Int J Clin Exp Pathol 2016;9(11):11499-11504
www.ijcep.com /ISSN:1936-2625/IJCEP0025930

Original Article

The expression of macrophage migration inhibitory factor in adenomyosis

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Received February 13, 2016; Accepted August 2, 2016; Epub November 1, 2016; Published November 15, 2016

Abstract: The aim of the present study was to investigate whether the expression of macrophage migration inhibitory factor (MIF) is altered in eutopic endometrium and ectopic lesion of women with adenomyosis. MIF expression was assessed by immunohistochemistry, western blotting analysis. Elevated expression of MIF protein was seen in eutopic endometrium and ectopic lesion of women with adenomyosis ($P<0.05$). There was a positive correlation between MIF protein expression and dysmenorrhea in women with adenomyosis ($P<0.05$). The study showed a significant increase of MIF expression in eutopic endometrium and ectopic lesion of women with adenomyosis, a relationship between MIF protein expression and dysmenorrhea. Those results suggest that MIF may play a role in the pathogenesis and progress of adenomyosis.

Keywords: Macrophage migration inhibitory factor, adenomyosis

Introduction

Adenomyosis is mainly caused by the invasion of the endometrial glands and mesenchyme to the myometrium, resulting in a diffusely enlarged uterus which microscopically exhibits ectopic non-neoplastic, endometrial glands and stroma surrounded by the hypertrophic and hyperplastic myometrium [1]. Uterine adenomyosis is relatively frequent, affecting 1% of females; its diagnosis is more often made in multiparous patients. Adenomyosis related uterus enlargements cause pelvic pain, dysmenorrhea and equally disturbs patients' micturition and defecation, thereby severely affecting patient's quality of life [2]. Although adenomyosis may be treated with several methods, such as hysterectomy, conservative surgery, drug therapy such as gonadotropin-releasing hormone agonist therapy (GnRHa), and uterine artery embolization, complete hysterectomy can thoroughly treat this disease. The ontogeny of adenomyosis is clearly important for the development of new alternatives to hysterectomy.

Recently, abnormal stromal cell invasion has been proposed in the etiology of adenomyosis [3], but the features in the microenvironment that regulate myometrial penetration by the overlying endometrium and the changes that trigger the development of uterine adenomyosis remain unclear. Macrophages and some cytokines derived from macrophages are suggested to be in contribution with the development of endometriosis and adenomyosis by promoting neovascularization, inflammation and attachment of endometrial cells [4]. Macrophage migration inhibitory factor suppressed the migration of macrophages and subsequently as a factor regulating macrophage host-defence functions [5, 6]. Forty years ago, MIF was firstly proposed by Bloom BR in a study assessing delayed hypersensitivity [5]. It is considered the first cytokine found in human, and is nick-named as IL-0 by some researchers. It is now known to be widely expressed in various cell types, including activated T cells, macrophages and anterior pituitary gland cells, etc [7-9]. However, whether MIF is involved in the pathology of adenomyosis has yet not been explored.

The aims of the present study were therefore to investigate whether there was a difference of MIF expression in adenomyosis, and to exam-

ine whether MIF expression in adenomyosis was associated with dysmenorrhea.

Materials and methods

Tissue sample colection

The ethical approval for the present study was obtained by the Ethics Committee of School of Medicine, Zhejiang University (Hangzhou, China). Written informed consent was obtained from all the patients prior to tissue collection. Endometrial tissue samples were collected at the time of surgery. The diagnosis of adenomyosis was confirmed by histological examination. Normal endometria were obtained from 21 women of reproductive age undergoing bilateral tubal ligation. Adenomyotic lesions were obtained from 23 patients with adenomyosis undergoing hysterectomy or subtotal hysterectomy. All of them had normal menstrual cycles (28-32 days) and had not received any anti-inflammatory or hormonal treatment for at least 6 months before inclusion in the study and surgery. Information about dysmenorrhea was taken from the patients' clinical records. Measuring pain intensity was performed using the standard visual analogue scale (VAS). The patients were taught to record their pain intensity from 1 to 10. For all the patients in the study, samples of eutopic endometrium were collected using a soft curette (Pipelle-de-Cornier, Laboratoire CCD, France). After the samples were collected, the endometrial tissues were either snap-frozen in liquid nitrogen and stored at -80°C for protein extraction, or fixed for 24 h in 4% paraformaldehyde for pathological examination and embedded in paraffin for immunohistochemical analysis.

