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Chapter

Proteomics Research and Its Possibility of Application in Endometriosis

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Abstract

The onset search for differential protein expression in endometriosis commenced more than 30 years ago, when the gel electrophoresis could not be available to distinguish serum from women with and without disease. Gradually as the proteomics allows the comprehensive analysis of peritoneal fluid, serum and tissue samples with good sensitivity and resolution, it has promised in delivering markers possibility associated with endometriosis. Cytokines and growth factors that are present in serum, peritoneal fluid, endometrium, endometriotic lesions tissues and involved in tissue implanting process including hormone regulation, angiogenesis, invading and malignancy may be the focus to develop the noninvasive diagnostic test and possible treatment target for endometriosis. Individual peptides or proteins that are present or absent (or up- or down-regulated) in various conditions can be assessed as possible biomarkers. Alternatively, proteomic profiling, using mass spectrometry in combination with bioinformatics software to identify the actual protein and peptide pattern can be used as a distinctive marker to diagnostic and treatment target contribution to the disease.

Keywords: diagnosis, treatment, endometriosis

1. Introduction

Endometriosis (EM) is defined as a benign condition of gynecological diseases when endometrial debris including gland and stroma components outside the uterus and spread to pelvis and extrapelvic sites. It affects at least 10% reproductive women with approximately 70% of cases developing pelvic inflammatory disease and 25–30% of cases associated with infertility [1]. The classical symptoms are dysmenorrhea, chronic pelvic pain, and ovarian chocolate cysts.

Till now, the pathogenesis of endometriosis is unclear. The retrograde menstruation is the basic theory which the menstrual fragments mostly attach to the peritoneum or ovaries area [2]. Whereafter endometriosis mainly responds to fluctuations in estrogen and progesterone by growth and inflammation. And endometriosis mostly involves in the reproductive tract components, such as ovaries, fallopian tubes, uterosacral ligaments, cervix, recto-vaginal septum and vagina. There are commonly exhibited with fibrous walls, adhering to the neighbor structures and usually containing chocolate-colored content. Therefore the affected organs are forming together with serial symptoms-dysmenorrhoea, ovarian chocolate cysts and infertility are often found in the patients. Recently, stem cell theory can be

considered as perspective view of the retrograde menstruation theory [3]. Other theories like immune system dysfunction, genetic susceptibility and exposure to the environment such as dioxins can devote to the progress of the disease [4, 5].

Generally, severity of endometriosis is classified by the revised American Fertility Society (rAFS) system [6], dividing patients into one of four stages (I–IV, minimal-severe) based on lesion size and pelvic adhesions associated with infertility. However, it remains uncertain whether the disease progresses through these stages. Actually some surgeon would prefer to stage on the basis of the sites and extent of the lesions under the laparoscopy or laparotomy and describe without AFS or r-AFS stage. Sometimes they may give some complementary suggestion [7]. Meanwhile, endometriosis has a variable symptom profile which does not relate with severity of this disease [8]. Furthermore, the patients often suffer from the symptoms of infertility or chronic pain for several years before the diagnosis is lately confirmed. So it makes the clinical diagnosis even more difficult. And actually there has been a lack of precise diagnosis method in previous researches. On the aspects of clinical application and financial consideration, more effective and noninvasive test will be needed in endometriosis disease.

Currently, laparoscopy offers the most widely accepted technique and method for evaluating and treating endometriosis. And most of endometriosis patients are treated by surgical removal of lesions and/or hormonal suppression focused on reducing estrogen, such as progestins, androgens, gonadotropin-releasing hormone (GnRH) agonists, and recent aromatase inhibitors. However, both approaches are associated with various side effects and a highly recurrent incidence [9, 10]. Therefore, identification of protein molecular mechanisms involved in the pathogenesis of endometriosis and strategic therapies for treatment are critical.

