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Research Paper

Investigation of Matrix Metalloproteinase -1, 2 and tissue inhibitor of matrix metalloproteinases-1 in Endometriosis

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Abstract

Objective: To explore the role of matrix metalloproteinase-1,2 (MMP-1, MMP-2) and tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) in endometriosis. **Methods:** The eutopic and ectopic endometria from 40 subjects suffering from endometriosis and regular endometria from 40 subjects (excluding endometriosis) were collected and examined by in situ hybridization technology and western blot assay. **Results:** Both expressions of MMP-1 and -2 were stronger in ectopic endometrium and eutopic endometrium than in normal endometrium. On the contrary, the expression of TIMP-1 in ectopic endometrium and eutopic endometrium was lower. The differences were significant ($P < 0.01$). Moreover, there was no relationship among the expressions of MMP-1, 2 and TIMP-1 in ectopic endometrium. **Conclusion:** The expressions of MMP-1, 2 and TIMP-1 lose balance and lack of periodic changes in ectopic endometrium , which explains the biological invasive behavior of endometriosis. It was suggested that regulating the balance between the MMPs and TIMP-1 should be an ideal therapeutic target to endometriosis.

Keywords: endometriosis; MMP-1; MMP-2; TIMP-1; in-situ hybridization; Western blot

INTRODUCTION

Endometriosis, a common disorder in gynecological practice, occurs in about 10% of women in reproductive age and up to 50% of women with infertility [1]. Despite benign development, this disorder has features of hyperplasia, infiltration and implantation metastasis like malignant tumor. Therefore, the role tumor associated factors which act in endometriosis, are issues requiring examination. Matrix metalloproteinases (MMPs) and tissue inhibitor of matrix metalloproteinases (TIMPs),which are the dominant enzymes to regulate synthesis and degradation of extracelluarmatrix (ECM), play a role in generation and proceeding of tumor [2]. This study serves to explore the association of MMP-1,-2 and TIMP-1 with endometriosis through detecting their genic and proteinic expressions in normal and abnormal endometrium, including ectopic and eutopic.

MATERIALS AND METHDODS

Patients and group dividing

All 80 patients were selected from the department of gynecology in Tongji Hospital from January to December in 2005. In those subjects, 40 ones with as average age of 36.5 ± 5.5 years, were victims by ovary-type endometriosis and were obliged to be operated; the others with average age of 37.5 ± 4.7 years, whose menstrual cycle was regular, suffering from intramural myoma and accepting uterectomy, were selected as control. In each group, half the subjects' endometria were in the proliferative phase and the others' were in secretory phase. All subjects had no other disease and were not treated by hormone or any drugs against endometriosis within 3 months preoperatively.

Hybridization in situ of the MMP-1,MMP-2

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and TIMP-1 mRNA expression

The endometria and dystopic endomembrane were sampled from the subjects with endometriosis, and the endometrial also were taken from controls for contrast. When the endometria were collected, the specimens were fixed by 4% paraformaldehyde including 0.1% diethyl pyrocarbonate(DEPC) and then processed by dehydrating, soaking with wax, embedding and eventually cutting into slices with 6 micrometer as thickness. In situ hybridization technology was employed to detect the mRNA on MMP-1, MMP-2 and TIMP-1. The digoxin-biotin labeled cDNA probe was produced by Genemed Synthesis Company and the related kit was applied by Maxin Biotech Company. The experimental process followed the instruction on the kit. The slices were analyzed by color image analysator made of Wei Niyand Company.

Identification of results in situ hybridization

The positive protein expression was judged by brownish yellow or sepia particles presented. A double-blind method and auto color assessment apparatus(microphotograph instrument of OLYMOUS BX50, digital camera of POLAROIDMC, plus HPIAS2000 image analysis software of TongJi QianPing Image Project Company) were adopted to analyze the stained results. Every slide was placed in the light in same intensity and three visual fields were picked up randomly to analyze. The mean optical density was looked as the expressional intensity of MMP-1, 2 and TIMP-1. All analysis processes were administered by the same person with same apparatus.

