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Original Article

Expression of nm23-H1, p53, and integrin β1 in endometriosis and their clinical significance

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Abstract: To investigate the expression and clinical significance of nucleoside diphosphate kinase A (nm23-H1), p53, and integrin β1 in endometriosis, normal and ectopic endometrial tissues were collected and the levels of nm23-H1, p53, and integrin β1 proteins were detected by western blotting. We also measured the mRNA expression of nm23-H1, p53, and integrin β1 in endometrial epithelial cells by droplet digital PCR, based on endometrial tissues using laser capture microdissection. Moreover, primary stromal cells from normal and ectopic endometrial tissues were also cultured and treated with different concentrations of estrogen. We assessed the mRNA levels of nm23-H1, p53, and integrin β1 by quantitative PCR. Compared with normal endometrial tissue, the levels of nm23-H1 and p53 proteins were significantly downregulated in ectopic endometrial tissues, while integrin β1 protein was upregulated. The same expression trend in the mRNA levels of nm23-H1, p53, and integrin β1 was also observed in both endometrial epithelial cells and stromal cells. In addition, with increasing estrogen concentration, nm23-H1 and p53 mRNA levels gradually decreased, while integrin β1 mRNA expression increased. Nm23-H1 and p53 may inhibit the progression of endometriosis, while integrin β1 has a promoting effect, and estrogen is involved in this process.

Keywords: Nm23-H1, endometriosis, p53, estrogen, integrin β1

Introduction

Endometriosis is a common benign gynecological disease [1-3] characterized by endometrial glands and interstitial cells appearing outside the uterine cavity [4, 5]. It manifests as chronic pelvic pain, dysmenorrhea, and other clinical symptoms [6, 7]. The incidence rate of endometriosis in women of childbearing age is approximately 10%, with a higher prevalence in women with infertility (~50%) [8]; however, the pathogenesis remains unclear [9, 10].

Ectopic endometrial tissue cells have strong metastatic and invasive ability [11]. Various genes involved in tumor metastasis may play an important role during this process. Evidence has confirmed that the nm23-H1 gene is an important tumor metastasis-related gene [12, 13]. On the one hand, it can activate the p53 gene and regulate the proliferation, differentiation, and apoptosis of tumor cells, thereby inhibiting the growth and metastasis of tumor cells [14, 15]. On the other hand, it also regulates the expression of integrin and further affects tumor invasion and metastasis [15, 16]. However, its relationship in endometriosis and its potential role are unclear.

In this study, we analyzed the expression of nm23-H1, p53, and integrin β1 in ectopic endometrial tissues, as well as in epithelial cells and stromal cells from endometrial tissues and observed the effects of estrogen on their expression. The results of this study help reveal the potential role of these genes in endometriosis.

Materials and methods

Human samples

This experiment was approved by the Medical Ethics Committee of Wannan Medical College Yijishan Hospital (Anhui Sheng, China) and strictly abided by the Helsinki Declaration. All tissue samples were collected from the Department of Obstetrics and Gynecology, Yijishan Hospital of Wannan Medical College, and informed consent was obtained from each

patient. Thirty patients with endometriosis were 24-48 years old at diagnosis with an average age of 39 years. No other disease was found in the patients, and no hormone therapy was used within 3 months before surgery. Patients underwent laparoscopic surgery to obtain ectopic tissue. All tissue samples were ovarian chocolate cysts. Thirty normal endometrial tissue samples were obtained from obstetrics and gynecology outpatients from the same hospital. The patients were 22-47 years old at diagnosis with an average age of 38 years. Tissue samples were obtained by diagnostic curettage. All samples were confirmed by two pathologists based on routine histology.