2. Protein molecular mechanism involving in endometriosis

There should be some factors present in the ectopic lesions of endometriosis, peritoneal fluid and blood that stimulate the lesion to proliferate, implant and invade [11, 12]. And comparing with eutopic endometrium, endometriosis lesions has different biochemical and functional expression including steroid receptivity and invasive potential [13]. Over all, the specific markers of endometriosis may be classified into three main categories: (i) serum and/or peritoneal: growth factors, cytokines, hormones, glycoproteins, proteolytic enzymes and their specific inhibitors, soluble adhesion molecules, autoantibodies and environmental contaminants. (ii) endometrial and biochemical-endometrial: stromal, glandular, neuronal, hormonal receptors, mesenchymal stem cells, proteolytic enzymes and their specific inhibitors, adhesion molecules, osteopontin.(iii) genetic types: oxidative stress genes, tumor suppressor genes, oncogenes, regulatory genes, DNA repair genes, chromosomal aberrations or amplifications, loss of heterozygosity, genetic polymorphism of variable genes, and genome-wide alterations. And we show the focus continuously.

2.1 Hormones regulation proteins in the pathogenesis of endometriosis

Steroid hormones play an important role in endometriosis physiology and pathology. The production of cytokines in endometriosis is the altered responsiveness to progesterone, showing a characteristic very low expression of progesterone receptor A (PR-A) and the absence of PR-B in rodent models, and with decreased PR-B/A ratio and reduced PR-B immunoreactivity, demonstrated in human endometriosis lesions [14, 15]. Hormonal dependence in endometriosis is demonstrated by an increased ER-β expression (approximately 100 times), due to altered

methylation in the ESR2 gene promoter [16]. In experimental model of endometriosis, ER-β may be involved in inhibiting apoptosis and increasing cytokine production in endometriosis, such as MCP-5, IL-1b and IL-16, which seem to enhance cells adhesion and proliferation [17]. The secretion of PGE2 is regulated by MIF through stimulating COX-2 activity. PGE2 is considered to be one of the main pro-inflammatory regulating factors which appears to be confirmed by its elevated level in endometriosis tissues of human being [18].

2.2 Proteins of angiogenesis process of endometrial tissue

On behalf of endometriosis lesions, neovascularization and angiogenesis factors should be introduced. Angiogenic factors such as VEGF [19], ENDO-I [20], angiogenin [21], pleiotrophin, midkine [22], PGF [23], angiopoietin [24], and glycodelin [25, 26] have been identified association with the lesions angiogenesis in endometriosis. Angiogenic activity is supplemented by the co-existence of pathologic angiogenesis, immune suppression, and immune activation. Study from human being indicated the correlation between high MVD and symptom of pelvic pain in patients with endometriosis by transvaginal color doppler ultrasound evaluation. MVD has been described using immunohistochemical evaluation with CD34-labeled endothelial cells of vessels [27, 28]. There was no significantly increased VEGF expression but it has been found in the involvement of other angiogenic factors with the active implants showing high mitotic index and increased MVD [27]. Further findings were reported in other articles that VEGF-A role was demonstrated by its increased concentrations in the endometrium of patients with endometriosis. Moreover, it was confirmed that the expression of VEGF-A gene was higher in peritoneal endometriosis compared with normal peritoneum [29].

2.3 Proteins in invading process of endometrial tissue

In endometriosis, after the attachment to the ectopic sites, the epithelial endometrial cells invade to the extracellular matrix with MMPs and TIMPs secretion playing an important role in degradation in extracellular matrix and basement membrane components [30]. It has been proved that this process is related to the involvement of MMPs, which is stimulated by TNF-a and IL-1 at high concentrations in peritoneal fluid. Meanwhile, TNF-a inhibits TIMP-1 and TIMP-2, leading to an imbalance of MMPs/TIMPs ratio. In patients of endometriosis, MMP-2 and membranous type 1 of eutopic endometrium have been found higher, TIMP-2 was lower than normal women [31]. Increased MT5- MMP expression and alterations of the balances between MMP-9/TIMP-1, MMP-9/TIMP-3, MMP-3/uPA, VEGF/MMP-3/uPA, VEGF/ /MMP-2/CD44/Ki67, PAI/TIMP-1, and IL-1/ /MMP-1 indicated that implantation and invasion might participate the mechanism of endometriosis [32–38].

