An Investigation of the Potential Malignant Characteristics of Endometriosis

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OBJECTIVE To investigate the potential malignant characteristics and pathogenesis of endometriosis as well as the role of human growth hormone and epidermal growth factor receptor in the pathogenesis of malignant transformation of endometriosis.

METHODS The immunohistochemical S-P method was used to examine the expression of human growth hormone and epidermal growth factor receptor in the eutopic and ectopic endometrium of 84 cases of endometriosis.

RESULTS The percentage of positive cases of human growth hormone in eutopic and ectopic endometrium was 90.77% (59/65) and 85.71% (72/84) respectively. The percentage of positive cases of epidermal growth factor receptor in eutopic and ectopic endometrium was 81.54% (53/65) and 89.29% (75/84) respectively. The positive rate of epidermal growth factor receptor in ectopic endometrium was higher than that in the eutopic endometrium but without a statistically significant difference ($P > 0.05$).

CONCLUSION Endometriosis is caused by multiple factors, which possesses some biologic features of neoplastic tissue such as invasion and metastasis. Both human growth hormone and epidermal growth factor receptor are highly expressed in eutopic and ectopic endometrium, which lead directly or indirectly to the pathogenesis of endometriosis and promote the initiation and malignant transformation of endometriosis.

KEYWORDS: endometriosis, growth hormone, epidermal growth factor receptor, malignant transformation.

Endometriosis (EMs) is a progressive, often debilitating disease with pelvic pain and infertility that affects 10-15% of women during their reproductive years.[1-4] It can be divided into external endometriosis (EEM) and adenomyosis. EEM is defined as the presence of endometrial glandular and stromal tissue outside of the uterus and attached to other organs in the abdominal cavity including ectopic lesions of the serosa and cervix of the uterus. Occasionally endometrial growths have also been found outside the abdomen, in the lung, arm, thigh, and other locations. In contrast, adenomyosis, also termed internal endometriosis (IEM), involves endometrial tissue growing into the myometrium with the lesion limited to the myometrium of the uterus. IEM used to be recognized as a distinct clinical entity.[1,2] Among gynecologic disorders, EMs are second only to leiomyoma in frequency and are common diseases that significantly impair health, quality of life and fertility. It has been noted that the prevalence of endometriosis is increasing.[2-6]

Although EMs are considered to be a benign disorder, which possess some malignant neoplastic features, such as invasion, metastasis etc, they often disrupt other organs. Some cases involve malignant

transformation, estimated to be about 1%.[7] Epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 (IGF-1) are highly expressed in many human tumors, which can promote hyperplasia, adhesion, invasion and metastasis of the tumors.[4,8,9] Since the level of IGF-1 is increased by human growth hormone (HGH), we assessed the expression of human growth hormone and epidermal growth factor receptor in eutopic and ectopic endometrium of EMs using the immunohistochemical Streptavidin Peroxidase (S-P) method. This study was designed to investigate the role of human growth hormone, epidermal growth factor receptor and other factors in the pathogenesis of malignant transformation of EMs.

MATERIALS AND METHODS

Materials

This research was a retrospective study of 84 cases of EMs confirmed by pathology, presented to the No. 4 Hospital of Wuhan between 1997 and 2003. There were 59 IEM cases (70%) and 25 EEM cases (30%), whose ages range from 19 to 60 years with a mean age of 41 years and a peak ages between 31and 50 years. Of these 65 cases (77%) were quite similar to a literature report.[1] The mean ages of the IEM and EEM patients were 43 and 37 years respectively, an obvious difference. The usual locations of the EEM in our cases were in the abdomen involving the ovaries (10 cases), laparotomy wound scars at Caesarean section or other operations in which the endometrial cavity had been opened (7cases), the cervix (6 cases), the wall of vagina (1 case) and lung (1 case). All histological evaluations were performed by a single pathologist.

Reagents

Ready-To-Use monoclonal antibodies against HGH, EGFR and a Streptavidin Peroxidase (S-P) kit were purchased from Maixin Bio Co.

Methods

Immunocytochemical staining of HGH and EGFR in eutopic and ectopic tissue of EMs

To determine the localization of HGH and EGFR in the eutopic and ectopic endometrium of EMs we performed immunocytochemical staining using an S-P method on the sections of the endometriosis. In brief, the tissue samples were formalin-fixed, embedded in paraffin, cut into 3~4 μm thick sections, deparaffinized in xylene and passed through an ethanol series. After the endogenous peroxidase activity was blocked with hydrogen peroxide, the sections were rinsed in 0.01 mol/L PBS (pH 7.4). Non-specific binding was blocked by treatment with non-immunone serum for 10 min at room temperature. Primary antibodies used were mouse monoclonal anti-HGH and EGFR antibodies. Each were applied to the different sections and incubated in a moist chamber overnight at 4℃. After the sections were washed in 0.01 mol/L PBS, biotinylated secondary antibody was added and incubated for 10 min. After washing, the sections were incubated with S-P solution for 10 min at room temperature and then washed again. The chromogen, 3-3'-diaminobenzidine (DAB), was added to the sections for 5 to 10 min until the desired reaction was achieved. Finally, the sections were washed, counterstained with haematoxylin and coverslips added. The sections with PBS instead of primary antibody served as negative controls.