Immunohistochemistry

Immunohistochemical analyses were performed on 3 cm tissue sections using specific antibodies. The anti-FAK antibody used in the present study was a rabbit polyclonal antibody of mouse origin, raised against amino acids 903-1052 of FAK (cat. no. sc-932; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Slides incubated with a rabbit immunoglobulin (IgG) antibody at the same dilution as the primary antibody were used as negative controls. Following three washes with PBS for 5 min each, slides were incubated with anti-IgG horseradish peroxidase (HRP)-conjugated secondary antibody (cat. no. P0488; Dako Cytomation, Inc., Carpinteria, CA, USA) for 30 min. After a further wash, the sections were treated with diaminobenzidine (Dako Cytomation, Inc.), counterstained with hematoxylin (Sigma-Aldrich, St. Louis, MO, USA), dehydrated and mounted in DPX mounting medium (Merck Millipore, Darmstadt, Germany). The experiments were repeated three times.

Western blotting analysis

After centrifuge at 13,000 × g for 10 min, the supernatant of lysates was denatured, loaded into the wells of the 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. The proteins were separated by electrophoresis, transferred to nitrocellulose membranes (Millipore), blocked with 5% nonfast milk in Tris buffered saline (TBS) buffer with 0.05% Tween-20 for 1 hour at room temperature, and then probed with mouse anti-MIF antibody (sc-20121; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and and β-actin (dilution, 1:400; sc-47778; Santa Cruz Biotechnology, Inc.) at 4°C overnight. After incubation for 2 hours with horseradish-peroxidase (HRP)-conjugated rabbit anti-mouse secondary antibody (1:10,000; P0488; Dako Cytomation, Inc., Carpinteria, CA, USA), bands were visualized by ECL plus western blot detection reagent (GE, USA). The house-keeping gene GAPDH was used as a loading control and detected by using mouse anti-GAPDH antibody (1:4000, abmart, China) plus HRP-conjugated goat anti-mouse secondary antibody (1:5000, Santa Cruz Biotechnology, USA).

Statistical analysis

All data were normally distributed and the results are expressed as the means ± standard deviation. Statistical analysis of the MIF to β-actin ratios was performed by one-way analysis of variance using SPSS software, version 11.5 (SPSS, Inc., Chicago, IL, USA). Linear regression was used to analyze the correlation between MIF protein expression and the score of dysmenorrhea. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Immunohistochemistry

MIF immunoreactive staining was observed in the cytoplasm of epithelial and stromal cells in

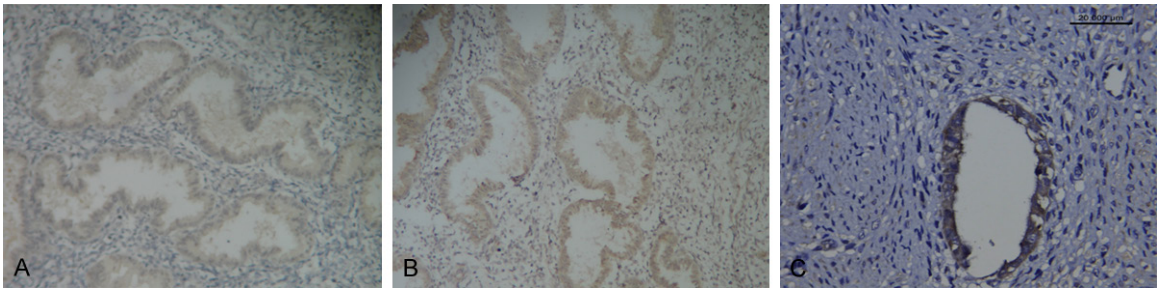

Figure 1. Immunohistochemical staining for MIF Original magnification 200×. A: Endometrium of women without adenomyosis. B: Endometrium of women with adenomyosis. C: Ectopic lesion of women with adenomyosis.