Western blot analysis

Sample tissues were stored in nitrogen canister after obtained. Those tissues were lysed by tissue lysate, total protein was extracted, and then was examined by protein quantitative kit(Bio-Rad) for protein concentration. 100 μg protein was taken from each sample and separated on 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% nonfat milk in PBS-Tween (0.1%) for 1 h and then incubated with monoclonal antibody for target protein. The sample protein was placed at 4℃ over night and then incubated with the secandary antibody for 2 h. At last, DAB coloration, picturing and scanning were performed and the optical density was analyzed by ZEISS automatic image analyzer. Relative value of protein expression on MMP-1,2 and TIMP-1 was measured as A value. Ratio of A values from ectopic or eutopic endometrium contrasted to nomal endometrium could elucidate the differences of protein expression. For example, when the ratio of A values was over 1, protein expression was noted as positive, while when the ratio was lower than 1, protein expression was noted as negative.

Statistical analysis

All data was noted as mean ± SD and performed by one-way ANOVA test. The correlation between different groups was analyzed by Spearman test. Whole data were dealed by SPSS 12.0 software.

RESULTS

Expression of mRNA on MMP-1, MMP-2 and TIMP-1 in different groups

The positive particles of MMP-1,2 and TIMP-1 were yellow brownish or sepia in cytoplasm, not in nucleus, of the glandular epithelium and of interstitial cells from endometrium (***Fig 1-3***). The expressions of MMP-1,2 in ectopic endometrium were significantly higher than that in eutopic or nomal endometrium ($P < 0.01$,or $P < 0.05$), while that of TIMP-1 was lower ($P < 0.01$), as shown in ***Tab 1***.

Expression of mRNA on MMP-1,2 and TIMP-1 in different phase of different endometrium

The expressions of MMP-1,2 in ectopic or eutopic endometrium were evidently higher than that in normal endometrium whatever in proliferative or in secretion phase ($P < 0.01$,or $P < 0.05$), while that of TIMP-1 was contrary ($P < 0.01$). In addition, expressions of MMP-1,2 in ectopic endometrium were higher than that in eutopic endometrium,but this feature presented only in proliferative phase. Furthermore, whatever in ectopic, eutopic or control endometrium, there were no significance between the proliferative phase and secretion phase ($P > 0.05$) on expressions of MMP-1, 2, as shown in ***Tab 2***.

Tab 1 **Expressions of mRNA on MMP-1, 2 and TIMP-1 in different groups** ($\bar{x} \pm s$)

Groups	Number	MMP-1	MMP-2	TIMP-1
Ectopic	40	$0.382 \pm 0.012^{*\Delta}$	$0.378 \pm 0.011^{*\Delta}$	$0.234 \pm 0.018^{*}$
Eutopic	40	$0.331 \pm 0.014^{*}$	$0.317 \pm 0.017^{*}$	0.251 ± 0.013
Normal	40	0.256 ± 0.015	0.263 ± 0.010	0.266 ± 0.010

Compared to the control, $^{*}P < 0.01$; compared to the eutopic endometrium, $^{\Delta}P < 0.05$.

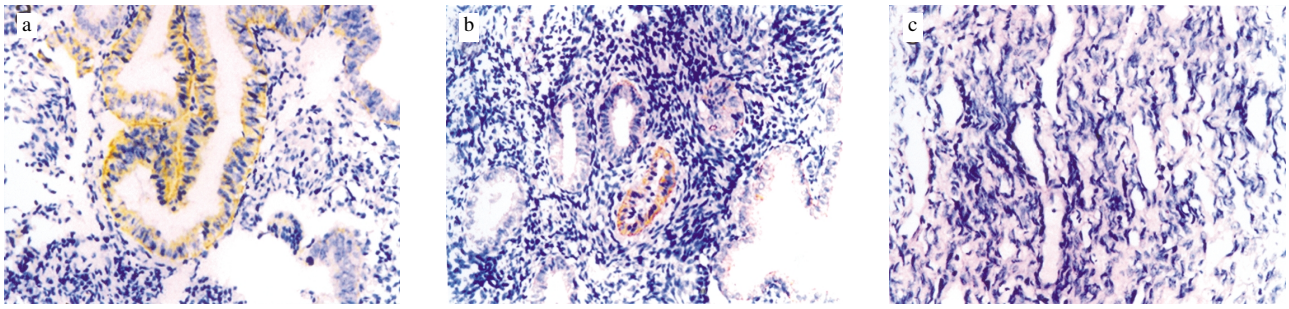


Fig 1 The expression of mRNA on MMP-1 (a), MMP-2 (b) and TIMP-1(c) in ectopic endometrium with hybridization in situ method. MMP-1,-2 mRNA were maldistribution and mainly in glandular epithelial cells. The expression of MMP-1,2 mRNA were strongly positive. Positive particles were obviously concentrated in the whole cell and showed buffy or brown. The expression of TIMP-1 decreased significantly and was nonspecific. (in situ hybridization, × 200)

