Arrays to detect Erring Factors of Endometrial Origin in Endometriosis

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ABSTRACT

Endometriosis is an estrogen-regulated chronic inflammatory disease, characterized by the presence and growth of endometrium-like tissue in extrauterine locations. Its prevalence is 6 to 10% of women in the general population, and 35 to 40% of women with pain and/or infertility. Endometriosis is manifested in different forms, of which peritoneal endometriosis, rectovaginal endometriosis and ovarian endometriosis are most common. Several investigations have been conducted to investigate the genetic basis of endometriosis. However, these studies have been unsuccessful in identifying robust genetic variants associated with endometriosis. On the contrary, the advent of whole genome cDNA microarray approach has allowed for the identification of genes that display modulation in their expression in an endometriotic tissue. Several biological pathways involved in the pathogenesis of endometriosis have been identified. This review article compiles the inferences drawn from high throughput investigations of endometriotic tissue.

Keywords: Endometriosis, Eutopic, Ectopic, Microarray.

INTRODUCTION

Endometriosis is an estrogen-dependent benign gynecological disease, characterized by the presence and growth of endometrial tissue (glands and stroma) in the extrauterine (ectopic) locations. It is a chronic inflammatory disease occurring in 6 to 10% of women in the general population and in 35 to 40% of women with pain and/or infertility. There are three clinically distinct endometriotic lesions, peritoneal implants that occur on the peritoneum, endometriomas that are cystic lesions sited in the ovary surrounded by endometrioid glands and rectovaginal nodules comprising fibrotic and endometriotic tissue that grow in the rectovaginal space. The etiology of endometriosis remains unclear. Retrograde menstruation is considered as one of the etiological factors contributing to endometriosis. However, retrograde menstruation occurs in 90% of women in the reproductive age, only 10% develop endometriosis. Plausible explanations for the occurrence of the disease in only some women could be increased exposure to menstrual debris (e.g. increased menstrual flow, shorter cycle length), abnormal eutopic endometrium, altered peritoneal environment, reduced immune surveillance, and enhanced angiogenic potential. There are other theories proposed to explain the origin of endometriosis. The coelomic metaplasia theory postulates that specialized cells transdifferentiate or transform into endometriotic cells giving rise to endometriosis. The induction theory proposes that the undifferentiated mesenchymal cells under the influence of factors secreted by the refluxed degenerated endometrium differentiate into endometrial epithelium and endometrium-like glands. Another theory attributes the origin of endometriosis to the stem cells in the endometrium. The basal layer of the endometrium, which is not shed during menstruation, is thought to be the source of stem cells. Recently, cells representing the stem cell population in the human endometrium have been identified and proposed to be involved in the generation of ectopic endometrial lesions. Owing to the fact that endometriosis is a multifactorial and polygenic disease, several, if not all, of these factors may play a role in the pathogenesis of endometriosis.

Gene mapping methodologies have been used to identify the genetic factors contributing to the pathogenesis of endometriosis. The candidate genes chosen for these studies were based on biological mechanisms believed to be of relevance in the pathogenesis/pathophysiology of the disease. Some of the candidate genes known to be involved in detoxification pathways, sex steroid synthesis pathways and cytokine signaling pathways, adhesion molecules and matrix enzymes, and cell cycle regulation were analyzed. Reports from these studies...
indicated the association of 76 genes with endometriosis susceptibility. Further, this information was used for linkage mapping analysis to search for genomic regions harboring genetic risk factors for endometriosis. One such large linkage study by Treloar et al combined Australian and British families and genotyped 4,985 women, including 2,709 with endometriosis. The study reported two loci of significant linkage on chromosome 10q26 and another region of suggestive linkage on chromosome 20p13. Although chromosome 10q26 had been implicated in a candidate gene study that reported aberrant endometrial EMX2 expression in women with endometriosis, variants of EMX2 have not been confirmed to contribute to the development of endometriosis. Therefore, although promising, linkage analysis has yielded few significant genetic markers.

Due to the unsuccessful attempts in identification of susceptibility genes for endometriosis, efforts were made to identify the gene(s) involved by utilizing hypothesis-free fine mapping association methods or genome-wide association (GWA) methods using very densely spaced SNPs. The methodology examines variation across the genome using computational models for comparison of the genotypes of people with and without disease to identify SNPs associated with disease. Some of the commonly reported endometriosis candidate genes from association studies are glutathione S-transferase 1, estrogen receptor-α, progesterone receptor, TNF-α, IL-6, IL-10, tumor suppressor p53, vascular endothelial growth factor alpha (VEGFA), hydroxysteroid 17-β dehydrogenase 1, etc. Despite these findings, to date, candidate gene studies have been unsuccessful in providing robust genetic variants associated with endometriosis. Both theoretical and empirical evidences suggest many genes or variants with small effects as risk factors.

