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Research on the Role of Ezrin Gene in the Pathological Process of Cervical Cancer

Duan H[1#], Zhang Z[2#], Shi J[3], Chen Y[4] and Li X[1*]*

[1]Department of Reproduction Gynecology, Northwest Women and Children's Hospital, China

[2]Department of Ultrasonography, The Affiliated Children Hospital of Xi'an, Jiaotong University Shaanxi, China

[3]Reproductive Center, Northwest Women and Children's Hospital, China

[4]Department of Reproductive Medical Center, Xi'an No. 4 Hospital, China

[#]These authors contributed equally to this work

Abstract

Aim: This paper is going to study the effect of Ezrin gene on the proliferation and migration of cervical cancer cells and to explore the key role of Ezrin gene in the pathogenesis of cervical cancer.

Method: RT-PCR and Western blot were used to detect the cervical cancer cell lines, HeLa and C33A, and the expression of Ezrin in cervical cancer tissue. After low-expression of Ezrin gene through RNA interfering technology, this paper observed the different expression of E-cadherin, MMP-2 and β-Catenin.

Result: The result from RT-PCR and Western blot showed that the expression of Ezrin protein was increasing in cervical cancer cell lines, HeLa, C33A and cervical cancer tissues, but not expressed in normal cervical tissues. After using the interfering RNA technology to express the Erzrin gene in the cervical cancer cell line, HeLa, the proliferative ability of HeLa cells was lower than that of the non-interference group. Meanwhile, the expression of E-cadherin protein was up-regulated after the low expression of Ezrin gene and the expression of MMP-2 and β-Catenin are increasing.

Conclusion: As a kind of cancer-promoting genes, Ezrin gene can activate EMT so as to induce proliferation, metastasis and infiltration of cervical cancer cells.

Keywords: Ezrin; Cervical cancer; EMT

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Correspondence:
Xiaojuan Li, Department of Reproduction Gynecology, Northwest Women and Children's Hospital, Shaanxi, 710003, Xi'an, China, E-mail: lxj197305@126.com
Haixia Duan, Department of Reproduction Gynecology, Northwest Women and Children's Hospital, Shaanxi, 710003, Xi'an, China, Tel: (+86-29)89622093; E-mail: hx840315@126.com

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Introduction

Cervical cancer is the second most common malignant tumor for women, which seriously threaten women's health [1]. Although early screening technology for cervical cancer and the widespread use of vaccines have greatly reduced the risk of death from cervical cancer, many patients still die from cervical cancer infiltration and metastasis [2]. Although various research institutions around the world have paid close attention to the pathogenic mechanism in this field and the pathological process of cervical cancer, the pathogenesis existing in the process of cervical cancer's occurrence and metastasis has not yet been elucidated. EMT (Epithelial-Mesenchymal Transition, EMT) was first put forward by Greenburg in 1982 [3]. EMT plays a key role in tumor in situ infiltration and distant metastasis [4]. By changing EMT, the adhesion between tumor cells can be reduced, the movement capacity can be enhanced, and the purpose of tumor infiltration and metastasis can be achieved finally. During the EMT process, specific changes may happen to some proteins, including the epithelial marker E-cadherin.

Ezrin protein is a highly homologous protein in the FERM (4.1-band ERM) superfamily [5]. It is an important linker for the cytoskeleton and a key protein molecule for the growth, adhesion and metastasis of tumor cell. Research by Li showed that the low expression of Ezrin gene resulted by RNA interfering technology could induce the change of EMT so as to affect the proliferation and metastasis of lung cancer cells. However, it has not been reported yet about its expression and function in in cervical cancer. To explore the expression of Ezrin protein in the treatment of cervical cancer and the function of molecules can provide theoretical support for the treatment of cervical cancer.

