Analysis of Differential Genes of Uyghur Women with Endometriosis in Xinjiang

Aixingzi Aili, Ding Yan, Hu Wenjing, Yang Xinhua and Hanikezi Tuexun
Xinjiang Medical University, China

1. Introduction

Endometriosis (EM) is a common and important health problem, it is estimated to be present in 10%-15% of women in the reproductive age group and 25%-35% of infertile women. In the First Affiliated Hospital of Xinjiang Medical University in China, 447 cases primarily diagnosed with surgically confirmed endometriosis between January 2000 to September 2005, among them 349 cases of endometriosis were Han Chinese (78.1%) and 69 cases Uyghur women with endometriosis (15.3%).

Fig. 1.

Xinjiang is the biggest province of China inhabited by ethnic minorities in which Uyghur people are accounted for more than 40% of the total population. In recent years, the number of the Uyghur women with endometriosis have been increased in Xinjiang, however still clearly less than Han chinese with endometriosis. The data from pathology departemnt of the First Affiliated Hospital of Xinjiang Medical University between 1992 and 1996 showed that there were only three Uyghur women with endometriosis (5.76%) among 52 cases. Between 2000 and 2001, only 4 Uyghur women with endometriosis (3.1%) among 128 patients. Between 2003 and 2010, there were 73 Uyghur women (13.45%) with endometriosis.
in 565 cases. In Kashi, the Uyghur is occupied more than 80% of population. In the last 8 years, there were only 16 Uyghur women with endometriosis among 600 cases of endometriosis in People's Hospital of Kashi. It was demonstrated that the number of Uyghur women with endometriosis dramatically lower than Han Chinese.

We performed AtlasTMcDNA Expression Arrays (Clontech # 7854-1) cDNA microarray (containing 22,000 DNA) to compare the differential expression genes between ectopic endometrium of Uyghur and Han Chinese women with endometriosis. Our study aimed to explore the molecular pathogenesis of endometriosis ethnic differences, so as to determine the cause of endometriosis of Uyghur women in Xinjiang.

Fig. 2. Total RNA results.

Fig. 3. Uyghur with and without endometriosis ectopic endometriosis hybrid.
Fig. 4. Han with and without endometriosis ectopic endometriosis hybrid.

Fig. 5. Uyghur with and without endometriosis ectopic endometriosis hybrid before and after correction signal strength distribution.
Fig. 6. Han with and without endometriosis ectopic endometriosis hybrid before and after correction signal strength distribution.

Fig. 7. Uyghur with and without endometriosis ectopic endometriosis hybrid before and after correction signal scatter.
Fig. 8. Han with and without endometriosis ectopic endometriosis hybrid before and after correction signal scatter.

| ID   | Name  | Cy5/Cy  | Description                                                                                       |
|------|-------|---------|---------------------------------------------------------------------------------------------------|
| 4340 | FOS   | 3.649786| V-fos FBJ murine osteosarcoma viral oncogene homolog                                           |
| 7224 | DCN   | 2.250099| Decorin                                                                                           |
| 10599| VIM   | 1.629836| Vimentin                                                                                         |
| 1900 | GNG5  | 1.211619| Guanine nucleotide binding protein (G protein), gamma 5                                           |
| 1527 | XCL1  | 1.169442| Small inducible cytokine subfamily                                                               |
| 13167| IGFBP7| 1.114798| Insulin-like growth factor binding protein 7                                                      |
| 1651 | RPS23 | 1.093641| Ribosomal protein S23                                                                            |
| 22665| TIMP3 | -1.15294| Tissue inhibitor of metalloproteinase 3                                                           |
| 13265| COL3A1| -1.55893| Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)                |
| 21389| RPL29 | -1.63396| Ribosomal protein L29                                                                           |
| 8626 | GAPD  | -2.01536| Glyceraldehyde-3-phosphate dehydrogenase                                                          |
| 22689| GAPD  | -2.30404| Glyceraldehyde-3-phosphate dehydrogenase                                                          |
| 22785| GAPD  | -2.71698| Glyceraldehyde-3-phosphate dehydrogenase                                                          |