Western blot analysis

Total protein from each sample was extracted and the concentration was quantified. Then equal amounts (10 μg) of protein were added and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto PVDF membranes. Membranes were incubated with 5% skim milk at 4°C overnight. After washing with TBST, membranes were incubated with primary anti-nm23-H1 (#3338; Cell Signaling Technology [CST], Danvers, MA, USA), p53 antibody (#9282; CST), and integrin β1 (D6S1W) anti-rabbit mAb (#34971; CST) antibodies for 1 h at 37°C, and then washed with TBST. Membranes were hybridized with horseradish-conjugated secondary IgG antibody for 1 h and developed with DAB solution (Cat. No. P0202; Beyotime) for 30 s. The images were quantified using ImageJ software (version 1.4.5).

Detection of nm23-H1, p53, and integrin β1 levels in epithelial cells

To analyze nm23-H1, p53, and integrin β1 in epithelial cells from ectopic endometrial cells, we employed the Arcturus® Laser Capture Microdissection (LCM) System to obtain endometrial cells from normal and ectopic endometrial tissues. Then droplet digital PCR (ddPCR) was performed to detect the mRNA levels of nm23-H1, p53, and integrin β1.

Culture and identification of endometrial stromal cells

After being washed three times with D-Hank's solution, the fresh endometrial tissue was cut into small pieces of 1-2 mm^3 and treated with 2 mg/mL collagenase for 30 min. After centrifugation of the digested single cell suspension for 700 rpm for 3 min, the supernatant was discarded, and the cell pellet was re-suspended in 1 mL DMEM/F12 (1:1) medium containing 10% fetal bovine serum. Then the cell suspension was filtered through a 40 μm sieve, and the filtered stromal cell suspension was cultured in a 6-well plate. Subsequently, stromal cells were identified by immunohistochemistry using vimentin monoclonal antibody (#XY-07-56R; Abcam, Cambridge, MA, USA).

Quantitative real-time polymerase chain reaction

Total RNA from the primary cultured normal and ectopic endometrial stromal cells was extracted and the concentration was detected by enzyme labelling. Total RNA was reverse transcribed into cDNA. Then quantitative PCR (qPCR) was used to analyze the expression levels of nm23-H1, p53, and integrin β1 in normal and ectopic endometrial stromal cells.

Effects of estrogen on the mRNA expression of nm23-H1, p53, and integrin β1

To investigate the effects of estrogen on the expression of nm23-H1, p53, and integrin β1 in stromal cells, we treated stromal cells cultured for 24 h with 0 mmol/L, 10^{-8} mmol/L, and 10^{-4} mmol/L estrogen. Cells were further cultured for 72 h, and total RNA was extracted according to the above-mentioned method, after which the mRNA levels of nm23-H1, p53, and integrin β1 were examined.

Results

Changes in the expression of nm23-H1, p53, and integrin β1 proteins in endometriotic tissues

To analyze the expression of nm23-H1, p53, and integrin β1 in endometriotic tissues, we analyzed their expression levels by western blotting. The results are shown in **Figure 1**. Compared with normal endometrial tissue, the expression levels of nm23-H1 and p53 in endometriotic tissues were significantly decreased (all $P < 0.01$, **Figure 1A**, **1B** and **1D**); however, the expression level of integrin β1 was significantly increased ($P < 0.01$; **Figure 1A** and **1C**).

