博士論文（要約）

Mechanism of exacerbation of endometriosis by local endocrine

and inflammation-related factors

（局所内分泌因子および炎症関連因子による子宮内膜症悪化

のメカニズムについて）

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Endometriosis is pathologically diagnosed by the presence of endometrium-like tissues outside the uterus. Endometriosis affects 10%-15% of reproductive-age women and 40% of women with infertility. Endometriosis can cause pelvic pain, menstrual pain, and infertility. Histologically, it consists of endometrial epithelial cells, stromal cells, and fibrotic tissues. There are three broad types of endometriosis: superficial endometriosis, ovarian endometrioma, and deep infiltrating endometriosis (DIE). The underlying mechanisms have not been clarified. In our study, we explored the expression and function of oncostatin M (OSM)-OSM receptor beta (OSMR) axis, phospho-estrogen receptor alpha (serine118) (p-ERα S118), and interferon-inducible transmembrane protein 1 (IFITM1) in endometriosis.

Part 1: OSM project

Endometriosis is associated with chronic inflammation and immune responses. Endometriosis-related pelvic inflammatory environment can lead to pelvic pain, infertility, and promote malignant transformation. Hormonal therapies are important in the treatment of endometriosis. However, women with pregnancy demands may be unable to conduct hormonal therapies, moreover, severe pain is refractory to hormonal therapies. Therefore, exploring non-hormonal novel therapeutic approaches is critically necessary for the treatment of endometriosis.

Our group succeeded in separating and culturing endometriotic epithelial cells, which was

considered to be extremely difficult. We also established our immortalized endometriotic epithelial cell lines. Meanwhile, to explore different expressed genes between endometriotic epithelial cells and endometrial epithelial cells, we also performed comprehensive gene analysis by RNA sequencing. We found 100 up-regulated genes and 48 down-regulated genes in endometriotic epithelial cells. OSM was in the upper ranks. Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis identified 7 pathways, which includes extracellular matrix (ECM) receptor reaction and focal adhesion. OSM is belonging to the IL-6 family, which play prominent roles in chronic inflammatory diseases (such as rheumatoid arthritis and inflammatory bowel disease), autoimmunity, infectious disease and cancer. However, there are no reports about OSM in endometriosis so far. Therefore, we decided to explore the expression of OSM in endometriosis, and the functions of OSM in endometriosis-related inflammation.

Western blotting, immunohistochemistry, immunofluorescence, quantitative PCR, and other experiments were used to explore the expression and function of OSM in endometriosis.

Our results showed that: (1) Both OSM and OSMR are expressed in human endometriotic epithelial cells. OSM and OSM receptors are also expressed in endometriotic epithelial cell lines (H1 and F3). (2) In addition to endometriotic epithelial and stromal cells, macrophages are the main source of OSM in the endometriotic microenvironment. (3)

Monocyte chemoattractant protein 1 (MCP1) secretion could be induced by OSM alone, and also OSM in combination with tumor necrosis factor alpha (TNFα) synergistically in the endometriotic epithelial cell line. However, Interleukin-8 (IL-8) couldn't be induced by OSM alone, but which could be induced by OSM in combination with TNFα in the endometriotic epithelial cell line. (4) Signaling pathways including STAT3, AKT, P44/42 MAPK, P38 MAPK, and JNK were activated by OSM in the endometriotic epithelial cell line. (5) Treatment of AKT, STAT3, JNK, and P38 MAPK inhibitors significantly reduced OSM-induced and OSM+TNFα-induced MCP1 secretion, and treatment of P44/42 MAPK, JNK and P38 MAPK inhibitors significantly reduced OSM+TNFα-induced IL-8 secretion. Which means, OSM or OSM+TNFα induced MCP1 secretion through STAT3, AKT, P38 MAPK and, JNK signaling pathways, and OSM+TNFα induced IL-8 secretion through P44/42 MAPK, P38 MAPK, and JNK signaling pathways. (6) OSM might induce epithelial-mesenchymal transition (EMT) and ECM deposition, and result in the progression of fibrosis. (7) Treatment of OSM promoted cell migration.

We also explored whether interleukin 31 (IL-31) has some similar or overlapping functions with OSM in endometriosis-related inflammation, because IL-31 receptor shares the same OSMR subunit with OSM receptor. However, the IL-31 Receptor alpha (IL-31RA) subunit was barely expressed in the endometriotic epithelial cells, and human recombinant IL-31 couldn't stimulate the secretion of typical inflammatory molecules in the endometriotic epithelial cell line.

In summary, our results showed that, among the two axes involved OSMR (OSM-OSMR axis and IL-31-OSMR axis), the OSM-OSMR axis mainly plays a role in the endometriosis. OSM had the potential to contribute to the exacerbation of inflammatory response, EMT, and fibrosis in endometriosis. Therefore, targeted blocking of the OSM or OSM-related pathways may provide novel strategies for the treatment of endometriosis.