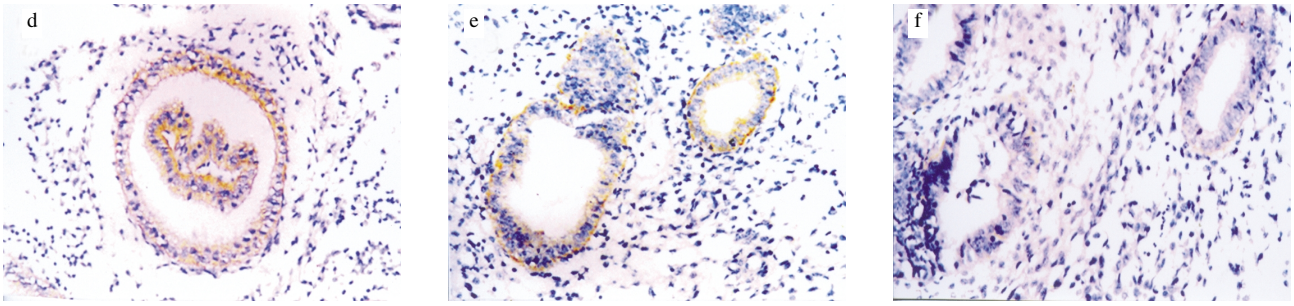


Fig 2 The expression of mRNA on MMP-1 (d), MMP-2 (e) and TIMP-1(f) in eutopic endometrium with hybridization in situ method. Their expression intensity were weakly positive or moderately positive. (in situ hybridization, × 200)

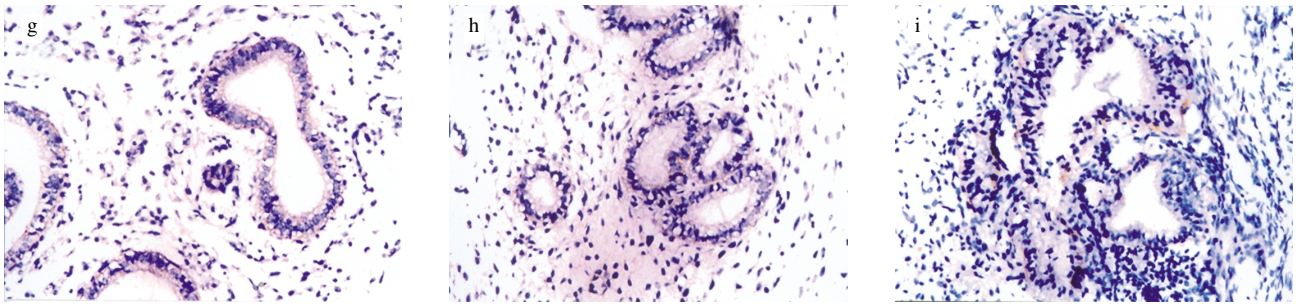


Fig 3 The expression of mRNA on MMP-1 (g), MMP-2 (h) and TIMP-1(i) in control with hybridization in situ method. The protein expressions were in glandular epithelial cells and interstitial cells of endometrium. Their expression intensity were weakly positive or moderately positive. (in situ hybridization, × 200)

Tab 2 **Expressions of mRNA on MMP-1, 2 and TIMP-1 in different phases of different endometriums** ($\bar{x} \pm s$)

Groups	Proliferative Phase			Secretory Phase		
	MMP-1	MMP-2	TIMP-1	MMP-1	MMP-2	TIMP-1
Ectopic endo.	$0.385 \pm 0.034^{*\Delta}$	$0.381 \pm 0.072^{*\Delta}$	$0.240 \pm 0.015^{*}$	$0.376 \pm 0.032^{*}$	$0.378 \pm 0.053^{*}$	$0.221 \pm 0.029^{*}$
Eutopic endo.	$0.330 \pm 0.032^{*}$	$0.322 \pm 0.067^{*}$	0.259 ± 0.023	$0.341 \pm 0.021^{*}$	$0.353 \pm 0.037^{*}$	0.246 ± 0.011
Normal endo	0.271 ± 0.019	0.258 ± 0.039	0.268 ± 0.130	0.269 ± 0.027	0.237 ± 0.063	0.267 ± 0.020

Compared to the control, $^{*}P < 0.01$; compared to the eutopic endometrium, $^{\Delta}P < 0.05$.