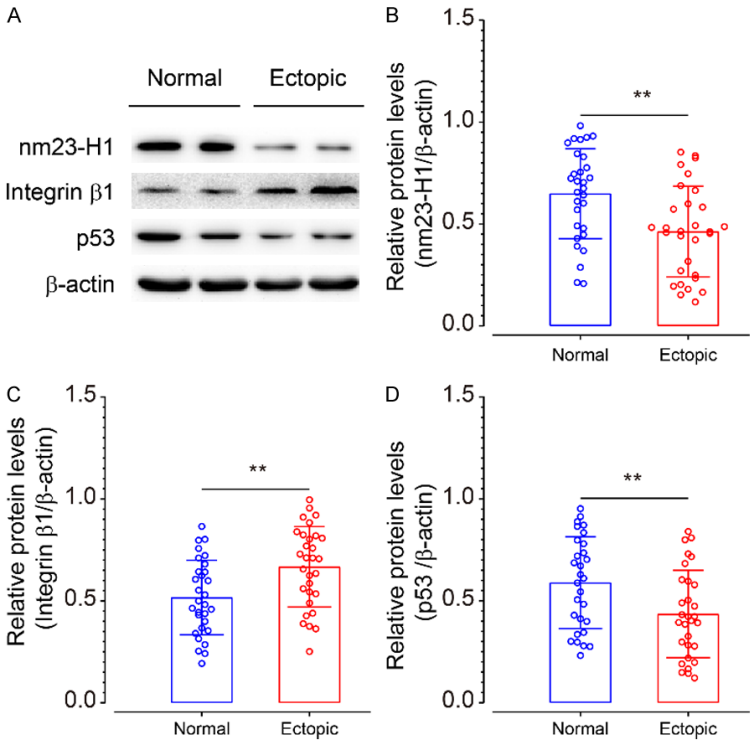

Figure 1. Expression of nm23-H1, p53, and integrin β1 proteins in normal and ectopic endometrial tissues. **P < 0.01.

Expression of nm23-H1, p53, and integrin β1 mRNA in endometrial epithelial cells

To accurately obtain epithelial cells in endometrial tissue, we used modified hematoxylin and eosin staining to determine whether the obtained cells were epithelial cells. The results showed that the LCM instrument successfully captured the endometrial epithelial cells (**Figure 2A**). The ddPCR results showed that the mRNA levels of nm23-H1 and p53 mRNAs were significantly downregulated in ectopic endometrial epithelial cells compared with normal endometrial epithelial cells (all P < 0.01; **Figure 2B** and **2C**), whereas mRNA levels of integrin β1 were significantly increased (P < 0.01; **Figure 2B** and **2C**).

Expression levels of nm23-H1, p53, and integrin β1 mRNAs in endometrial tissue stromal cells

First, we identified the stromal cells obtained by immunohistochemistry. The results showed that endometrial stromal cells were successfully isolated and cultured with a purity greater than 95% (**Figure 3A**). The results of qPCR showed that the mRNA expression of nm23-H1 and p53 in ectopic endometrial stromal cells was significantly downregulated compared with the normal endometrial stromal cells, but the expression of integrin β1 mRNA was significantly upregulated (**Figure 3B**).

In addition, in estrogen-treated ectopic endometrial stromal cells, estrogen inhibited the mRNA expression levels of nm23-H1 and p53, and their expression was gradually downregulated with increasing estrogen concentration, whereas the level of integrin β1 was elevated and estrogen-dependent (**Figure 3C**).

Discussion

Endometriosis is an estrogen-dependent chronic inflammatory disease [17]. Endometriosis causes severe physical and mental illness in women of childbearing age due to chronic pain [18]. Surgical resection is a routine treatment for endometriosis. However, its pathogenesis is still unclear, leading to its high incidence and recurrence rate [19]. Therefore, knowing its pathogenesis and mining key molecules can help prevent the occurrence and development of the disease.

Numerous studies have confirmed that nm23-H1 not only has nucleoside diphosphate kinase and 3'-5' exonuclease activity but also plays an important role in DNA synthesis, replication, and repair [20]. In addition, nm23-H1 is downregulated in some malignant cells and was the first identified tumor metastasis-suppressor gene [21]. Additional studies [22] found that nm23-H1 interacts directly with p53, an important tumor suppressor gene [23], thereby regulating the function of p53 [24]. Our results indicate that nm23-H1 and p53 gene expression levels were lower in the endometriosis group than in the normal control group. We hypothesized that the decrease in p53 gene expression may be regulated by nm23-H1, but additional studies are needed to determine the detailed mechanism.