Table 1. Uyghur with and without endometriosis ectopic endometriosis differential genes.
| ID     | Name    | Rate of Cy5/Cy3 | Description                                                                                                                                                                                                 |
|--------|---------|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 18428  | FTL     | 4.340706       | ESTs, Weakly similar to FRHUL ferritin light chain [H.sapiens]                                                                                                                                              |
| 8586   | APOE    | 3.768085       | Apolipoprotein E                                                                                                                                                                                            |
| 7998   | CD74    | 3.623251       | CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated                                                                                                                                                      |
| 21034  | CTSD    | 2.992721       | Cathepsin D (lysosomal aspartyl protease)                                                                                                                                                                   |
| 20900  | IGL1    | 2.941947       | H.sapiens mRNA for IgG lambda light chain V-J-C region (clone Tg19)                                                                                                                                         |
| 18699  | BIN3    | 3.235432       | Bridging integrator 3                                                                                                                                                                                       |
| 10633  | CTSB    | 2.736133       | Cathepsin B                                                                                                                                                                                                |
| 16743  | IGKV1-9 | 2.482954       | Immunoglobulin kappa variable 1-9                                                                                                                                                                           |
| 10901  | IGHG3   | 2.24961        | Immunoglobulin heavy constant gamma 3 (G3m marker)                                                                                                                                                          |
| 17716  | ZFHX1   | 2.221742       | Zinc finger homeobox 1 b                                                                                                                                                                                   |
| 15732  | ACTA2   | 1.939791       | Actin, alpha 2, smooth muscle, aorta                                                                                                                                                                       |
| 6876   | HLA-G   | 1.795164       | HLA-G histocompatibility antigen, class I, G                                                                                                                                                               |
| 9464   | HLA-A   | 1.617767       | Major histocompatibility complex, class I, A                                                                                                                                                               |
| 12611  | ITM2C   | 1.60875        | Integral mendometriosisbrane protein 3                                                                                                                                                                     |
| 9202   | DLGAP4  | 1.56221        | KIAA0964 protein                                                                                                                                                                                          |
| 6875   | WNT7A   | 1.55996        | Wingless-type MMTV integration site family, mendometriosisber 7A                                                                                                                                            |
| 6494   | FTH1    | 1.55145        | Ferritin, heavy polypeptide 1                                                                                                                                                                              |
| 6952   | TMSB10  | 1.539032       | Thymosin, beta 10                                                                                                                                                                                          |
| 22794  | ACTB    | 1.51553        | Actin, beta                                                                                                                                                                                                |
| 22769  | ACTB    | 1.495315       | Actin, beta                                                                                                                                                                                                |
| 10474  | FLJ14950| 1.489821       | Hypothetical protein FLJ14950                                                                                                                                                                              |
| 13167  | IGFBP7  | 1.486395       | Insulin-like growth factor binding protein 7                                                                                                                                                               |
| 18071  | HUMMHC  | 1.460991       | Cw1 antigen                                                                                                                                                                                                |
|        | W1A     | 1.441213       | Secreted protein, acidic, cysteine-rich (osteonectin)                                                                                                                                                      |
| 22698  | ACTB    | 1.45868        | Actin, beta                                                                                                                                                                                                |
| 12651  | SPARC   | 1.441213       | Secreted protein, acidic, cysteine-rich (osteonectin)                                                                                                                                                      |
| 22696  | RPL5    | 1.421712       | Ribosomal protein L5                                                                                                                                                                                       |
| 13180  | LOC51237| 1.416254       | Hypothetical protein                                                                                                                                                                                        |
| 14701  | HSU79274| 1.391079       | Protein predicted by clone 23733                                                                                                                                                                           |
| 22793  | ACTB    | 1.374773       | Actin, beta                                                                                                                                                                                                |
| 5712   | GPX3    | 1.370492       | Glutathione peroxidase 3(plasma)                                                                                                                                                                            |
| 22985  | ACTB    | 1.342603       | Actin, beta                                                                                                                                                                                                |
| 22674  | ACTB    | 1.333726       | Actin, beta                                                                                                                                                                                                |
| 22697  | ACTB    | 1.33342        | Actin, beta                                                                                                                                                                                                |
| 21176  | KPTT13  | 1.327325       | Keratin 13                                                                                                                                                                                                  |
| 22889  | ACTB    | 1.325337       | Actin, beta                                                                                                                                                                                                |
| 22700  | CYCI    | 1.325323       | Cytochrome c-1                                                                                                                                                                                             |
| 23193  | LOC389643| 1.323453   | LOC389643                                                                                                                                                                                                  |

