Expression of HIWI in endometrial carcinoma tissues and its clinical significance

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Summary
Objective: This study aims to investigate the expression of the HIWI gene and explore its relationship with endometrial carcinoma. Materials and Methods: This study included 72 endometrial carcinoma (EC) cases. The study also analyzed 68 cases of endometrial benign disease and 78 cases of normal endometrium. Immunohistochemistry and western blot were used to detect the expression of HIWI in different tissues. Results: The expression level of HIWI was significantly higher in endometrial carcinoma than in benign endometrium and normal endometrium (P < 0.05). In addition, HIWI expression was significantly correlated with tumor myometrial invasion depth, cervical invasion, lymph vascular invasion, lymph node metastasis and clinical stage (P < 0.05), but was not correlated with the age or menstrual period of patients (P > 0.05). Conclusion: HIWI is correlated to the occurrence and development of endometrial carcinoma, and may be one of the factors that affect the prognosis of endometrial carcinoma.

Keywords: HIWI; Endometrial carcinoma; Immunohistochemistry; Western blot.

Introduction
HIWI is one of the members of the PIWI gene family [1], and has been proven to play an important role in the regulation of stem cell self-renewal, proliferation and differentiation [2-5]. In recent years, it was found that the occurrence and development of malignant tumors is closely correlated to the existence of tumor stem cells (TSC) [6, 7]. HIWI is located in chromosome 12 [8], and this can also be detected in hematopoietic progenitor cell CD34+ [2]. Further studies have revealed that the expression of HIWI is associated with DNA methylation, and that the downregulation of HIWI expression can reduce DNA methylation and inhibit tumor growth [9]. HIWI protein has been detected to be overexpressed in bladder cancer, prostate cancer, gastric cancer, ovarian cancer, liver cancer and other malignant tumors [10-15], and may play a pivotal role in colorectal cancer [12, 16]. However, it is underexpressed in normal cells, and the relationship between HIWI and endometrial cancer remains unclear. The present study aims to investigate the relationship between HIWI and the occurrence and development of endometrial carcinoma, and its influence on the prognosis of endometrial carcinoma.

Materials and Methods
A total of 218 specimens were taken from the Cancer Hospital Affiliated to Harbin Medical University from January 2015 to June 2015 (72 endometrial cancer patients, 68 endometrial benign diseases and 78 normal endometrium).

The paraffin embedded tissue sections were deparaffinized with xylene, and dehydrated using graded ethanol. For tissue repair, the paraffin sections were placed in sodium citrate solution at 100°C for five minutes, washed with phosphate buffered saline (PBS) for three times, and treated with 3% H2O2 for 15 minutes. The primary antibody (rabbit anti-human polyclonal antibody HIWI, 1:300) was incubated at 4°C overnight, and washed by PBS for three times. The secondary antibody (IgM/Rabbit Anti-Goat IgM/HRP, 1:1,000) was incubated at room temperature for an hour, and washed for three times with PBS. After washing, the slides were incubated with DAB (3,3-diaminobenzidine tetrahydrochloride) and immediately washed with tap water after color development. Then, the slides were counter stained with hematoxylin, mounted with dibutyl phthalate xylene (DPX), and were observed under a light microscope.

The HIWI protein was mainly expressed in the cell membrane and cytoplasm. An intensity percentage (IP) score was used to distinguish the stained results. A simple algebraic formula was conceptualized for score assignment to the IHC images. (1) Staining intensity: 0 point for unstained cells, 1 point for yellow -stained cells, 2 points for brown-stained cells, and 3 points for dark-brown stained cells. (2) The number of stained cells: 0 point for < 10%, 1 point for 10%-30%, 2 points for 31%-60%, and 3 points for > 60%. Positive: score = staining power × the number of stained cells ≥ 2. Negative: score = staining power × the number of stained cells ≤ 2.

Endometrial carcinoma, endometrial benign disease and normal uterus tissues were weighed. The same weight was used to organize the block by liquid nitrogen with the addition of 1%
PMSF and RIPA lysis buffer (50 mM of Tris-HCl [pH 7.4], 150 mM of NaCl, 1% NP-40, and 0.1% SDS). Then, these were mixed with Polytron as a homogenate (15,000/minute × 1 minute) at 4°C. After 30 minutes of incubation on ice with 1% PMSF, the cell lysates were isolated by centrifugation for 30 minutes. The cell lysates was obtained to determine the protein concentration using the Bradford colorimetric method. After boiling with the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer for five minutes, these samples underwent SDS-PAGE. Then, the proteins were transferred onto a polyvinylidene difluoride membrane. After blocking for one hour at room temperature, the membrane was incubated with the primary antibody (rabbit anti- human polyclonal antibody HIWI, 1:400; GAPDH, 1:400) overnight at 4°C. Before detection using an ECL chemiluminescence detection kit, the proteins were incubated with the corresponding secondary antibody for 1.5 hours at room temperature. Software Quantity One was used to analyze the ECL results.

The experimental data were analyzed by rank-sum test using SPSS 19.0. \( P < 0.05 \) was considered statistically significant.

### Results

The immunohistochemistry results revealed that the HIWI protein was mainly distributed in the cell membrane and cytoplasm, and the protein expression mainly manifested from the intensity of the staining (Table 1, Figure 1). The expression of HIWI was significantly higher in the endometrial carcinoma than in the endometrial benign disease and normal endometrium, and the difference was statistically significant \( (P < 0.05) \). However, there was no significant difference in HIWI expression between the endometrial benign disease and normal endometrium \( (P > 0.01) \), Figure 2).