Assessment of immunocytochemical results

Referring to the literature methods [10,11] the results of the immunochemistry were graded according to: (1) the percentage of positively stained cells: - less than 5%; + 5%~25%; ++ 25%~50%; +++ above 50%; (2) the color (yellowish-brown) of staining intensity was graded as -negative (none), + weakly, ++ moderately and +++ strongly staining respectively.

RESULTS

The results of immunohistochemical staining for HGH and EGFR in eutopic and ectopic endometrium of EMs are presented in Table 1.

The results showed that the number of HGH positive cases in the eutopic and ectopic endometrium were 59 and 72 with a percentage of 90.77% (59/65) and 85.71% (72/84), respectively. The percentage of positive cases of ectopic endometrium was slightly lower than that of the eutopic endometrium. The number of EGFR positive cases in the eutopic and ectopic endometrium were 53 and 75 with a percentage of positive cases was 81.54% (53/65) and 89.29% (75/84), respectively. The percentage of positive cases of ectopic endometrium was slightly higher than that of the eutopic endometrium which varied from a previous report.[12] There was no difference in the positive rate of EGFR expression between the tissue of normal endometrium and those of EMs. There was no statistically significant difference for either HGH or EGFR ($P>0.05$).

Table 1. The detection of HGH, EGFR in eutopic and ectopic endometrium of endometriosis.

		HGH					EGFR				
Locations	*n*	(-)	(+)	(++~+++)	Positive	PR**	(-)	(+)	(++~+++)	Positive	PR**
Eutopic	65*	6	29	30	59	90.77	12	33	20	53	81.54
Ectopic	84	12	48	24	72	85.71	9	44	31	75	89.29
P value			>0.05					>0.05			

* Including the eutopic endometria of 59 cases of IEM and 6 cases of EEM. ** Positive rate

DISCUSSION

EMs were first described by Rokitansky over 100 years ago (1860). From that time numerous studies have been conducted on Ems but the exact mechanism of their development has not been clearly established. Unfortunately views often have been contradictory and confusing. For many years IEM and EEM were regarded as the same disease entity. Later many researches showed that they differed to some degree, in etiology, histogenesis and pathogenesis, and suggested that IEM is a distinct clinical disease. Recently there have been many reports indicating that the two diseases are variants of the same disease process and that both IEM and EEM resulted from dislocation of basal endometrium. Furthermore those reports indicated that, with respect to the main morphological components, i.e. endometrial epithelium, endometrial stroma and smooth muscular tissue, no principal differences exists between EEM and IEM.[11,13]

With regard to the histogenesis of IEM, the most widely accepted theory has been that the basal endometrium directed infiltration into the myometrial wall affected by high levels of estrogen, an intrauterine device[1,14,15] and as indicated by the present study, HGH and EGFR. The continuity of the ectopic lesion with the basal endometrium of the uterus also has been confirmed by means of our serial sections. During pregnancy the stromal cells around the acini had developed into decidual cells, which had been observed as well. Concerning the histogenesis of EEM, three theories have dominated current thinking.[16] These theories include (1) Sampson's theory of retrograde menstruation (2) Meyer's theory of totipotential cells undergoing metaplasia and (3) embryonic rests or rudimentary cells of mullerian origin. Of course hematogenous dissemination, lymphatic spread, immune and genetic factors also play important roles in the pathogenesis of EEM.[1,4,17,18]

Like the normal endometrium, ectopic tissue is sex steroid-dependent, but many studies examining estrogen receptor (ER) and progesterone receptor (PR) status and the proliferative activity of endometriotic lesions had produced conflicting reports.[18] Some research indicated that ER expression in both epithelium and stroma of ectopic endometrium was significantly higher than in eutopic endometrium throughout the cycle. In contrast, PR expression tended to be reduced in ectopic endometrium compared with eutopic tissue. Other reports suggested that estrogen receptors were reduced in ectopic tissue.[4]

In the development of EMs, malignant transformation forms the greatest risk to the patients. In 1925 Sampson first reported in that EMs may give rise to malignant change, and proposed criteria for diagnosis of malignancy arising in endometriosis. Since that time extensive evidence for an association between endometriosis and cancer (especially ovarian) has accumulated. In resent years with the development of molecular biology it has been demonstrated that, similar to the malignant tumors, there are mutations, deletion of a tumor suppressor gene, PTEN,[19] and overexpression of the heparanase gene and matrix metalloproteinases.[2,4] Malignancy often displayed overexpression of survivin, anti-apoptotic (Bcl-2) and under-expression of pro-apoptotic (BAX) factors. Similarly, endometriotic lesions have also evolved strategies to evade apoptosis by: (1) increased Bcl-2, and decreased BAX; (2) up-regulation of survivin.[4] There have been some reports concerning EGFR in EMs but the results have not been consistent, while the studies of HGH in EMs have scarcely been reported.