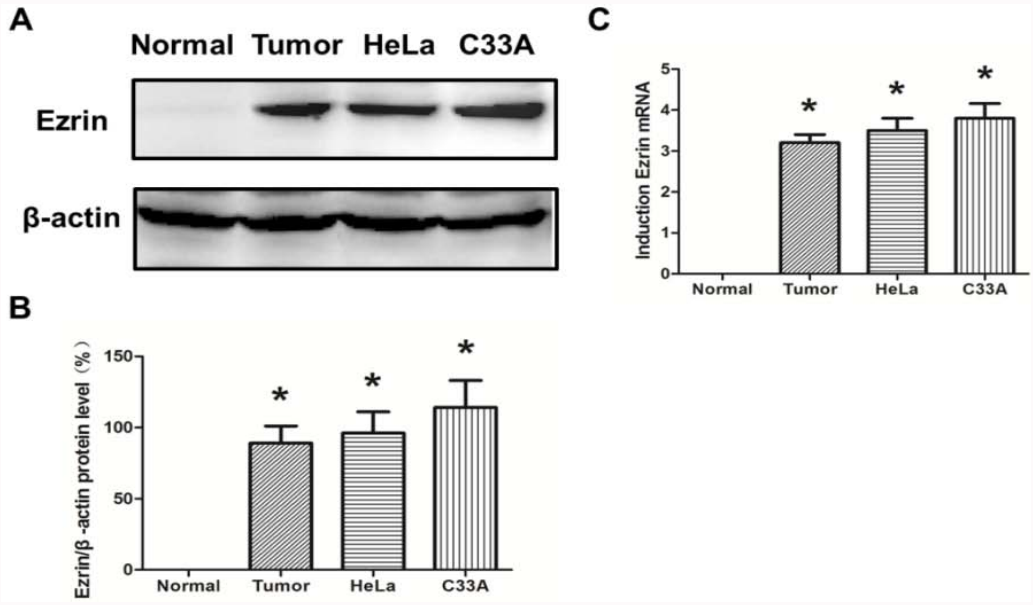

Figure 1: Expression of protein Ezrin in specimens of HeLa, C33A cells, cervical cancer tissues and normal cervical tissues. At the end of the culture period, western blotting was performed to assess the expression of Ezrin (A). The values from densitometry of Ezrin was normalized to the level of β-actin protein and expressed as fold increase (B). RT-PCR was performed to assess the level of Ezrin mRNA (C). The data were expressed as means ± SD of three independent experiments. *p<0.05, compared with control group.

Material and Method

Material

HeLa, C33A cells were provided by Department of Obstetrics and Gynecology, First Affiliated Hospital of Air Force Military Medical University. Aralia saponin was provided by the Department of Pharmacy, First Affiliated Hospital of Air Force Military Medical University. Culture media, RPMI-1640, was bought from Invitrogen company. Actin, E-cadherin, β-Catenin and MMP-2, Akt were bought from Abcam company. MTT (3-(4,5-dimethylthiazole-2) -2,5-Diphenyltetrazolium bromide) were bought from Amressco company; DMSO (Dimethyl Sulfoxide) was bought from Tianjin BASF Chemicals Company, Limited. All procedures were approved by the Animal Research Ethics Board of The Fourth Military Medical University.

Method

HeLa Cell culture: Cultured HeLa cells under conventional conditions, 37°C, 5% saturated humidity condition.

HeLa Detection of inhibition on cell growth: Normal HeLa group, interfering group and si-Ezrin processed group were be clarified in the experiment. After 48 h, a new configuration of 5 mg/mL MTT 20 μL was added, and then put it into the incubator for 4 h. After discarding the supernatant, a 150 μL DMSO was added into each hole, shaking mixture. The absorbance was detected by a 490 nm microplate reader.

Western blot: Western blot was used to test EMT relative protein and the signal pathway changes of PI3K/Akt. After that, Protein samples were collected and protein levels were measured by method of quantitative protein detection. 50 μg of protein was added to the sample buffer, followed by gel electrophoresis, and then transferred into PVDF (Polyvinylidene Fluoride) membrane. The primary and secondary antibodies were incubated, with the help of chemiluminescence method to color, expose, develop, and fix. Actin is used as an internal reference. The strips were scanned with a thin layer scanner, and the gray values of each strip were analyzed by software to calculate the relative contents of each protein (E-cadherin, β-Catenin, MMP-2).

Statistical treatment

The data were processed by SPSS19.0 statistical analysis software.