Correlation between expression of MMP-1, MMP-2 and TIMP-1 mRNA in different groups

In normal endometrium, the expression of mRNA on TIMP-1 had negative correlation with that on MMP-1, 2 ($P < 0.01$), but that correlation did not exist in ectopic or eutopic endometrium ($P > 0.05$), as shown in ***Tab 3***.

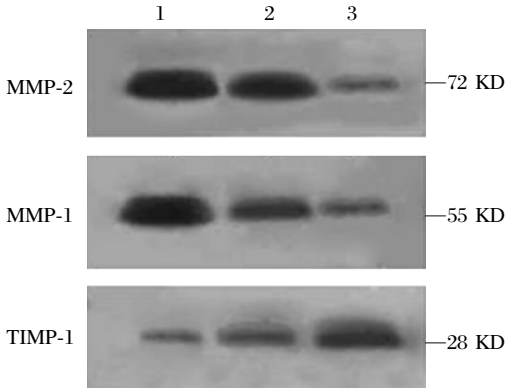
Expressions of MMP-1, 2 and TIMP-1 protein in different groups

The positive rates of protein expression on MMP-1, MMP-2 and TIMP-1 were 87.5%(35/40), 82.5%(33/40), 10.0%(4/40) in ectopic endometrium and

Tab 3 **Correlation between expression of mRNA on MMP-1, MMP-2 and TIMP-1 in different groups**

Different object	TIMP-1		
	Ectopic endo.	Eutopic endo.	Normal endo.
MMP-1	R = 0.067	R = 0.059	R = −0.932
	P = 0.764	*P* = 0.719	*P* = 0.000
MMP-2	R = 0.024	R = 0.021	R = −0.938
	P = 0.925	*P* = 0.897	*P* = 0.000

67.5% (27/40), 60.0% (24/40), 30.0% (12/40) in eutopic endometrium, while 10.0% (4/40), 15.0% (6/40), 82.5% (33/40) in control, respectively. The protein expression on MMP-1, MMP-2 in ectopic endometrium was significantly higher than that in eutopic and control endometrium, while the expression of TIMP-1 was lower than that in ectopic and control endometrium, which was in accordance with the results of mRNA expression (***Fig 4***).



1: ectopic endometrium, 2: eutopic endometrium, 3: control endometrium.

Fig 4 Expression of protein on MMP-1, MMP-2 and TIMP-1 in different groups

DISCUSSION

Matrix metalloproteinases (MMPs) belonging to the family of Zn^{+} dependent protease have the ability to degrade collagen, fibronectin, laminin and mucoprotein which are the main constituents in extracellular matrix (ECM)[3], MMPs participate in this remodeling of basement membranes and ECM [4]. 18 members of the MMP family have been described[5]. Because of their key roles in matrix degradation, activated members of the MMP family are involved in many physiological processes such as blastocyst implantation and trophoblast invasion[6]. In contrast, excessive or inappropriate expression of MMPs contributes to tissue-destructive diseases including arthritis, multiple sclerosis, atherosclerosis, gastric ulcers, fibrotic lung disease, and tumor progression [7]. In normal homeostatic tissue MMPs rarely express, but increase when they are stimulated by proinflammatory cytokines, hormones, growth factors and so on. MMPs play a important role in many physiological and pathological progress, such as embryonic development, angiogenesis, involution of uterus, tissue repair and remodeling, inflammation, tumorous invasion and metastasis[8]. MMP-1 (whose substrate is reticular fiber) is a main member in MMPs family and is the essential barrier for cellular invasion. MMP-2 is a gelatinase with the largest molecular weight in MMPs family and its hydrolysates include degenerative collagen, Ⅳ and Ⅴ collagen, which participate to compose basal lamina of ECM, fibronectin and laminin belonging to gly coprotein. TIMPs is the chief essential to suppress MMPs through inhibiting not only activated MMPs but also interaction of MMPs and related enzymes[9]. It is considered that the metabolism of ECM can be carried out when the ratio of MMP and TIMP approaches to one [10]. MMPs, recently, have been implicated in the invasion and development of adenomyosis. Matrix metalloproteinase inhibitor may suppress the invasiveness of stromal cells with high ECM-degrading activity in mouse adenomyotic tissues[11, 12].

