Suppression of Hiwi inhibits the growth and epithelial-mesenchymal transition of cervical cancer cells

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Abstract. Cervical cancer is a common gynecological malignancy. Hiwi exhibits a high level of expression in cervical cancer cells. However, the effects of Hiwi expression in cervical cancer cells remain unresolved. In the present study, the effects of Hiwi downregulation on the growth and epithelial-mesenchymal transition of cervical cancer cells were investigated. The results of the present study revealed that the suppression of Hiwi was able to inhibit the proliferation of cervical cancer cells and arrest cell cycle at G1 phase. The downregulation of Hiwi was also revealed to inhibit the epithelial-mesenchymal transition process of cervical cancer cells by regulating the expression of E-cadherin, N-cadherin, vimentin, and snail. The present study demonstrated that the suppression of Hiwi was able to inhibit the growth and epithelial-mesenchymal transition of cervical cancer cells. Therefore, the results suggest that Hiwi may function as an oncogene in cervical cancer cells and may become a potential target for cervical cancer therapy.

Introduction

Cervical cancer is one of the most common malignancies globally and is one of the leading causes of mortality resulting from gynecologic malignancies (1). In recent years, the incidence of cervical cancer has decreased. However, the rate of cervical cancer-associated morbidity in young adult women has increased.

The members of the Piwi protein family are highly conserved during evolution and serve notable roles in cell proliferation, gametogenesis, germ cell proliferation and translational regulation (2-4). Hiwi is a human homologue of the Piwi family, located on chromosome 12q24.33 and encoding a 98.5 kDa protein. Hiwi is expressed in CD34+ hematopoietic stem cells, but not in well-differentiated cell populations (5). Hiwi serves a function in the development of hematopoietic stem cells (5). Prior studies have demonstrated that Hiwi is also expressed in various types of cancer cells and affects the differentiation and proliferation of tumor cells (2,6-11). Overexpression of Hiwi is reported to lead to tumors (11).

Hiwi exhibits an increased level of expression in cervical cancer cells (12,13). However, the function of Hiwi in cervical cancer cells remains unclear. In the present study, the effects of siRNA induced-knockdown of Hiwi on the growth and epithelial-mesenchymal transition of cervical cancer cells were investigated. The present study reveals that Hiwi may act as an oncogene in cervical cancer cells and may therefore be a novel target for the treatment of cervical cancer.

Materials and methods

Cell culture. Human cervical cancer cells, HeLa, were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and cultured in a humidified atmosphere at 37°C with 5% CO2.

Transfection. Hiwi specific small interfering RNA (siRNA) (sequence, 5’-GCCGUUCAUACAAGACUATT-3’ and 5’-UUA GUC UUG UAU GAA CGG CTT-3’) and a scrambled negative control (sequence, 5’-UUC UCC GAA CGU GUC AC G UTT-3’ and 5’-ACGUGACACGUCCAGAATT-3’) were obtained from Biomics Biotechnologies Co., Ltd. (Nantong, China). The cells were seeded into 6-well plates (1x105 cells/well). After 24 h, the cell medium was changed to serum-free medium for an additional 6 h. Then 100 pmol siRNA or its corresponding negative control was transfected into the cells using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's
Western blot analysis. The cells transfected with negative control or siRNA were harvested and proteins in cells were extracted on ice using radioimmunoprecipitation assay lysis buffer. The concentration of the proteins was measured using a Enhanced BCA Protein Assay kit (Beyotime Institute of Biotechnology, Haimen, China). 40 μg proteins were separated using 12% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). Following blocking with 5% skimmed milk at 37˚C for 1 h, the membranes were incubated with primary antibodies against Hiwi (1:1,000; cat. no. sc-22685; Santa Cruz Biotechnology, Inc.), vimentin (1:1,000; cat. no. sc-5565; Santa Cruz Biotechnology, Inc.), E-cadherin (1:1,000; cat. no. sc-71009; Santa Cruz Biotechnology, Inc.), N-cadherin (1:1,000; cat. no. sc-59987; Santa Cruz Biotechnology, Inc.), cyclin A (1:1,000; cat. no. sc-751; Santa Cruz Biotechnology, Inc.), cyclin D1 (1:1,000; cat. no. sc-70899; Santa Cruz Biotechnology, Inc.), E-cadherin (1:1,000; cat. no. sc-71009; Santa Cruz Biotechnology, Inc.), PCNA (1:2,000; cat. no. sc-25280; Santa Cruz Biotechnology, Inc.), cyclin A (1:1,000; cat. no. sc-751; Santa Cruz Biotechnology, Inc.), vimentin (1:1,000; cat. no. sc-5565; Santa Cruz Biotechnology, Inc.), snail family transcriptional repressor 1 (SNAIL; 1:1,000; cat. no. sc-28199; Santa Cruz Biotechnology, Inc.) and β-actin (1:2,000; cat. no. sc-130065; Santa Cruz Biotechnology, Inc.) at 4˚C overnight. Following washing with Tris-buffered saline with Tween-20 (TBST), the membranes were incubated with corresponding horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (H+L) (cat. no. A0208), HRP-labeled goat anti-mouse IgG (H+L) (cat. no. A0216), or HRP-labeled donkey anti-goat IgG (H+L) (cat. no. A0181) (1:5,000; Beyotime Institute of Biotechnology) at 37˚C for 1 h. Following washing with TBST, the targeted proteins were visualized using an Chemiluminescent HRP substrate (ECL) (EMD Millipore). The relative protein level was analyzed by Quantity One 4.6 (Bio-Rad, Hercules, CA, USA) and calculated as targeted protein level/reference protein level.