Human growth hormone, a complex protein of 191 amino acids, is critical for tissue repair, healing, muscle growth, bone strength, brain function, physical and mental health. Of course HGH stimulates the tissue and stromal cell proliferation of the endometrium as well. Once secreted by the pituitary gland, circulating

levels of HGH stimulate the production of insulin-like growth factor-1 (IGF-1) from the liver and other tissues. A majority of the growth-promoting effects of HGH are actually due to IGF-1 acting on its target cells. Plasma IGF-1 levels are higher in cases of severe Ems and IGF-1 has been implicated in endometriosis and cancer development, appearing to be a prerequisite for ectopic implantation and malignant transformation. A higher risk for cervical, ovarian and endometrial cancer was related to high IGF-1 levels in post- and premenopausal women.[4] Recent research has demonstrated that the ovary itself has the ability to synthesize HGH which could promote the synthesis of estrogen and progesterone. The results of the studies have shown that the positive rates of HGH expression in cutopic and ectopic endometrium were 90.77% (59/65) and 85.71% (72/84) respectively, suggesting that HGH may promote the secretion of estrogen and progesterone and stimulate the proliferation of endometrial cells. Those results confirm that HGH directly and indirectly participates in the development and progression of EMs. There are data, suggesting that high level of HGH which are secreted during operations, trauma, stress and some irritable conditions may be related to endometriosis and are often found in women in their reproductive years, who have had a history of miscarriage, abortion or an intrauterine operation.

EGFR is the prototype of a family of receptors that consist of four known members (EGF receptor/HER1, neu/erbB2/HER2, erbB3/HER3, and erbB4/HER4). These receptor tyrosine kinases are characterized by an extracellular ligand-binding domain, an internal kinase domain, and a carboxyl-terminal domain that contains multiple tyrosine residues.[20] Epidermal growth factor (EGF) is a single polypeptide of 53 amino acid residues which is involved in the regulation of cell proliferation and has shown to induce proliferation in endometrial cell culture. Upon binding of EGF, EGFR dimerizes and becames phosphorylated on the carboxyl-terminal tyrosyl residues. These residues act as docking sites for multiple signaling proteins that contain SH2 domains resulting in stimulated DNA and protein synthesis.[21,22] EGFR was found to be widely distributed in the epithelium and stromal cells of normal endometrium. EGFR does not change with the menstrual cycle,[22] and plays a variety of roles in normal development, cell mitosis, and proliferation.[20]

Extensive experimental data indicate that estrogen effects the endometrium via several types of growth processes such as EGF stimulation or an autocrine or paracrine stimulus, which may be derived either from ectopic endometrial cells itself or from invading inflammatory cells. EGF can transcriptionally activate genes that contain estrogen response elements and estrogen in turn may be able to activate many of the effectors classically thought to be part of the EGFR signaling pathway and modulate EGFR levels so as to maintain the growth of endometriotic implants.[23]

This study reveals that the positive rate of EGFR in cutopic and ectopic endometrium is 81.54% (53/65) and 89.29% (75/84), respectively. The percentage of positive cases of ectopic endometrium is slightly higher than that of the cutopic endometrium and confirms that EGFR is involved in the occurrence and development of endometriosis through the mechanisms mentioned above. In human tumors, expression or high expression of EGFR and IGF-1 have been widely reported and are associated with the metastasis of tumors.[4] Enhanced EGFR and IGF-1 seem to promote tumor growth by increasing cell proliferation, motility, adhesion, and invasive capacity. Thereby, like malignancy, endometriosis displayed features of adhesion, invasion, metastases and high expression of EGFR and HGH, as indicated, supporting the hypothesis that endometriosis is a neoplastic process with a potential for malignant transformation[4,8] although a benign disorder.

The present study indicates that EMs are caused by multiple factors and that although it is a benign disorder, it possesses some neoplastic features, such as invasion metastasis etc. Both HGH and EGFR are highly expressed in endometriosis, resulting in stimulation of mitosis, proliferation, synthesis of DNA or protein of the epithelial/stromal cells. This action plays an important role in maintaining growth of endomeiotic implants, and directly or indirectly leads to the development and progression of endometriosis. Nevertheless the various types and presentations of endometriosis also suggest that more than one of the above mechanisms are responsible for the development of EMs and malignat transformation. Therefore the exact pathogenesis of EMs still remains to be elucidated.

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