The expression of HIWI was correlated with histological type, pathological grade, depth of myometrial invasion, clinical stage and lymph node metastasis, and the difference was statistically significant \( (P < 0.05) \). However, there was no significant effect with the patient’s age or menopause state \( (P > 0.05) \), Table 2.

### Discussion

The PIWI gene was found in lower organisms for the first time [17]. The PIWI family was involved in the regulation of stem cell proliferation and self-renewal, while merely the PIWI L1 (HIWI) gene was first found in human testicular tissues [2]. HIWI is necessary to maintain the self-renewal and asymmetric division of stem cells. Therefore, it was detected to be highly expressed in bladder cancer tissues, but lowly expressed in normal bladders [18]. In a study conducted on prostate cancer, HIWI was highly expressed in prostate cancer cell line pc -3m-2b4, but lowly expressed in normal prostate [19].

Silencing the expression of HIWI could inhibit the proliferation of lung cancer stem cells and promote the apoptosis of cancer cells, suggesting that HIWI may be a new target for the treatment of lung cancer [20-22]. The high expression of HIWI was also found in colorectal cancer, which was an independent prognostic factor for colorectal cancer [12]. The expression of HIWI in hepatocellular carcinoma was higher than that in normal livers, and was also closely correlated to the recurrence of liver cancer and the five-year survival rate [13]. In the study of pancreatic ductal adenocarcinoma, the expression level of HIWI may be correlated to the invasiveness of tumor cells, thereby affecting the prognosis of patients with pancreatic ductal adenocarcinoma [23].

Hu et al. [24] reported that HIWI was antagonistic to Ki67, but had a positive correlation with clinical stage. Above all, HIWI was highly expressed in a variety of malignant tumors, and is closely correlated to the occurrence, development and prognosis of tumors.

In the present study, 72 cases of endometrial carcinoma, 68 cases of endometrial benign disease, and 78 cases of normal endometrial tissue were detected using the immunohistochemical

| Table 1. HIWI expression in endometrial carcinoma, endometrial benign disease and normal endometrium |
|---------------------------------------------------------------|
| Positive (n) | Negative (n) | Positive rate (%) |
|----------------|-------------|-------------------|
| Endometrial carcinoma (72) | 59 | 13 | 81.94%*# |
| Endometrial benign disease (68) | 13 | 55 | 19.12% |
| Normal endometrium (78) | 6 | 72 | 7.69% |

*\( p < 0.01 \) (compared to normal endometrium), #\( p < 0.01 \) (compared to endometrial benign disease).
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Table 2. Positive expression of HIWI in endometrial carcinoma.

| Items                        | Cases (n) | HIWI protein Positive rate (%) | $X^2$ | $p$ |
|------------------------------|-----------|--------------------------------|-------|-----|
| Age (years)                  |           |                                |       |     |
| < 50                         | 28        | 21 (75.0)                       | 0.753 | 0.682 |
| $\geq$ 0                     | 44        | 38 (86.4)                       |       |     |
| Menopausal state             |           |                                |       |     |
| Premenopausal                | 15        | 11 (73.3)                       | 0.423 | 0.421 |
| Postmenopausal               | 57        | 48 (84.2)                       |       |     |
| Histological type            |           |                                |       |     |
| Endometrial carcinoma        | 53        | 41 (77.4)                       | 3.642 | 0.042*|
| Others                       | 19        | 18 (94.7)                       |       |     |
| Pathological grading         |           |                                |       |     |
| G1                           | 25        | 18 (72.0)                       | 4.524 | 0.025*|
| G2                           | 31        | 25 (80.6)                       |       |     |
| G3                           | 16        | 16 (100.0)                      |       |     |
| Myometrial invasion          |           |                                |       |     |
| $< 1/2$                      | 40        | 29 (72.5)                       | 3.124 | 0.043*|
| $\geq 1/2$                   | 32        | 30 (93.7)                       |       |     |
| Pathological staging         |           |                                |       |     |
| I                            | 49        | 38 (77.6)                       | 7.532 | 0.009*|
| II                           | 14        | 13 (92.9)                       |       |     |
| III                          | 7         | 6 (85.7)                        |       |     |
| IV                           | 2         | 2 (100.0)                       |       |     |
| Lymph node metastasis        |           |                                |       |     |
| Yes                          | 13 12 (92.3) | 6.428 0.016*                   |       |     |
| No                           | 59 47(79.7) |                                |       |     |

*p < 0.05, *p < 0.01

method. These results show that the expression of HIWI was significantly higher in endometrial carcinoma than in benign endometrium and normal endometrium. However, when this expression increased in endometrial benign diseases, no significant difference was found when compared with the normal endometrium. This reveals that HIWI, as a kind of oncogene, influences the development and progression of endometrial carcinoma. In order to further explore the relationship between HIWI and clinical factors, the present study conducted an in-depth analysis of the clinical data obtained from 72 patients. The results revealed that there was no significant relationship between HIWI with the age of patients and menopause. However, this was correlated to histological type, pathological grade, pathological stage, myometrial invasion depth, and lymph node metastasis. The typical case of patients with endometrial cancer was vaginal bleeding after menopause. Hence, most patients choose surgical treatment in the early stage. Therefore, the later the pathological stage, the higher the degree of malignancy. In the present study, the high gene expression of HIWI was caused by the high pathological grade and late pathological stage of patients, suggesting that HIWI is closely correlated to the prognosis of endometrial carcinoma. In order to reduce the risk of recurrence, patients should be treated at the early stage. Thus, most cases in the present study were in surgical pathological stage I, which was the main limitation of the present experiment. It remains unknown whether exogenous HIWI can affect the proliferation and differentiation of normal endometrial tissues.

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