Transwell assay. Following transfection with Hiwi siRNA or negative control, the cells were collected and suspended (1x10^5 cells/ml). The Transwell inserts (Corning Life Sciences, Tewksbury, MA, USA) were inserted into a 24-well plate. Subsequently, 200 μl cell suspension was added into the upper chambers, and 600 μl DMEM containing 20% FBS was added into the lower chambers. The cells were allowed to migrate at 37˚C for 24 h. Subsequently, the cells above the membranes were removed with cotton swabs. The cells below the membranes were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet at room temperature for 15 min. The cells were observed via light microscopy using a x200 magnification. The cells in each field of view were counted, and the average number of cells in five randomly selected fields of view was calculated as the number of migratory cells.

Statistical analysis. The results are presented as the mean ± standard deviation. Differences between groups were analyzed using Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Hiwi siRNA decreases the level of Hiwi in cervical cancer cells. Hiwi siRNA was used to investigate the effects of Hiwi on cervical cancer cells. Following transfection with Hiwi siRNA or the negative control, the relative level of Hiwi mRNA was detected using RT-qPCR. The results revealed that the relative level of Hiwi mRNA was decreased to 11.01±4.78% following transfection with Hiwi siRNA (Fig. 1A). Western blot analysis demonstrated similar results to RT-qPCR. Compared with the control group, the relative protein level of Hiwi was decreased from 1.64±0.28 to 0.33±0.09 following transfection with Hiwi siRNA (Fig. 1B and C). These results demonstrated that Hiwi siRNA was able to effectively decrease the levels of mRNA and protein in cervical cancer cells.
Downregulation of Hiwi inhibits the proliferation of cervical cancer cells. Following transfection with Hiwi siRNA or the negative control, the proliferation of cervical cancer cells was detected using MTT assay. As presented in Fig. 2A, compared with the control group, the proliferation of the cells was significantly inhibited by transfection with Hiwi siRNA. The level of PCNA protein was detected by western blotting. The results of western blot analysis revealed that, following transfection with Hiwi siRNA, the relative level of PCNA protein was decreased from 1.30±0.178 to 0.49±0.06 (Fig. 2B and C). These results support the hypothesis that transfection with Hiwi siRNA inhibits the proliferation of cervical cancer cells.

Downregulation of Hiwi inhibits the cell cycle of cervical cancer cells. The cell cycle has an important function in the growth of cancer cells, thus the effects of Hiwi siRNA on the cell cycle of cervical cancer cells were investigated using flow cytometry. The results of flow cytometry revealed that following transfection with Hiwi siRNA, the percentage of cells in the G1 phase was increased from 53.37±2.87 to 64.78±3.49% (Fig. 3A and B). The levels of cyclin A and cyclin D1 proteins, which are important cytokines regulating the cell cycle progress, were also detected using western blotting. Western blot analysis revealed that following transfection with Hiwi siRNA, the relative level of cyclin A protein was decreased from 0.66±0.16 to 0.17±0.03 (Fig. 3C and D). The relative level of cyclin D1 protein was also decreased from 0.77±0.15 to 0.12±0.02 (Fig. 3E and F). These results demonstrated that Hiwi siRNA may have an inhibitory effect on the cell cycle of cervical cancer cells.

Hiwi siRNA inhibits the epithelial-mesenchymal transition of cervical cancer cells. Following transfection with Hiwi siRNA, the migration of cervical cancer cells was evaluated using Transwell assay. The results of the Transwell assay identified that the number of migratory cells was decreased...
significantly in cells transfected with Hiwi siRNA compared with the control cells (Fig. 4A and B). The levels of E-cadherin, N-cadherin, vimentin and snail proteins were detected using western blotting. The results of western blotting revealed that following transfection with Hiwi siRNA, the relative level of E-cadherin protein was increased from 0.22±0.05 to 0.84±0.17 (Fig. 5A and B). However the relative level of N-cadherin protein was decreased from 0.96±0.14 to 0.17±0.03 (Fig. 5C and D).
The relative level of vimentin protein was decreased from 0.77±0.14 to 0.24±0.05 (Fig. 5E and F), and the relative level of snail protein was decreased from 0.26±0.04 to 0.14±0.04 (Fig. 5G and H). These results indicate the inhibitory effects of...
Hiwi siRNA on epithelial-mesenchymal transition of cervical cancer cells.

Discussion

In the present study, the effects of downregulating Hiwi in cervical cancer cells were investigated. The results of the present study demonstrated that suppression of Hiwi was able to inhibit the proliferation of cervical cancer cells and arrest cell cycle at the G1 phase. The downregulation of Hiwi was also able to inhibit the epithelial-mesenchymal transition process. Taken together, these results suggest that Hiwi may be a potential target for cervical cancer therapy.

