 (0.000) |
| ≥ 1/2 | 33 | 25 | 75.8% | 8 | 24.2% | |
| Menopause | | | | | | |
| No | 21 | 11 | 52.4% | 10 | 47.6% | 0.30 (0.543) |
| Yes | 31 | 17 | 54.8% | 14 | 45.2% | |

cancer and normal endometrium, and found that the high Pin1 expression rate in endometrial cancer was significantly higher compared with normal endometrium ($\chi^2$ = 12.497, P = 0.000), suggesting an overexpression of Pin1 in endometrial cancer. Cyclin D1 stimulates the phosphorylation of tumor suppressor Rb and its family members, and therefore plays a key regulatory role in the pathogenesis of cancer [25]. Overexpression of cyclin D1 has been reported in breast cancer, lung cancer, melanoma and oral squamous cell cancer [6]. In this study, we also compared the expression of Pin1 in endometrial cancer and normal endometrium, and confirmed a higher rate of positive cyclin D1 expression in endometrial cancer compared with normal endometrium ($\chi^2$ = 8.914, P = 0.020) and adjacent non-cancerous endometrium ($\chi^2$ = 6.000, P = 0.021). Our results suggested that abnormity of Pin1 and cyclin D1 expression might be associated with the occurrence of endometrial cancer. It has been suggested that overexpression of Pin1 and cyclin D1 may trigger cell metabolism imbalance, interrupt cell cycle, and eventually stimulate the transformation of normal cells into cancerous cells [21].

We further compared the Pin1 and cyclin D1 expression in endometrial cancer with different characteristics. It was found that the expression of both proteins was associated with clinical stage, pathological grade, lymphatic metastasis, and depth of myometrial invasion (P < 0.05), but was irrelevant to age, histological type and menopause (P > 0.05). The findings suggested a close association between the expression of both proteins and the development of endometrial cancer.

It has been known that cyclin D1 gene is one of the specific downstream targets of Pin1. Pin1

**Table 4.** Comparison of cyclin D1 expression in endometrial cancer with difference clinical characteristics

| Clinical characteristics | n | Cyclin D1 Positive expression | Ratio | Cyclin D1 Negative expression | Ratio | $\chi^2$ (P) |
|---|---|---|---|---|---|---|
| Age (years) | | | | | | |
| < 50 | 21 | 10 | 47.6% | 11 | 52.4% | 0.105 (0.483) |
| ≥ 50 | 31 | 12 | 38.7% | 19 | 61.3% | |
| Histological type | | | | | | |
| Adenocarcinoma | 38 | 15 | 39.4% | 23 | 60.6% | 1.841 (0.502) |
| Adenocarcinoma with squamous differentiation | 10 | 4 | 40% | 6 | 60% | |
| Serous adenocarcinoma | 4 | 1 | 25% | 3 | 75% | |
| Clinical stage | | | | | | |
| I | 23 | 3 | 13.0% | 20 | 87.0% | 16.789 (0.000) |
| II | 18 | 10 | 55.6% | 8 | 44.4% | |
| III/IV | 11 | 9 | 81.8% | 2 | 18.2% | |
| Pathological grade | | | | | | |
| G1 | 20 | 5 | 25.0% | 15 | 75.0% | 11.916 (0.020) |
| G2 | 22 | 8 | 36.4% | 14 | 63.6% | |
| G3 | 10 | 9 | 90.0% | 1 | 10.0% | |
| Lymphatic metastasis | | | | | | |
| Yes | 5 | 5 | 100.0% | 0 | 0.0% | 7.544 (0.010) |
| No | 47 | 22 | 36.3% | 30 | 63.8% | |
| Depth of myometrial invasion | | | | | | |
| < 1/2 | 19 | 2 | 10.5% | 17 | 89.5% | 12.389 (0.000) |
| ≥ 1/2 | 33 | 20 | 60.6% | 13 | 39.4% | |
| Menopause | | | | | | |
| No | 21 | 8 | 38.1% | 13 | 61.9% | 0.256 (0.414) |
| Yes | 31 | 14 | 45.2% | 17 | 54.8% | |

**Table 5.** Positive association between Pin1 and cyclin D1 expression in endometrial cancer

| The number of cases | Cyclin D1 positive expression | Cyclin D1 negative expression | $\chi^2$ (P) | r (P) |
|---|---|---|---|---|
| Pin1 high expression | 18 | 10 | 12.006 (0.001) | 0.480 (0.001) |
| Pin1 low expression | 4 | 20 | | |

can stimulate cyclin D1 overexpression, and thus induce cell cycle disruption, cell proliferation abnormity, and eventually the occurrence of cancer [20]. In this study, the positive cyclin D1 rate in patients with high Pin1 expression was significantly higher than that in those with low Pin1 expression ($\chi^2$ = 12.006, P = 0.001). Moreover, the Pin1 expression was positively correlated with cyclin D1 expression in endometrial cancer (r = 0.480, P = 0.001), which is in good agreement with the literature on the close association between the two proteins [20]. Nevertheless, it is worth noting that 10 out of 28 cases of endometrial cancer with high Pin1 expression were cyclin D1-negative, suggesting that Pin1 might regulate the occurrence and development of endometrial cancer without involving the cyclin D1 pathway. Further studies are needed to elucidate the relevant molecular mechanisms.

## Conclusion

This study have shown that Pin1 and cyclin D1 are highly expressed in endometrial cancer, and is associated with clinical stage, pathological grade, lymphatic metastasis and the depth of myometrial invasion, suggesting their regula-

tory role in the occurrence and development of endometrial cancer. This study will not only shed light on the pathogenesis of endometrial cancer but also provide a theoretical basis for the selection of potential molecular targets for anti-tumor therapies.

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## Disclosure of conflict of interest

None.

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