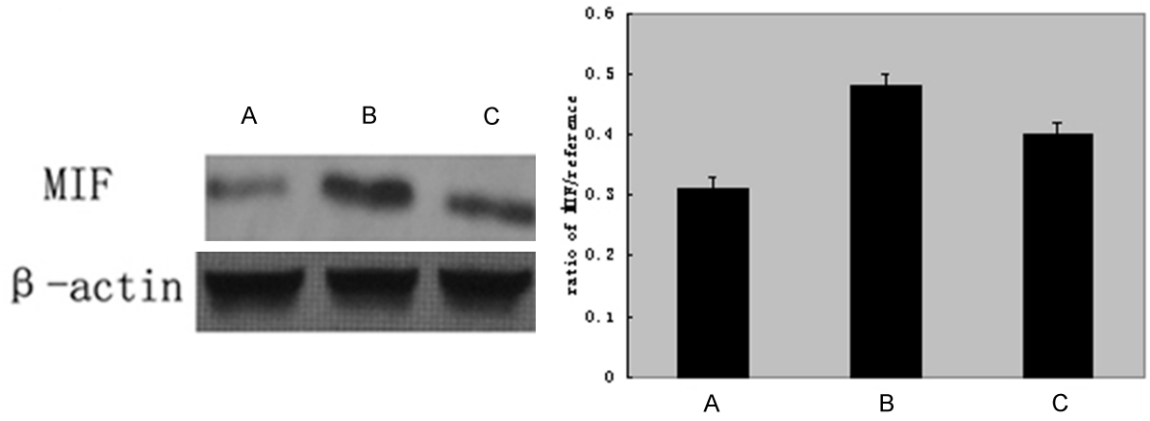

Figure 2. MIF protein expression in endometrial tissues was assessed by Western blotting (**Figure 2**). Lane A: Eutopic endometrium from women without adenomyosis. B: Eutopic endometrium from adenomyosis. C: Ectopic lesion of women with adenomyosis. Normalized density was analyzed using the internal β-actin as reference (means ± SD).

endometrium (**Figure 1A**) in control group and adenomyosis (**Figure 1B**). MIF expression was also positive in ectopic lesion in adenomyosis (**Figure 1C**).

Western blotting analysis

The anti-MIF antibody detected a band in protein extracts from all tissues (**Figure 2**). After normalizing each band of MIF protein with β-actin from different samples, MIF expression in eutopic endometrial of adenomyosis (0.48± 0.09) was significantly higher than that in eutopic endometiurm of control group (0.31± 0.10) ($P<0.01$). And MIF expression in ectopic lesion of adenomyosis (0.40±0.05) was also higher than that in eutopic endometiurm of control group (0.31±0.10) ($P<0.05$) (**Figure 2**).

Correlation of MIF expression and dysmenorrhea

A positive correlation was observed between MIF protein expression in eutopic endometrial tissues and dysmenorrhea ($r^2=0.135$, $P=0.006$; **Figure 3**) in patients with adenomyosis. And a positive correlation was also observed between MIF protein expression in ectopic lesion and dysmenorrhea ($r^2=0.125$, $P=0.019$; **Figure 4**) in patients with adenomyosis.

Discussion

MIF is a non-glycosylated 12.5-kDa protein composed of 114 amino acids. Crystal structures demonstrate that MIF is a homotrimer with structural homology to three bacterial enzymes; oxalocrotonate tautomerase, 5-carboxymethyl-2-hydroxymuconate isomerase, and chorismate mutase [10-14]. It was first described as a soluble factor responsible for the inhibition of emigration of macrophages during DTH in 1966 [5]. MIF is highly expressed in various tumors, including lung [15], liver [16], breast [17], gastric [18], colon [19], and prostate [20] cancers, and overexpression of MIF stimulates proliferation and inhibits apoptosis

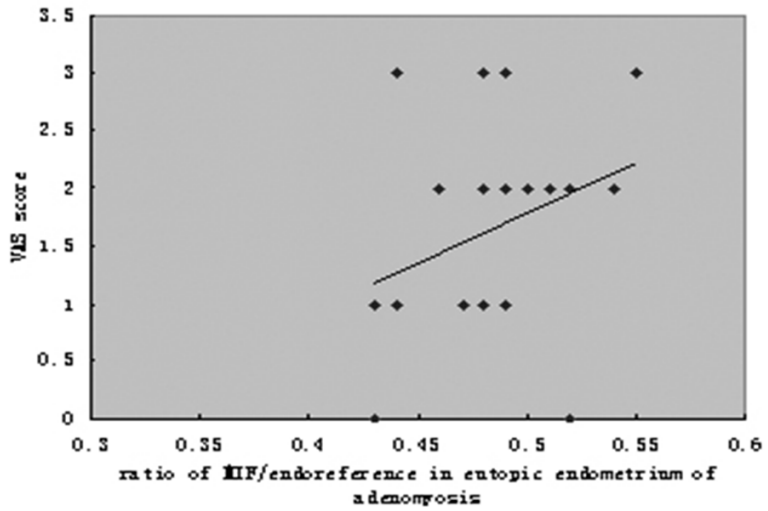

Figure 3. There was a positive correlation between the normalized densities of endometrial MIF proteins and pain intensity score of dysmenorrhea (n=23, r^2=0.135, P=0.006).