**GENE EXPRESSION STUDIES: cDNA MICROARRAYS**

Several studies have been undertaken using the cDNA microarray approach in an attempt to elucidate the mechanisms involved in the pathogenesis of endometriosis. However, the data obtained from these studies are incomplete owing to usage of chips containing limited number of human cDNAs. Hence, came the advent of using a whole genome cDNA microarray approach to address this issue. The main advantage of using a whole-genome cDNA microarray approach is that it allows identification of families or pathways of genes that change in concert in a disease state rather than the 'hit or miss' approach inherent in the microarrays that contain only a fraction of the genome. Previously, Eyster et al used a microarray platform that contained approximately 3000 human genes to compare gene expression in ectopic vs eutopic endometrium of women with endometriosis. In another study, Eyster et al (2007) screened for 53000 human genes/transcribed sequences for analysis of paired eutopic and ectopic endometrium from the same patients.

CDNA microarray analysis was also found useful for validation of animal model for human endometriosis. Using cDNA microarray technology compared gene expression patterns of ectopic and normal endometrium in a rat model for intestinal endometriosis. Four implants of the uterine horn measuring (2 × 2 mm) were sutured next to the mesenteric vessels of the small intestine in the experimental animals; while in the control animals, four silk sutures without the implants were attached to the mesentery of the intestine. All the animals were sacrificed 60 days postautotransplantation. A total of 168 genes (123 upregulated genes and 45 downregulated) were found to be differentially expressed in ectopic endometrium. Significantly, enriched gene ontology categories were identified. Of these, in particular, genes involved in immune response, inflammatory, chemotaxis, angiogenesis, response to wounding, programmed cell death, metalloproteinase function, cell adhesion, extracellular matrix and collagen metabolism were enriched significantly while downregulated genes were involved in processes like tube development, regulation of ossification and G1/S phase transition of mitotic cell cycle reached statistical significance. These data was found to be comparable with that reported for human disease.

Attempts were made to investigate and characterize the biochemical changes that accompany early stages of development of endometriosis. In one such study, using a mouse model of endometriosis, the peritoneum excluding the transplant was sampled 24, 48 and 96 hours postautotransplantation of endometrium and subjected to microarray analysis. Since, endometriosis is known to be an extraterine condition, the molecular profiling of the peritoneum may provide answers to a higher proportion of women developing peritoneal and ovarian endometriosis as opposed to the rare cases of thoracic endometriosis, colorectal obstructive endometriosis, etc. Results from their data demonstrated an upregulation of adhesion molecules and genes involved in inflammatory response implying the significance of these during the early stages of endometriosis progression.

**Eutopic Endometrium of Women with Endometriosis behaves Differently**

Investigations have been carried out to examine the potential role of eutopic endometrium in the pathogenesis of endometriosis. In fact, there is growing evidence that eutopic endometrium of patients with endometriosis may
be different from that of women without endometriosis. Matsuzaki et al compared eutopic endometrium of women with deep endometriosis was compared with that of women without endometriosis across late proliferative, early-, mid- and late-secretory phases of menstrual cycle. Several genes involved in two important signaling pathways: RAS/RAF/MAPK and PI3K, such as RON, SOS, 14-3-3 protein eta and uPAR in epithelial cells and KSR and PI3Kp85 regulatory subunit alpha in stromal cells were found differentially expressed. Although, several of genes were found to be differentially expressed following cycle-phase matched comparison, these differences were not consistent across different patients with endometriosis when compared with women without endometriosis. The authors defended their data by proposing that the eutopic endometrium may be different among patients presenting different forms of endometriosis.

However, there is an exception to this observation. Sharpe-Timms et al reported no significant differences in gene expression of eutopic endometrium of controls vs that of patients with endometriosis. These contrasting observations could be attributable to differences in the study design and/or the methodology used in different studies.

Analysis of Paired Eutopic and Ectopic Endometrium of Women with Endometriosis

The gene expression profiles of paired eutopic and ectopic endometrium of women with endometriosis have been compared. Several genes were found to be differentially expressed between paired eutopic and ectopic endometrium of women with endometriosis. Eyster et al reported differential expression of eight genes namely β-actin, α-2 actin, vimentin, 40S ribosomal protein S23, Ig-γ light chain, Ig germ line H chain G-E-A region γ-2 constant region gene, major histocompatibility complex class 1, C and complement component 1S subcomponent in endometriosis implants compared with uterine endometrium. Out of the eight genes that were differentially expressed, six were unique to the endometrium, with β-actin and α-2 actin genes common to other tissues (lung, placenta and uterus) as well. Ectopic endometrium is considered as an invasive tissue requiring cytoskeleton rearrangement that facilitates cell attachment and movement. It is known that vimentin and some forms of actin form cytoskeleton links. This probably explains the upregulation of β-actin, α-2 actin and vimentin expression in endometriosis implants. Also, vimentin has been shown to have an indirect role in steroidogenesis. The lipid droplets containing cholesterol are connected with the cytoskeleton via vimentin, allowing increased recruitment of these droplets by mitochondria during higher need of ectopic endometrium for estrogen synthesis. Although ribosomal proteins are generally considered being housekeeping genes, their expression has also been found to be regulated by estrogen. Increased estrogen synthesis implies differential expression of estrogen-regulated genes and, therefore, an increased need for translation machinery. This justifies the upregulation of 40S ribosomal protein S23 in endometriosis implants compared to eutopic endometrium. Dysfunction of immune system has also been implicated in the etiology of endometriosis. Upregulation of immune-related genes like Ig-λ light chain, Ig germ line H chain G-E-A region γ-2 constant region gene, major histocompatibility complex class 1, C and complement component 1S subcomponent in ectopic endometrium further supports the role of immune system in the development of endometriosis.