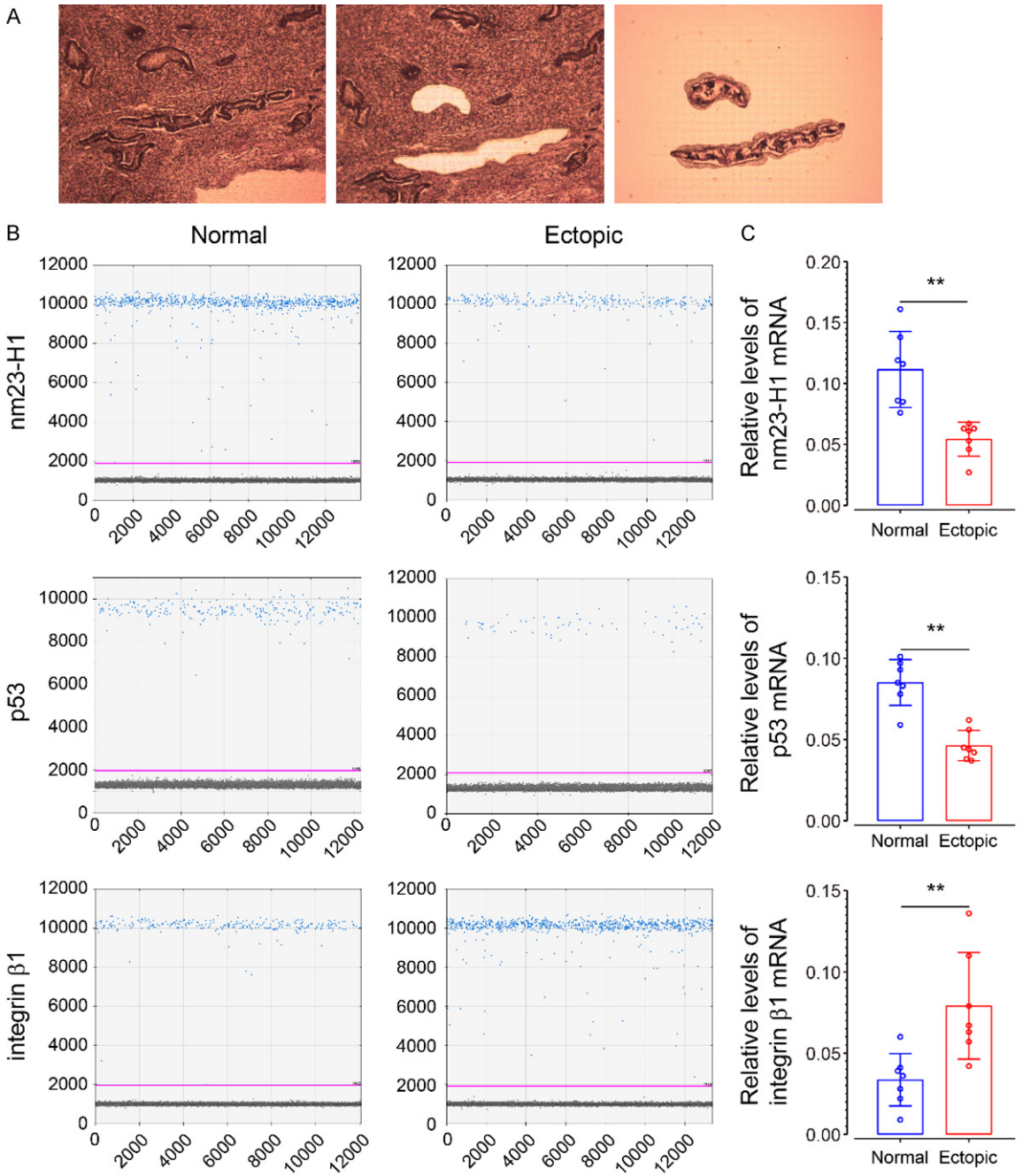

Figure 2. Change in mRNA levels of nm23-H1, p53, and integrin β1 in endometrial epithelial cells obtained by LCM. A. Epithelial cells were obtained by LCM (hematoxylin and eosin staining, 100×) (left panel). Representative section before dissection by LCM (middle panel); Representative section after dissection by LCM (right panel); Epithelial cells were obtained by LCM. B. Representative image of ddPCR. C. Relative levels of nm23-H1, p53, and integrin β1 mRNAs in endometrial epithelium. **P < 0.01.