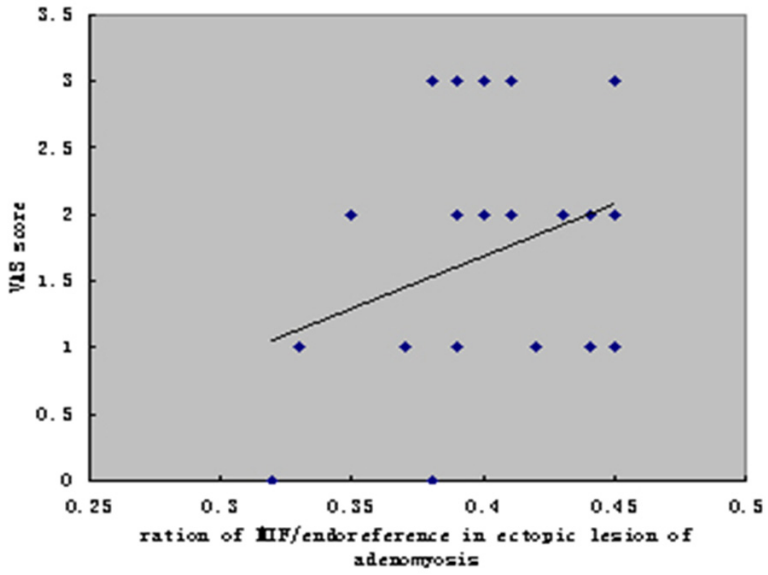

Figure 4. There was a positive correlation between the normalized densities of eMIF proteins in ectopic lesion and pain intensity score of dysmenorrhea (n=23, r^2=0.125, P=0.019).

in cancer cells via the paracrine pathway [21, 22]. These findings suggest that MIF plays an essential part in the development of chronic inflammation and cancer, and possibly in carcinogenesis related to chronic inflammation.

In our study, an elevation of MIF expression was observed in the eutopic and ectopic endometrium of patients with adenomyosis and an association was identified between MIF protein expression in adenomyosis and the scores of dysmenorrhea. These results provide further evidence that the eutopic endometrium of patients with adenomyosis is aberrant [23]. Our findings support two models for the role of MIF in the transformation of the eutopic endometrial cells of endometriosis. Firstly, Those local traumas at the endometrial-myometrial interface caused by a direct trauma (birth, cesarean section, curettage) lead activation of the mechanism of 'tissue injury and repair' (TIAR) [23]. Macrophages and some cytokines derived from macrophages are suggested to be in contribution with the development of adenomyosis by promoting eovascularization, inflammation and attachment of endometrial cells [24]. MIF, as termed can inhibit the aggregating of macrophage, could effect the progress of trauma repair, which will be helpful to the downgrowth and invagination of the basalis endometrium into the myometrium. Secondly, MIF has been shown to modulate p53 protein stability [25-27], effect migration in tumor cells [28]. MIF phosphorylates ERK1/2 and activates cPLA2 and cyclooxygenase-2 (Cox-2), blocking p53-induced apoptosis. Higher expression of MIF may be helpful to the invasion and metastasis of endometrial cells in adenomyosis. Therefore, it's very likely that MIF plays a role in the pathogenesis adenomyosis.

But we found the expression of MIF protein in ectopic lesion of adenomyosis was associated with the vas scores of dysmenorrhea. Previous research had found that MIF overexpressed in malignant human NSCLC cell lines was necessary for the maximal expression and secretion of vascular-endothelial cell growth

factor (VEGF) and IL-8 [29]. Elevated levels of MIF also result in reduced p53 accumulation in the cytoplasm, thereby blocking p53-mediated cell death and leading to continuous production of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and prostaglandins [30-32]. Those factors could induce the secretion of NGF, which will enhance the symphtom of adenomyosis.

In conclusion, our study showed for the first time a significant increase of MIF expression in the secretory endometrial tissues of women with endometriosis as well as a relationship between FAK expression and disease stage, pelvic pain, and serum steroid hormones. These findings suggest that MIF may contribute to the pathogenesis of endometriosis and be regulated by steroid hormones. Further investigation of MIF as a clinical marker and as a therapeutic target appears to be warranted.

Acknowledgements

This study was supported by a grant from the National Natural Science Foundation of China (grant number: 81100407).

Disclosure of conflict of interest

None.

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