Khan et al compared paired eutopic and ectopic endometrium of 18 fertile women with ovarian endometriosis for genome-wide expression profiles. Of the many genes, around 75 and 50% of expressed genes showed marked signals in autologous eutopic and ectopic samples, respectively. Genes that were differentially expressed by three-fold among different categories of comparison were used for generation of differential expression (DE) patterns. These DE patterns were used to highlight the enriched categories of pathways that could be involved in the pathogenesis of ovarian endometriosis. Among the enriched pathways, several signaling pathways involved in immune responses were commonly affected in eutopic and ectopic endometrium of women with endometriosis. Other than these, pathways related to cellular signaling, apoptosis and survival, cytoskeleton remodeling, chemotaxis, cell adhesion and several neurophysiological processes were also affected in accordance with several reports based on different experimental models. Collectively, these studies indicate that eutopic endometrium which is transcriptionally dysfunctional in mediating immune and endocrine responses may be vulnerable to a transformation into endometriotic lesions. Studies also indicate a risk for malignant transformation in endometriosis.

Gene expression profiles of paired early proliferative phase eutopic and ectopic endometria of patients with endometriosis were compared in patients having ovarian as well as peritoneal endometrioma. Using rapid subtractive hybridization (RaSH) method, a total of 291 genes were found to be differentially expressed, out of which 191 were upregulated and 100 were downregulated in lesions. Out of these, 17 have been reported
previously for their association with endometriosis. These include c-MYC, MMP3, IGFBP1, CTGF, and PAEP while 274 genes have not been known for their association with endometriosis. Also, when this data was compared with that obtained from comparative genome hybridization (CGH) studies in endometriotic lesions, an overlap of 36 upregulated genes and 16 downregulated genes with the regions of gain and loss of genomic material respectively was observed. This further demonstrates the contribution of genomic alterations to the pathophysiology of endometriosis.

**Gene Expression Analyses: A Tool for Classification of Endometriosis**

There are different types of endometriosis known and various theories of histogenesis have been proposed based upon the location of endometriotic lesion. It is therefore important to identify factors associated with different forms of endometriosis and their role in the clinical outcome of the disease. Till date, few attempts have been made to classify endometriosis on the basis of their expression profiles. In one such attempt, Wu et al taking into account the menstrual phase and location of the lesion, identified differentially expressed 904 genes/ESTs in eutopic and ectopic endometrium of women with endometriosis. These differentially expressed genes were found to be enriched in different pathways which include platelet-derived growth factor receptor α (PDGFRA), platelet-derived growth factor β (PDGF), rapidly accelerated fibrosarcoma (RAF1), mitogen-activated protein kinase (MAPK6), DUSP5 (a member of the MAPK phoshatase family), PLA2G5 (a member of cytosolic phospholipase A2 family), MKNK1 and RPS6KA3 (a member of ribosomal S6 kinase 2), transforming growth factor beta-3 (TGFβ3), RCA1 (a member of Cdo42/Rac family), AKT1, HSPB2, GSTM1, GGTLA1, GSTD1, and GXP4 (involved in glutathione metabolism), SOD1, cytochrome C (involved in oxidative stress), HSD2 mRNA was detected in epithelial cells from both OE and DIE. Few genes like PDGFRA and members of the downstream RAS/RAF/MAPK signaling pathway were differentially expressed between stromal cells from DIE and matched eutopic endometrium. These findings suggest that hormonal microenvironments, in association with aberrant expression of other genes, could influence the development of a particular form of endometriosis.

Zhao et al performed gene set enrichment analysis (GSEA) of six independent publicly available gene expression data sets to identify the common biological pathways involved in endometriosis. Gene set enrichment analysis method identifies differences in expression between normal and patient samples. Gene set enrichment analysis is likely to be a more powerful tool than conventional single-gene analysis in the study of complex diseases to investigate the subtle contribution of group of genes. Comparisons of the gene expression between lesion locations (ovarian vs peritoneal), phases of the uterine cycle (proliferative to midsecretory), and cell types (endometrial endothelial cells vs whole tissue) as well as overall eutopic vs ectopic endometrium were selected for the analyses. The transcriptomes of eutopic and ectopic endometrium suggested that ovarian endometriosis and peritoneal endometriosis are two different diseases.
CONCLUSION

A deeper understanding into the pathogenesis of the disease can be achieved by focusing on deregulation of gene sets or pathways rather than on individual genes. Microarray is indeed an effective tool to study the differential expression of genes in biological tissues exposed to different microenvironments. Microarray based data have indicated differences in the gene expression profiles, depending upon the location of an ectopic endometrium. However, more such studies are needed in a larger sample size to deduce the specific role and interactions of the genes included in the related pathways. Such studies will expand our understanding of the etiology and pathogenesis of endometriosis and also will assist in the eventual development of novel treatments for endometriosis.

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