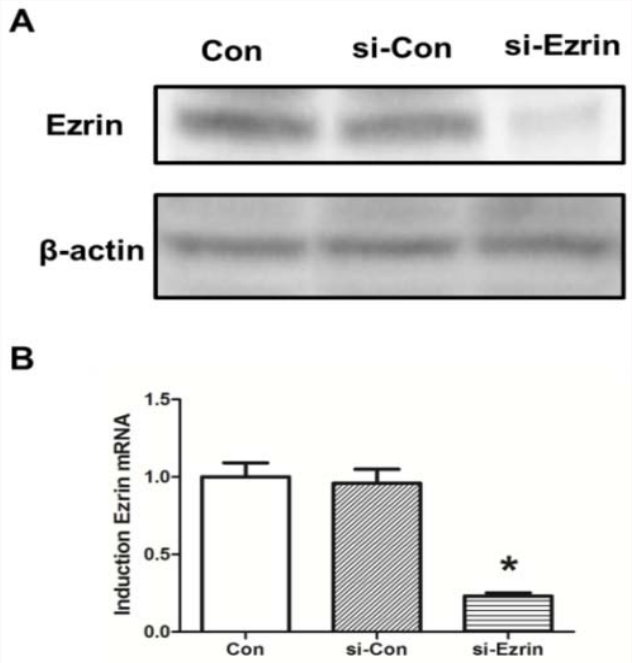

Figure 2: Changes of Ezrin protein and RNA level after transfection of Ezrin-shRNA by HeLa cells. HeLa cells were transfected for 24 h with Ezrin-shRNA. At the end of the culture period, western blotting was performed to assess the expression of Ezrin (A). RT-PCR was performed to assess the level of Ezrin mRNA (B). *p<0.05, compared with control group.

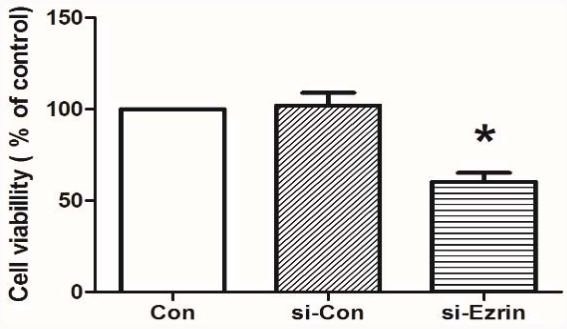

Figure 3: The changes of proliferation viability after Ezrin-shRNA transfected by HeLa cell. At the end of the incubation period, cell viability was determined with the tetrazolium salt assay. The data were expressed as means ± SD of three independent experiments. *p<0.05, compared with control group.

The measurement data were expressed as ± s. The *t*-test was used for comparison between groups. The difference was statistically significant at P<0.05.

Results

Difference comparison of Ezrin protein expression between tumor tissue and normal tissue. Extracting specimen proteins of HeLa, C33A cells, cervical cancer tissues, and normal cervical tissues, using Western blot to detect the Ezrin protein expression from four specimen, we found that compared with the control group, the expression of Ezrin protein was increased in HeLa, C33A cells and cervical cancer tissues. Gray scale analysis showed that P<0.05 was statistically significant, as shown in Figure 1. The changes of Ezrin's RNA level were detected by RT-PCR, which indicated that the level expression of Ezrin's RNA was higher in HeLa, C33A cells and cervical cancer tissues than in the matched group. P<0.05 was statistically significant, as shown in Figure 1.

Effects of Hela cells after interfering Ezrin protein. We used RNA interfered by Ezrin protein to transfect HeLa cells. Western blot and RT-PCR were used to detect the changes of Ezrin protein from protein and RNA level respectively. We found that Ezrin-shRNA can inhibit the level of Ezrin protein and RNA. P<0.05 was statistically significant, as shown in Figure 2.

Comparison of proliferation viability after Ezrin protein being interfered by HeLa cells. In the experiment of MTT cell proliferation, it was found that compared with the matched group, the viability of HeLa decreased evidently after its cell diameter (si-Ezrin) being transfected for 48 h. P<0.05 was statistically significant, as shown in Figure 3.