2.4 Proteins in carcinogenesis possibility of endometriosis

Clinicopathological, molecular, and genetic evidences support the hypothesis of endometriosis as a neoplastic process, with a potential to malignant transformation. Polypoid endometriosis, premalignant changes, borderline tumors and malignant tumors were described. Except for an increased MMPs expression associated to deregulation of the intercellular adherence signaling, tumor suppressor genes, oncogenes, CAMs, furthermore LOH and inflammatory immunomodulation were detected [39, 40]. The progressive accumulations of genetic alterations of tumor-suppressing genes and oncogenes are probably responsible for endometriosis development and its possible association with the development of malignancies [41–45].

2.5 Proteins involving in peritoneal fluid and plasma of endometriosis

Most of researches have demonstrated that macrophages, lymphocytes, endometrial cells and mesothelial cells are able to produce cytokines and inflammatory mediators such as ILs [3, 46, 47], TNF-a [48], PGF2, PGE2 and thromboxane B2 [49], MCP-1 [50], RANTES [51], eotaxin [52], GROa [47], SDF1 [53], and MIF [50, 54]. The main process is leading to the stimulation of endometriotic cells proliferation and adhesion to ectopic sites, angiogenesis, and stimulation of the release of other cytokines and chemokines, later amplifying their effects.

Macrophages can amplify the activity of COX-2 and PGE2, which results in VEGF stimulation in endothelial cells from endometriotic lesions, together with factor StAR, association with an increased estrogen level in the endometrial tissue [55–57]. PGE2 suppresses the activity of phagocytes, that allowing endometriotic implants formatting [58]. Estrogens and PGE2 can induce FGF-9 expression which can further activate endometrial cells proliferation, paralleling to the stimulation of angiogenesis and apoptosis inhibition at the same time. Lymphocytes are involved in various cytokines production with potential role in endometriosis lesions implanting. Whether in vitro study or in human endometriosis, Th2 cells of peritoneal fluid were shown to stimulate the secretion of IL-4 and IL-10. NK cell-mediated cytotoxicity, commonly manifested by lymphocytes adherence to endometrial cells through LFA-1 — ICAM-1 pathway and their presentation as targets to NK cells, may fail in endometriosis [59, 60]. This indicated the possible mechanism that sICAM-1 may bind to LFA-1 expressing in lymphocytes can prevent endometrial cells recognition involved in endometriosis pathogenesis.

TNF-a is a typical pro-inflammatory cytokine produced by macrophages, which can exhibit high levels in serum and peritoneal fluid of patients with endometriosis [61, 62]. Recent studies have shown that TNF-a-induced activation of IKKb complex leads to the initiation and progression of endometriosis by enhancing the survival rate of ectopic epithelial cells rather than stromal cells and not eutopic epithelial cells [63]. MIF, another cytokine, has been shown a high level in the peritoneal fluid, in serum samples, and in peritoneal macrophages, its secretion being regulated by estrogens in endometriosis [85]. MIF can stimulate endothelial cell proliferation, endometriotic lesions survival, expressing VEGF, IL-8, PGE2, COX-2, MCP-1, aromatase, and resulting back in stimulating TNF-a in endometrial cells [18, 64]. That is why in experimental models, MIF antagonist significantly reduces lesions size of endometriosis by inhibiting cell adhesion, tissue remodeling, angiogenesis, and inflammation, in addition to routine alteration of the balance between pro- and anti-apoptotic factors [65].