Part 2: p-ERα S118 project

Endometriosis is a highly estrogen-dependent chronic inflammatory disease. Endometriotic tissues can synthetize estrogen locally via aromatase. Elevated estrogen exposure and estrogen hypersensitivity are thought to be important endocrine features of endometriosis. The normal endometrium is affected mainly by systemic estrogen, while endometriotic tissues are thought in response to systemic and local estrogen. Even though endometriosis is characterized by increased expression of ERβ and decreased expression of ERα, the importance of ERα in endometriosis has been demonstrated by the endometriotic mouse model using estrogen receptor 1 (ESR1) knockout mice. Therefore, there remains the possibility for the existence of ERα activation pathways in the endometriosis.

Activation of ERα is initiated by ligand binding, while the function of ERα is also regulated by post-translational modifications, including phosphorylation and

ubiquitination. S118 is the most-well studied phosphorylated site. Phosphorylation of ERα S118 is regulated by kinases including MAPK, CDK7, and mTOR without binding to estradiol (E2). However, as far as we know, there are no reports about ERα phosphorylation in endometriosis. Therefore, we decided to explore the phosphorylation of ERα S118 in the endometrium and endometriosis.

In our study, we examined the expression of p-ERα S118 in 49 cases of endometriosis, which included 17 cases of endometrium without endometriosis, 8 cases of endometrium with endometriosis, 18 cases of ovarian endometrioma, and 6 cases of DIE. We found that: (1) The phosphorylation level of ERα S118 was lower in endometrium and DIE, compared to a high frequency of p-ERα S118 expression in ovarian endometrioma. (2) Co-localization of P44/42 MAPK phosphorylation and ERα S118 phosphorylation was observed in ovarian endometrioma. (3) E2 treatment enhanced the phosphorylation of ERα S118 in the endometrial epithelial cell line. (4) Phosphorylation of ERα S118 was also enhanced by TNFα treatment, and addition of P44/42 MAPK inhibitor partially suppressed phosphorylation of ERα S118, which suggested that TNFα-induced ERα S118 phosphorylation was mediated by P44/42 MAPK signaling pathway.

In summary, we found for the first time that phosphorylation of ERα S118 was elevated in ovarian endometrioma compared to the endometrium and DIE. Local high E2 level and elevated TNFα environment might be two of the reasons for the higher ERα S118

phosphorylation and P44/42 MAPK phosphorylation in ovarian endometrioma, and which might lead to E2 hypersensitivity and ERα pathway activation without E2 in ovarian endometrioma. This will provide a new perspective in understanding the pathological mechanism of ER in endometriosis.

Part 3: IFITM1 project

Endometriosis commonly forms lesions, such as peritoneal, ovarian, and deep-filtrating endometriosis, and less commonly involves the bowel, bladder, or rarely distant sites from the uterus, such as the navel and lung. When the presence of endometriotic lesions are not evident by hematoxylin and eosin staining, cluster of differentiation 10 (CD10) has been used as a useful marker to highlight and confirm the presence of endometriotic stroma. However, CD10 is not specific only to the endometrial stroma but is also expressed in many other cells. Therefore, for better accuracy of diagnosis, development of a better indicator of endometriotic stroma would be beneficial. IFITM1 was identified as an efficacious immunohistochemical marker of normal endometrial stroma and endometrial stromal neoplasm by bioinformatics approaches in a public database of protein expression profiles. However, there are no reports on its status in endometriosis. So, we comprehensively analyzed IFITM1 expression in endometriosis and extragenital endometriosis.

In our study, we examined the expression of IFITM1 and CD10 in 18 cases of ovarian

endometriosis and 44 cases of extragenital endometriosis, which included 19 intestinal, 8 bladder, 3 ureteral, 3 inguinal, 1 abdominal wall, and 10 diaphragmatic endometrioses. Among the 62 patients, all (100.0%) were positive for IFITM1 and 60 (96.8%) for CD10, and CD10 was negative in 2 cases that were positive for IFITM1. To confirm this, we verified that these endometriotic lesions were also positive for ER, PR, and PAX8. Preoperative hormonal therapies, such as GnRH agonists and dienogest, are often used to reduce the size of the lesion or control the condition before surgery. Therefore, it is important to verify that hormonal therapies do not alter the expression of endometriosis markers (eg, IFITM1 and CD10). Our results showed that among the 62 cases of endometriosis, 39 patients did not undergo preoperative hormonal therapy, while 16, 5, and 2 patients used GnRH agonist, dienogest, and oral contraceptives, respectively. No significant difference was observed in frequency of positive staining for IFITM1 and CD10. Therefore, IFITM1 sensitivity was unaffected by the presence or absence of hormonal therapy.

In summary, IFITM1 is a highly sensitive stromal marker of endometriosis and extragenital endometriosis, which is comparable or possibly superior to CD10. IFITM1 can be a useful addition in immunohistochemical examination of the disease, particularly in the accurate diagnosis of endometriosis in cases of ambiguous or unexpected CD10 expression.