Adenomyosis is characterized by an abnormal growth of stroma and glands into and beyond the myometrial layers of which the pathological change is certainly associated with the degradation and reconstruction of the ECM. In normal uterus, the intrusion of endometrial cells is blocked by the musculature consisting of smooth muscle cell layers with ECM. In general, it is known that the degradation and reconstruction of the ECM be related to the expression of MMPs. Some study revealed that the stromal cells of adenomyotic uterus especially invade gels containing type IV collagen, and did not invade those consisting of other types of collagen [13]. Type IV collagen, which presents in the basement membrane of blood vessels in human and animals [14], is degraded by gelatinase A (MMP-2) and gelatinase B (MMP-9). Endometrial expression of the MMP family is normally tightly regulated by physiological factors such as ovarian estradiol and progesterone production, and it has been detected in different phase of menstrual cycle [15-17]. Through detecting the expressions of MMP-1, 2 and TIMP-1 in gene and protein level, this experiment demonstrates there were hardly expressions of MMP-1, 2 normally present. The higher expression of MMP-1, 2 in ectopic endometrium than that of normal can account for the progressional characteristic that ectopic endometrium

has. Because the overexpression of MMP-1, 2 can hydrolaze proteins, the endometrium can stride over and degenerate the ECM(extra cellular membrane), peritoneum and other peripheral collected tissues. Meanwhile, MMP-1,2 promote neovascularization, which help ectopic endometrium implantation and aggression. Similarly, the obviously lower expression of TIMP-1 in ectopic endometrium than that in normal endometrium indicates there is stronger function of MMP-1, 2 because of the inhibitory loss of TIMP-1. That the correlationship between MMP-1, 2 with TIMP-1 was found positively in normal endometrium, not in ectopic or eutopic endometrium and without any periodic variations, is consistent with the known reports of other researches[18,19]. This also explains that the malajusment of MMP-1,2 and TIMP-1 plays a pivotal role in promoting ectopic endometium degenerating ECM and implanting.

Though there are many theories about the occurrence of endometriosis, the convincing evidence is still based on endometrial implantation. Therefore eutopic endometrium must break through some barrier like ascites, celiac cells and ECM to finish the "adhesion-invasion-neovascularization" process. A new therory of "eutopic endometrium deciding"[20] that there are differences between ectopic and eutopic endometrium, and between the eutopic endometrium of the patients with endometriosis and eutopic endometrium of normal women, explains why the incidence of menstrual backflow is 70%~90% in women, but only 10%~15% suffer from endometriosis. Similarly, this new theory also illuminates transmissibility and familiar assembly of this disease [20]. In this study,the expressions of MMP-1,2 were obviously higher in eutopic endometrium of the patients with endometriosis than that in normal endometrium, but lower than that in ectopic endometrium of the patients with endometriosis, which indicated that the function of hydrolyzation of endometrium was most effective in ectopic endometrium, and more in eutopic endometrium than in normal endometrium. The feature may be the special biological characteristic of endometriosis.

Furthermore, this study shows, whatever in proliferative phase or secretion phase, expressions of MMP-1, 2 in eutopic and ectopic endometrium were higher than that in normal endometrium while that of TIMP-1 in ectopic endometrium were prominently lower than that in normal endometrium, which also showed that eutopic endometrium of patients with endometriosis have a stronger ability to degenerate ECM as compared to a normal endometrium. In addition, a stronger expression of MMP-1,2 in ectopic endometrium than that in eutopic endometrium shows that the ectopic implantation and proliferation of endometiosis may happen simultaneously and interactionally to progress the state of this illness.

Nevertheless, the complete pathogeneses has not been clear so far. Because of its complicated mechanisms, increased incidence, and no radical cure, it becomes the focus of research of clinical workers. Inhibiting the activity of MMP-1 and promoting the activity of TIMP-1 to adjust their equilibrium may be a reasonable target at endometriosis.

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