3. Progress in proteomics

It is recognized that gene expression changes does not reflect the changes in protein expression within the cell. Better tools are needed to accurately probe the protein activities and levels. Protein arrays are arguably underachieving their potentials as they are perceived not as precise as DNA arrays. Functional proteomics still holds great promises that it could result in greater understandings of the mechanism of disease. The goals proteomics of diseases are to improve molecular classification of diseases and to discover molecular biomarkers for their diagnosis, treatment target and following up [66–68]. Better tools are needed to accurately calculate the protein activity levels. Protein arrays (used synonymously with protein microarrays or protein biochips) are one of the solutions to the high throughput study of protein interaction networks or immune reactivity [69–71]. Mass spectroscopy (MS) [72], relies mainly

on the mass-to-charge ratios to distinguish different proteins. It is widely used as a discovery tool of diagnostic, prognostic, and therapeutic protein biomarkers. Moreover, it does not need to identify molecules. And protein arrays depend on the availability of specific recognition molecules. MS-based methods can simultaneously screen numerous proteins, but its sensitivity is approximately 1 ng/mL. Emerging protein arrays based on magneto-nanosensors enable to study many proteins to proteins interactions simultaneous easily with sensitivity low than about 1 pg/mL. Proteomic researches mainly includes the 2-DE reference map and a database by proteomic comparison between healthy persons and patients. Comparing to western blot, 2-DE can be used to find out autoantigens that may induced autoantibodies in some diseases.

3.1 Protein arrays

Protein arrays with antibodies are developing a tool for rapid measurement of abundance of proteins expression. It makes the possibility to screen the aimed proteins changes in different diseases and biological processes. In most cases, this approach depends on exposing serum samples from patients to an ordered array of antigens, capturing those antibodies that bind the antigens on the arrays. Immobilization of proteins on the surface of arrays and neutralizing reactive areas after the immobilization are important practical issues in protein array. Many different types of proteins arrays such as antibody arrays and peptide arrays have been reported [73]. It starts research in breast cancer and leukemia [74, 75]. Recently, one of the protein array technologies is the magneto-nanosensor array where giant magnetoresistive (GMR) [76] sensors are used to quantitatively measure analyte of interest proteins which are labeled with magnetic nanoparticles (MNP). Another emerging protein array technology is Nucleic Acid Programmable Protein Arrays (NAPPA) [77], which have thousands of protein features directly expressed by nucleic acids on array surface.

More attentions have been paid to the role of protein arrays in medicine. They can be used for early detection of diseases, diagnosis of stages, stratification of patients, and prediction of therapeutic effects, and are increasing realization of the vision of personalized medicine. For cytokine measurements, protein arrays should be improved in both functional sensitivity and probe density. Till now, the arrays have two major drawbacks: first, they are biased, because antigen selection is based on their potential to play a role in disease. Secondly, the analytical comprehensiveness of this technique is limited because only the molecules represented on the array can be identified.

3.2 Mass spectrometry (MS)

In the past few decades, the paradigm of biomarker research has shifted from a hypothesis-driven approach to a discovery-driven approach. Mass spectrometry and separation techniques and proteomics methods have been fully developed and have been common. Particularly when two-dimensional liquid chromatography/tandem mass spectrometry, or two-dimensional gel electrophoresis (2-DE) and matrix laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) combination, followed by database search (sequence tags or peptide mass fingerprints) are widely used. These methods greatly increase the comprehensiveness of protein identification.

Identify disease-associated antigens can elicit through immune responses by combining protein separation (2-DE, gel-free separation), immunological detection (Western blotting) and MS, or combining immunocapture and MS [78]. Proteins derived from cells or tissues (e.g., cancer cells) are separated by 2-DE, and antigen proteins are detected by applying patient serum, which may include disease-specific antibodies followed by enzyme-labeled secondary antibodies. In order to identify

immunogenic proteins, the corresponding spots are separated from the gel and gels were digested. MS or tandem mass spectrometry is used for analysis and then analyzed by peptide fingerprinting or sequence tags method.

3.2.1 Isotope-coded affinity tagging multidimensional LC-MS

LC–MS makes identification and quantification of target protein possible in a large and complex sample and most of the time it may not be achieved in the clinical work. However, it will be useful when proteins are limited in a sample and at present this method will be considered as a supplement to 2-DE gel [79, 80].