Table 2. Han with and without endometriosis ectopic endometriosis differential genes.
| ID   | Name       | Rate of Cy5/Cy3 | Description                                                                 |
|------|------------|-----------------|-----------------------------------------------------------------------------|
| 9988 | ACTB       | 1.293784        | Actin, beta                                                                 |
| 20299| IL1 RN     | 1.283803        | Interleukin 1 receptor antagonist                                            |
| 16687| TP73       | 1.277275        | Tumor protein p73                                                           |
| 22986| ACTB       | 1.267406        | Actin, beta                                                                 |
| 22770| ACTB       | 1.258614        | Actin, beta                                                                 |
| 10599| VIM        | 1.251847        | Vimentin                                                                    |
| 22865| ACTB       | 1.233357        | Actin, beta                                                                 |
| 22345| LOC440552  | 1.232367        | similar to OK/SW-CL.16                                                      |
| 8173 | MARK2      | 1.227909        | ELKL motif kinase                                                           |
| 22962| ACTB       | 1.219306        | Actin, beta                                                                 |
| 21216| SLPI       | 1.215856        | Secretory leukocyte protease inhibitor                                       |
| 22690| ANKT       | 1.213863        | Nucleolar protein                                                           |
| 23200| LOC389622  | 1.212664        | LOC389622                                                                   |
| 22961| ACTB       | 1.212137        | Actin, beta                                                                 |
| 22673| ACTB       | 1.210056        | Actin, beta                                                                 |
| 22866| ACTB       | 1.208872        | Actin, beta                                                                 |
| 19718| SULT1C2    | 1.207869        | Sulfotransferase family, cytosolic, 1C, mendometriosisber 2                  |
| 12903| HSPA5BP1   | 1.179906        | Hypothetical protein FLJ20539                                               |
| 22785| PDGFRA     | 1.159421        | Platelet-derived growth factor receptor                                     |
| 1900 | GNG5       | 1.125384        | Guanine nucleotide binding protein (G protein), gamma 5                      |
| 20231| SERF2      | 1.112907        | Small EDRK-rich factor 2                                                    |
| 15491| ZNF14      | 1.104864        | Zinc finger protein 14 (KOX 6)                                             |
| 9735 | KIAA0635   | 1.100348        | Hypothetical protein FLJ13621                                              |
| 16540| CDW92      | 1.092801        | CDw92 antigen                                                               |
| 22762| COPEB      | 1.08944         | Core promoterelendometriosisent binding protein                             |
| 22866| ACTB       | 1.083347        | Actin, beta                                                                 |
| 8626 | GPX3       | 1.078504        | Glutathione peroxidase 3(plasma)                                           |
| 1401 | ELAVL3     | 1.077059        | ELAV (endometriosisbryonic lethal, abnormal vision, Drosophila)-like 3 (Hu antigen C) |
| 19581| GNG5       | 1.057676        | Guanine nucleotide binding protein (G protein), gamma 5                      |
| 7186 | ID3        | 1.057184        | Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein       |
| 17362| FKBP14     | 1.036957        | Hypothetical protein FLJ20731                                              |
| 9824 | MTBP       | 1.030879        | Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)     |
| 8364 | ARHGDI     | 1.029915        | Rho GDP dissociation inhibitor (GDI) alpha                                  |
| 21961| RPS23      | 1.025033        | Ribosomal protein S23                                                      |
| 1580 | NELF       | 1.017617        | DKFZP586j1624 protein                                                       |
| 22665| GAPD       | -1.003765       | Glyceraldehyde-3-phosphate dehydrogenase                                   |
| 22761| GAPD       | -1.001544       | Glyceraldehyde-3-phosphate dehydrogenase                                   |
| 17133| GAPD       | -4.29046        | Glyceraldehyde-3-phosphate dehydrogenase                                   |