Hiwi is overexpressed in various types of cancer, including colon (15,16), liver (17), stomach (2) and pancreatic cancer (7), and is associated with the growth, migration and angiogenesis of cancer cells (11,18). Hiwi contributes to tumorigenesis, serves as a potential biomarker of certain types of cancer (17) and is regarded to be an indicator of poor prognosis (2,6-8,19). Previous studies have demonstrated that the expression of Hiwi is associated with the histological grade of cancer, however associations with age, gender, tumor size or location were not observed (10,17). Additionally, Hiwi was revealed to be highly expressed in cervical cancer tissues (12,13), which indicates that Hiwi may exhibit an association with cervical cancer progression. The results of the present study demonstrated that the downregulation of Hiwi was able to inhibit the growth and epithelial-mesenchymal transition of cervical cancer cells. These data indicate that Hiwi may serve an oncogenic function in cervical cancer cells. However, the present study was performed in only one cervical cancer cell line, which is a limitation of the study. Additional in vitro and in vivo experiments are required to further strengthen the findings of the present study.

Liu et al (12) demonstrated that Hiwi may be involved in cervical cancer carcinogenesis and may be a potential therapeutic target for the treatment of cervical cancer. Liu et al (12) demonstrated that the expression of Hiwi was associated with the stage of cervical cancer, but exhibited no association with the other clinical characteristics. Furthermore, another study (2) also demonstrated that by blocking endogenous Hiwi expression, it was able to inhibit the proliferation of gastric cancer cells. Liang et al (11) demonstrated that the proliferation of lung cancer cells was inhibited by downregulating Hiwi.

In the present study, the effects of silencing Hiwi on the growth of cervical cancer cells were investigated. The results revealed that the proliferation of cervical cancer cells was inhibited by transfection with Hiwi siRNA, with decreased levels of PCNA following transfection. Consistently, Liu et al (12) demonstrated that Hiwi promoted tumorigenicity of cervical cancer in vitro and in vivo. However, when it came to the growth of cervical cancer cell lines in vitro, Hiwi was revealed to inhibit the viability of the cervical cancer cell lines, SiHa (12). The results of the present study did not verify these findings, but the results were consistent with the results of previous studies (2,11,18). This difference may require further investigation.

Cell cycle analysis of the cervical cancer cells was also undertaken in the present study. The results of the present study demonstrated that cell cycle was arrested upon silencing of Hiwi. Additionally, the levels of cyclin A and cyclin D1 proteins, which are regulators of the cell cycle (20), were also decreased following transfection with Hiwi siRNA. This suggests that the cell cycle of cervical cancer cells was arrested at the G1 phase by downregulation of Hiwi. Consistent with the present study, the results of Wang et al (18) also identified that the downregulation of Hiwi was able to induce cell cycle arrest of glioma cells. However, the results of Liu et al (2) demonstrated that the cell cycle of gastric cancer cells was arrested by Hiwi suppression at the G2/M phase. The molecular mechanisms underlying these differences should be investigated further.

The assays investigating the effects of Hiwi downregulation on the proliferation and cell cycle demonstrated that the growth of cervical cancer cells was inhibited by silencing of Hiwi. In addition to the effects on proliferation and cell cycle, Hiwi is also able to affect cell apoptosis. The results from Wang et al (18) demonstrated that Hiwi suppression induced cell apoptosis in glioma cells. Liang et al (11) also reported that cell apoptosis in lung cancer cells was promoted by suppression of Hiwi.

In the present study, the effects of Hiwi downregulation on the epithelial-mesenchymal transition process in cervical cancer cells were also detected. The results of the present study demonstrated that the downregulation of Hiwi was able to increase the level of E-cadherin, and decrease the level of N-cadherin, vimentin and snail, which are hallmarks of epithelial-mesenchymal transition (EMT) (21-25), suggesting that suppression of Hiwi was able to inhibit the EMT process. The results of Wang et al (18) demonstrated that suppression of Hiwi was also able to inhibit the migration and invasion of glioma cells with decreased expression of matrix metalloprotein-2 and matrix metalloprotein-9 which are associated with degradation of the extracellular matrix, thus contributing to the migration and invasion of cells (26,27). Jiang et al (17) demonstrated that the expression of Hiwi was associated with metastasis to intrahepatic tissue, local lymph nodes and remote organs. Raeissosadati et al (15) also demonstrated that Hiwi expression was associated with tumor invasion depth. Hiwi is also associated with the angiogenesis of cancer (9).

To conclude, in the present study, the effects of Hiwi on the growth and epithelial-mesenchymal transition process of cervical cancer cells was investigated. The results of the present study indicated that the suppression of Hiwi was able to inhibit the growth of cervical cancer cells and arrest the cell cycle. The epithelial-mesenchymal transition was also inhibited by the downregulation of Hiwi. The present study indicates that Hiwi may act as an oncogene in cervical cancer cells, and that it may be a target for novel cervical cancer therapy.

Competing interests

The authors declare that they have no competing interests.

References