3.2.2 MALDI-MS

Antigen analysis of immunocapture MS is derived from the immobilization of antibodies in the serum of patients. Almost all antibodies are captured on protein A or G, which is a bacterial derived protein with specific affinity for the Fc domain of the antibody. Protein mixtures (cell or tissue lysates) are applied to a column or bead immobilized with antibodies to capture specific antigens of antibodies present in patient samples. Finally, the proteins were identified by MALDI-TOF-MS or surface enhanced laser desorption/ionization mass spectrometry (SELDI-MS). In SELDI-MS, the protein is enzymatically cleaved into a peptide mixture and undergoes MS. It can be a fully automated system allowing high-throughput and rapid identification. MS afford a method to identify a protein even from a complex mixture of proteins [81, 82]. It is preferred and an applicable for a pure protein or a single spot for 2-DE gel.

3.2.3 Surface-enhanced laser desorption/ionization (SELDI-MS)

Chemical defined or antibodies-coated protein biochip arrays for rapid protein detection. This system is used when small amount of samples is available. Very high surface expression of the immunoglobulin binding protein (proACTR) as the antigen capture and transfer reagent [83]. ProACTR can immobilize the antibody through the Fc region of antibody, and allows for higher capture capacity than antibody-coated beads. Captured antigens can be directly transferred to a platform for MS analysis (SELDI-TOF-MS). It starts its diagnosis research with post-translationally modified proteins and high-throughput technique in breast cancer, lung cancer and prostate cancer. Unfortunately, it does not allow reliable protein sometimes [84, 85]. To the best of our knowledge, immunoproteomics with proACTR has not yet been applied to profile antigens associated with a certain disease, but mainly to the quantification of a single target [86, 87].

4. Applications and experiences in the past

The 2-DE for tissue sample is more complex than that for serum. Tissue from ovarian chocolate cysts or from eutopic endometrium contains connective tissues, red blood cells, epithelial cells and stromal cells. Stringent protocols are adopted to ensure uniformity throughout the process to facilitate the protein maps. However, the development of robust assay platforms and standardized protocols are required before MS-based antigen profiling can be performed in the clinical setting.

4.1 Expression and significance of peritoneal fluid protein in patients with endometriosis

In 2003, Tabibzadeh et al. [88] used two-dimensional gel electrophoresis to analyze the protein profile of peritoneal fluid in 12 patients with EMs (6 cases were mild and 6 cases were severe degree). However, 12 cases of non-EMs ascites were analyzed as control (6 cases of infertility, 6 cases of normal fertile). There was no significant difference between the infertile controls and the normal fertile control group. However, the patients with mild EMs had protein reductions associated with several peritoneal protein spots of approximate molecular weights of 35–40 kD and pI close to 5.7–6.0. and the reduction in severe EMs cases was more markedly apparent. Consistent with these data, enzyme-linked immunosorbent assay showed that severe endometriosis was associated with markedly elevated levels of IL-10 in the peritoneal fluid. Endometriosis maybe associated with disturbed secretion of proteins into the peritoneal cavity and with an elevated level of IL-10 in the peritoneal fluid. Most of these proteins have not been further described in the existing literatures, so it is still unclear whether the aforementioned results can be used as diagnostic markers for EMs.