Table 2. Han with and without endometriosis ectopic endometriosis differential genes. (Continuation)
2. The incidence of endometriosis of Uyghur ethnic group in Xinjiang

The incidence of endometriosis has no precise information. Researchs have found that Asian women with endometriosis have a higher prevalence, and its disease risk OR:8.6 (95% CI 1.4 – 20.7). There was an exploratory study suggested that there might be an associated risk of endometriosis for those women who have worked as a flight attendant, service station attendant, or health worker, particularly a nurse. But they have not been reported the correlation between nationality, religion and other factors with endometriosis.

Clinical and epidemiological survey found that endometriosis has a genetic predisposition and significant family aggregation, and it loss of heterozygosity of 40% -70%. Dingyan (researcher in Xinjiang) found that no evidence was found to suggest an association between GSTM1-null genotype and endometriosis in the Hans chinese and Uyghurs. An association was found between GSTT1 -null genotype and endometriosis in the Hans chinese, but not in the Uyghurs. The two ethnic groups have different genetic predisposing factors to the development of endometriosis. There were significant difference in the frequencies of these two points among the Han chinese, European and Uyghur in Xinjiang. In Uyghur the distribution of CYP 1 A 1 /MspI genotypes were different from Han chinese and European.

3. The spectrum of microarray applications on endometriosis

A large number of microarray gene-specific cDNA are fixed on a glass or silica using the hybridization principles to detect the mRNA of the different sources. This study shows the different organization, different cells and tissues in different developmental stages that have differentially expressed genes. Development of molecular mechanisms provide theoretical basis for gene diagnosis and treatment of cutting-edge biotechnology. The theory proposed by Sampson in 1927 suggests that endometrial tissue is released into the peritoneal cavity via retrograde menstruation. The shed tissue then implants and grows ectopically. This theory is supported by the fact that up to 76% -90% of women experience retrograde menstruation; and yet, endometriosis only affects 10% -15% of women. Reference to foreign literature, different individuals sample of patient with endometriosis geometric mixed, different individuals sample of patient without endometriosis geometric mixed, to eliminate non-specific genetic differences between individuals, and search for specific associated genes with endometriosis. By the gene microarray expression profiling 22,000 points compare ectopic endometrium and normal ectopic endometrial of the Uyghur and Han chinese with endometriosis, 11 differential genes expressed in ectopic endometrium were
screened out between Uyghur women with or without endometriosis respectively, FOS, DCN, VIN, GNGS, XCL 1, IGFBP7, PRS23, TIMP3, COL3A1, PRL29, GAPD; GAPD expression in the three loci, including FOS, DCN, VIN, GNGS, XCL1, IGFBP7, PRS23 were up-regulated, and TIMP3, COL3A1, PRL29, GAPD were down-regulated. The Han chinese group were significantly different genes, 58 of which TIMP3, PAEP, GAPD were down-regulated, but GAPD expressed in three loci shows different range. And from a different CD74, ACTA2, GPX3 and other 55 genes were upregulated, ACTB appear in 17 loci, GNGS appear in two loci. The same genes difference between the two groups is VIM, GNGS, PRS23, GAPD, TIMP3, including GAPD, TIMP3 are down-regulated. We get different genes according to their main function and are divided into the following categories: immune-related genes, proto-oncogenes and tumor suppressor genes, cell receptor, ion channels and transport protein, cytoskeleton and sports-related protein, apoptosis-related protein, DNA synthesis and repair, recombinant protein, DNA binding, transcription factors, cell signaling and transmission white and some unknown functional genes.