As a member of the integrin family, integrin β1 is involved in the regulation of pathophysiological processes such as cell adhesion and migration [25], but the role of integrin β1 in endometriosis has been less studied. Our results showed that integrin β1 is highly expressed in ectopic endometrial tissues, suggesting that it may be involved in the occurrence of endometriosis. In addition, it is negatively correlated with nm23-H1 and p53. These results suggest that nm23-H1 may also affect the adhesion and invasiveness of endometrial cells by regulating the expression of integrin β1 in endometriosis.

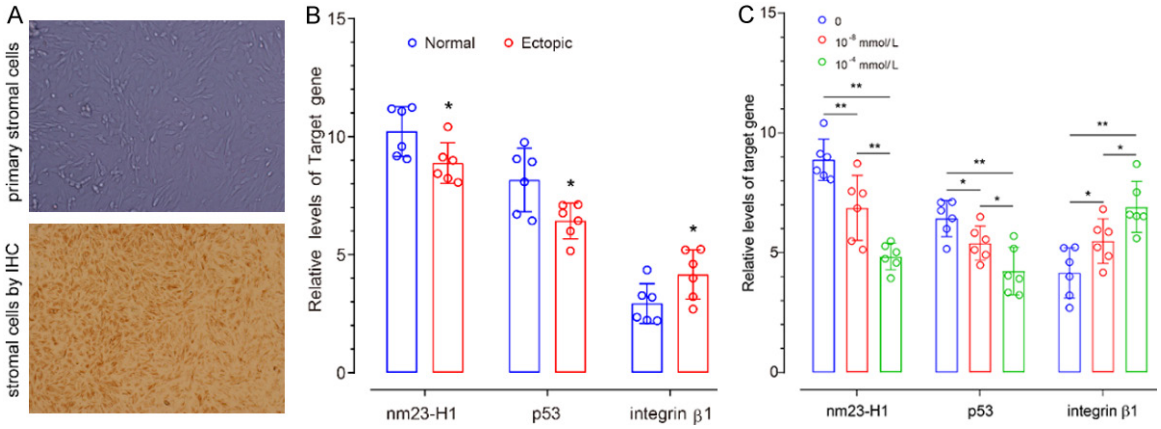

Figure 3. Change in mRNA levels of nm23-H1, p53, and integrin β1 in stromal cells. A. Culture of stromal cells derived from the endometrium (upper panel) Representative picture of stromal cells under light microscopy (lower panel); Representative image of stromal cells derived from the endometrium by immunohistochemistry. B. Relative mRNA levels of nm23-H1, p53, and integrin β1 in normal and ectopic endometrial stromal cells. C. Effects of estrogen on the mRNA expression of nm23-H1, p53, and integrin β1 in ectopic endometrial stromal cells. **P < 0.01; *P < 0.05.

Estrogen is a driving factor in maintaining the normal menstrual cycle [26], and its secretion disorder (especially abnormal increase) is a predisposing factor for endometriosis [27]. A previous study [28] showed that estrogen stimulates endometrial ectopic tissue-derived stromal cells to downregulate the expression of nm23-H1, which is consistent with our results. Our results showed that estrogen inhibited the expression of nm23-H1 in ectopic endometrial stromal cells and was negatively correlated with dose dependence; and p53 had the same expression trend. However, estrogen promoted the expression of integrin β1 in a dose-dependent manner. The detailed mechanism is still unclear and needs further study.

In summary, nm23-H1 and p53 were downregulated in ectopic endometrial tissues compared with normal endometrial tissues. In addition, estrogen inhibited their expression in ectopic endometrial stromal cells. Integrin β1 showed the inverse trend, and this was estrogen-dependent.

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Disclosure of conflict of interest

None.

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