Ferrero et al. [89] used two-dimensional gel electrophoresis, silver stained, semiquantitative computerized analysis the changes of protein expression profile in the peritoneal fluid and plasma of 72 patients with EMs and 35 infertile control patients. Compared with the controls, one beta chain isoform (HpbetaE; molecular weight 38.40 ± 0.94 kD; and isoelectric point, 5.63 ± 0.17) had significantly higher expression in women with endometriosis. HpbetaE level was found no significant difference between mild endometriosis (rAFS, stage I-II) and severe endometriosis (rAFS, stage III-IV). But the expression of HpbetaE in the control group was obtained to be related to the stage of menstrual cycle. The above studies indicate that changes in the protein expression profile of patients with endometriosis. In 2007, Liu et al. [90] used SELD I-TOF-MS technology and its associated protein chip to detect the plasma protein profiles from 36 patients of endometriosis and 35 healthy individuals. 21 differentially expressed protein peaks were found and three protein peaks were established. The endometriosis diagnostic model had a sensitivity of 91.7% and a specificity of 82.9%, and was performed on 16 healthy subjects and 15 patients. The sensitivity was 87.5% and the specificity was 80%. It provides an approach for screening the plasma markers of endometriosis. In 2007, Ferrero et al. [91] used two-dimensional gel electrophoresis; protein spots of interest were identified by liquid chromatography tandem mass spectrometry to study the differential expression of peritoneal fluid proteins in patients with and without endometriosis. Several molecules had aberrant expression in peritoneal fluid of women with endometriosis may be useful for a better understanding of the pathogenesis of this disease.

In 2006, Zhang et al. [92] applied two-dimensional gel electrophoresis (2-DE), Western blotting, and mass spectrometry (MS) technology to study proteins in endometriosis and normal controls, and analyzed differences using Western blots. The normal human serum and patient serum were compared with the total protein of endometriosis. In patients with endometriosis, 13 protein spots were associated with 11 known proteins, while 11 protein spots were found differently expressed in the endometrium of patients with and without endometriosis. Some proteins may be cytoskeleton, some may regulate in cell cycle, signal transduction or immune function participation. The hybridization of vimentin, beta-actin and ATP synthase beta subunit in serum of patients with endometriosis was significantly different from that of normal serum. Three different points were used to determine the protein expression profile, vimentin, β-actin, and ATP synthase β subunits respectively.

ATP synthase may play an important role in ectopic endometrium as it needs invasive, and cell adhesion and cytoskeletal remodeling. Vimentin and β-actin is a cytoskeletal protein. Studies have shown that the expression of these proteins is up-regulated in the endometrium in patients with endometriosis. These proteins have a certain effect on the formation of endometriotic lesions. Given that the occurrence of endometriosis may be due to an abnormality of eutopic endometrium itself. In 2007 and 2008, Liang [93] and others used surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) technology to study serum protein expression in patients with endometriosis and healthy controls. A diagnostic model consisting of 5 protein peaks was established with a sensitivity of 91.7% and a specificity of 90.0% provides new prospect for screening endometriosis markers. In 2007, Liu et al. [90] also used SELDI-TOF-MS technology to study plasma protein expression in patients with endometriosis and healthy controls. It was found that 20 protein peaks were elevated or decreased in both of them. The sensitivity was 87.5% and the specificity was 80.0%.

4.2 Expression and significance of tissue protein in patients with Endometriosis

In 2006, Kyama et al. [5] used SELD I-TOF-MS technology to find that the expression of proteins and peptides in the eutopic endometrium of patients with endometriosis weight range of 2.8–12.3 kDa was 3–24 times lower than the molecular with non endometriosis. The expression of proteins and peptides in the lesions of patients with endometriosis had a tendency to increase within the molecular weight range of 3–96 kDa, and especially an up-regulated cluster of proteins between 22 and 23 kDa, identified to be transgelin, a smooth muscle actin-binding protein. In 2007 Fowler et al. [94] used 2-DE and peptide mass (MS) technology to confirm that the protein associated with endometriosis. Several deregulated proteins are identified: (1) chaperones and calcium binding proteins include heat shock protein 90 (HSP90) and annexin A2. (2) Cell oxygenation status related to protein, such as peroxiredoxin and thioredoxin-1, -2 (thioredoxin reductase-1, -). (3) Proteins associated with protein/DNA synthesis/decomposition, such as nucleoside diphosphate reductase, prohibitin, and proline-4-hydroxylase. (4) Secreted proteins, such as apolipoprotein A1. (5) Structural proteins such as vimentin and actin, whose function suggests that they play a role in the pathogenesis of endometriosis. At the same time, it was believed that the differentially expressed protein spots produced are identical whether the lysate of the endometrium was aggregated or isolated. Immunohistochemistry, Western blotting, and biological effects were also used to validate differential proteins and achieve desirable results. This study demonstrated that 2-DE gel analysis and mass spectroscopic protein identification are suitable for the identification of proteins with candidate associations with endometriosis.