4. The possible role of clinically relevant different gene in endometriosis pathogenesis

The difference in the screened genes, tissue inhibitor of metalloproteinase 3 (TIMP-3) both in the Han chinese and Uyghur with endometriosis were down-regulated. The study of Zhou Honghui found that TIMP-3 down-regulation is remarkable in the secretory phase than proliferative phase. TIMP is a metalloproteinase (MMPs) inhibitors by the endometrial cells of MMPs which plays an important role in the invasion of the peritoneum and other connective tissue. Increased endometrial MMPs and TIMP down-regulation with the development of endometriosis is closely related. Because of TIMP up-regulation and MMPs down-regulation, ectopic endometrial of endometriosis is more invasive than normal force, and develop to the peritoneal endometriotic lesions. Angiogenesis is considered as a major process in the pathogenesis of endometriosis. Many factors are involved in this complex mechanism, and the vascular endothelial growth factor (VEGF) is an important mediator of angiogenesis; it is a potent endothelial cell mitogen, morphogen, and vascular permeability-inducing agent. VEGF binds to either of two tyrosine kinase receptors, the fm5-like tyrosine kinase (flt) and the kinase domain receptor (KDR or Flk-1). Peritoneal endometriotic lesions with high proliferative activity are also accompanied by high angiogenic activity, as reflected by higher expression of VEGF-A in stroma and glandular epithelium and VEGFR-2 in blood vessels. In our recent study, we showed that the vascular density and the expression of VEGF and its receptor VEGFR-2 (Flk-1) are significantly higher in deeply infiltrating endometriosis affecting the ovary, bladder and mainly the rectosigmoid, compared with the ectopic endometrium.

Controlled clinical analyses of angiogenesis in human endometriotic lesions are limited, because it is not possible to monitor the lesions without repeated laparoscopies. Thus, research into the fundamental mechanisms by which menstrual endometrium adheres, invades and establishes a functional vasculature to persist in an ectopic site, as well as the development of new therapeutical approaches, is best performed in experimental animal models. In contrast to humans and non-human primates, estrous animals do not shed their endometrial tissue and therefore do not develop endometriosis spontaneously. However,
Endometriosis can be induced by transplanting endometrial tissue to ectopic sites, and the establishment of an experimental model of endometriosis may be a good way to study the endometriosis angiogenesis process, and allow evaluation of the balance of the many factors involved.

This study by glyceraldehyde 3-phosphate dehydrogenase (GAPD) gene in Han Chinese and Uyghurs with endometriosis group are down-regulated, GAPD genes are housekeeping gene family, Gene bank No. NM-002046, is a basic enzyme in the human body. It is a key enzyme of a series of biochemical reactions of the glycolysis, which generate ATP for the source of human cells energy, a variety of cells are present in the body, involved in glucose metabolism in glycolysis, in 12p13.

In Han and Uyghur groups the same set of common up-regulated genes are GNGS, VIM and PRS23. Abundance or localization changes in endometrial tissue were validated by immunohistochemistry and Western blotting. In addition, multiple charge and size isoforms were observed for VIM in endometriosis patients that was below the level of detection in healthy women.

Our experiment confirmed endometriosis may be related to multiple factors similar as diabetes, asthma, cancer-related disease, genetics and aberrant regulation in the endometrium and endometriotic. Lower different genes expression on Uyghur women with endometriosis compared to Han Chinese women with endometriosis may be the essential factor for relatively lower incidence of endometriosis on Uyghur women. Most genes we found on the endometrium of both Uyghur and Han Chinese women with endometriosis were the cytoskeleton, adhesion, invaded and immune related gene, partially explained the mechanism of malignant biological behaviors.

5. References

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This book provides an insight into the emerging trends in pathogenesis, diagnosis and management of endometriosis. Key features of the book include overviews of endometriosis; endometrial angiogenesis, stem cells involvement, immunological and hormonal aspects related to the disease pathogenesis; recent research reports on infertility, endometrial receptivity, ovarian cancer and altered gene expression associated with endometriosis; various predictive markers, and imaging modalities including MRI and ultrasound for efficient diagnosis; as well as current non-hormonal and hormonal treatment strategies. This book is expected to be a valuable resource for clinicians, scientists and students who would like to have an improved understanding of endometriosis and also appreciate recent research trends associated with this disease.

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