Changes of EMT related proteins after interference of Ezrin protein in HeLa cells. After 48 h of Ezrin-shRNA transfection, the changes of E-cadherin, CK-18, beta-Catenin and MMP-2 were detected by Western blot. Compared with the matched group, the expression of E-cadherin, beta-Catenin and MMP-2 decreased after Ezrin-shRNA transfection. P<0.05 was statistically significant, as shown in Figure 4.

Discussion

Cervical cancer is the second most common malignant tumor for women [1]. Many patients die of cervical cancer infiltration and metastasis, which is a serious threat to women's health [2]. At present, it is unclear about the pathogenesis of cervical cancer occurrence and metastasis. Ezrin protein is an important connectionist of cytoskeleton, and is the key protein molecule for the growth, adhesion and metastasis of tumor cell. It was found in our study that the expression of Ezrin protein was increasing in cervical cancer cell lines and tissues, which means that Ezrin protein is a key protein for the survival of cervical cancer cells and tissue [6-9]. Ezrin protein is closely related to EMT in tumor cells. Studies have shown that Ezrin gene can induce the changes of EMT after low expression, which affects the proliferation and metastasis of lung cancer cells; however, it has not been reported about its expression and role in cervical cancer [10-12]. We used RNA technology to low express Ezrin protein in HeLa cells. Firstly, we observed the changes of the proliferation viability of HeLa cell. The results showed that when Ezrin protein expression decreased, the proliferation viability of HeLa cells decreased obviously, suggesting that Ezrin protein plays an important role in maintaining the proliferation of tumor cells [13]. At the same time, we also observed the changes of EMT in HeLa cells. Our results showed that the expression of EMT related protein

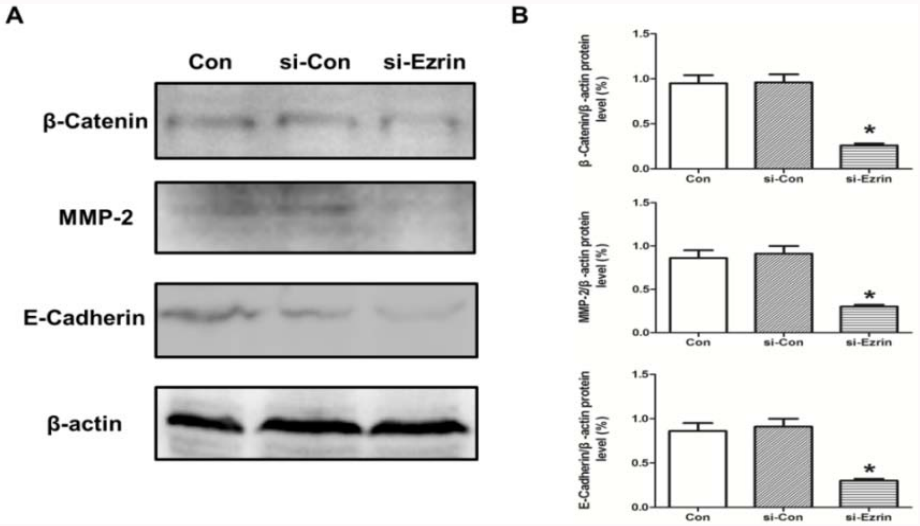

Figure 4: The changes of EMT related proteins after HeLa transfecting Ezrin-shRNA. At the end of the culture period, western blotting was performed to assess the expression of β-Catenin, MMP-2, E-Cadherin (A). The values from densitometry of β-Catenin, MMP-2, E-Cadherin were normalized to the level of β-actin protein and expressed as fold increase (B). The data were expressed as means ± SD of three independent experiments. *p<0.05, compared with control group.

E-cadherin, β-Catenin, MMP-2 in HeLa cells decreased after the low expression of Ezrin protein, which means that EMT was inhibited. Therefore, we suspect that the Ezrin protein is likely to be involved in the EMT pathological process of cervical cancer cells and it plays an important role in the occurrence and metastasis of cervical cancer. The results of our study reveal that Ezrin protein plays an import role in the pathological process of cervical cancer cell EMT. Although this result needs further clarification and demonstration, it can provide important clues and theoretical basis for the clinical treatment of cervical cancer.

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