4.3 Endometriosis infertility protein detection

Previous result of our group indicated [95] that 76 eutopic endometrial polyps cases of endometriosis group histologically resembled endometrial polyps but the majority of endometrial polyps with endometriosis occurred in primary infertility cases and in fewer pregnancy rate women who had stable and smaller EPs without association with the AFS stage. The recurrence rate of endometrial polyps with endometriosis group was higher than that in non endometriosis group. In 2009, Ferrero et al. [96] reported the peritoneal fluid proteome collected under laparoscopy from 26 fertile women and 26 infertile ones With endometriosis. One isoform of immunoglobulin light chain spot and 9 protein spots had been found significantly higher expression in PF of infertile patients than infertile controls by applied with

2-dimensional gel electrophoresis (2-DE) with computerized analysis and protein spots were identified by liquid chromatography tandem mass spectrometry (MS). No protein spots had significantly lower expression and 3 protein spots remain unidentified.

4.4 Proteomics detection of animal models of endometriosis

Many animals such as rhesus monkeys, rabbits, and nude mice have been used as models for studying endometriosis, but most of these animals have no menstrual cycle, and only primates can spontaneously produce endometriosis with regular menstrual cycles and menstrual blood flow. Their pathogenesis and pathological features are similar to those of humans. Monkey experiments have confirmed that current menstrual bleeding can cause pelvic lesions. In 1991, Sharpe et al. [97] first reported using two-dimensional electrophoresis (2-DE) to observe that surgically induced ectopic endometrium in rats lacked progesterone-induced secretory uterin protein 21 (PUP21, MW 70 kDa, p I 5. 7), whereas normal endometrium expression of the protein suggests that PUP21 deficiency is associated with reduced fertility in patients with endometriosis. In 1993, Sharpe et al. [98] also used two-dimensional electrophoresis to study the rat endometriosis implanted by surgery. It was found that ectopic endometrium specifically expressed two groups of proteins: ENDO I (MW 40–50 kDa, p I 4. 0–5. 2) and ENDO II (MW 28–32 kDa, p I 7.5–9.0). Further studies using amino acid sequence analysis confirmed that ENDO-2 is TIMP-1 and ENDO-1 is haptoglobin-like. Studies have shown that endometriotic lesions secrete haptoglobin in combination with macrophages to reduce their adherent phagocytic capacity, so that intimal ectopic cells cannot be eliminated, but haptoglobin can stimulate macrophages to secrete inflammatory cytokines such as IL-1, − 6 and TNF, IL-6 can up-regulate ectopic endometrium cells to express haptoglobin, forming a positive feedback in the lesion, thereby promoting the progression of endometriosis. In the peritoneal fluid and serum of patients with endometriosis, the concentration of TIMP-1 was significantly reduced. Therefore, the abnormal expression of TIMP-1 may be one of the causes of the onset of endometriosis, and may become a potential marker for diagnosing this disease.

5. Possible directions in the future

Once we have discovered one or more proteins that are specifically expressed in patients with endometriosis, the next step is to develop them as a diagnostic test for it. The diagnostic method must have good sensitivity, specificity, positive predictive value, and negative predictive value. What we need to overcome is not only the individual differences in the population, but also the differences in specific tissue protein components and the effects of natural menstrual cycles or hormone effects. Protein chip, protein array mass spectrometry technique have been used to perform a comprehensive search of protein expression profiles of endometriosis patients in order to find a group of proteins with high sensitivity and specificity. Applying the changes in the expression of this group of proteins to diagnose and predict disease will undoubtedly bring about a new endometriosis diagnosis field.

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Conflict of interest

None.

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