Original Article

Persistent dysregulation of genes in the development of endometriosis

Yanli Chen[#], Yanqun Ma[#], Yanzhi Zhai[#], Haiyan Yang, Chunlan Zhang, Yingxin Lu, Wei Wei, Qing Cai, Xuewen Ding, Shan Lu, Ziyu Fang

Department of Obstetrics and Gynecology, The Fourth Affiliated Hospital of Guangxi Medical University, Liuzhou, China

Contributions: (I) Conception and design: Y Chen, Y Ma, Y Zhai; (II) Administrative support: H Yang, W Wei; (III) Provision of study materials or patients: C Zhang, Y Lu; (IV) Collection and assembly of data: Y Zhai, Q Cai, X Ding; (V) Data analysis and interpretation: S Lu, Z Fang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work and should be considered as co-first authors.

Correspondence to: Ziyu Fang. Department of Obstetrics and Gynecology, The Fourth Affiliated Hospital of Guangxi Medical University, Liuzhou, China. Email: fangziyu2009@163.com.

Background: Endometriosis is a chronic condition that affects women of child-bearing age. Since the etiology and pathogenesis of endometriosis have not been fully elucidated, it is important to investigate the mechanisms that lead to the deterioration of endometriosis.

Methods: In this study, the transcriptome data of patients with normal, mild, and severe endometriosis were examined using the GSE51981 dataset obtained from the Gene Expression Omnibus database. Short Time Series Expression Miner (STEM) was used to screen the genes with continuous expression disorder in the development process, and the core genes were identified by constructing a protein-protein interaction network. The molecular mechanisms of endometriosis were examined using enrichment analysis. Finally, the transcription factors that regulate the core genes were predicted and the comprehensive mechanisms involved in the development of endometriosis were examined.

Results: A total of 3,472 differentially expressed genes were identified from the normal, mild, and severe endometriosis samples. These were allocated into 12 modules and *HRAS*, *HSP90AA1*, *TGFB1*, *TP53*, and *UBC* were selected as the core genes. Enrichment analysis showed that the genes in modules 6, 7, and 9 were significantly related to oxygen levels, metallic processes, and hormone levels, respectively. Transcription factor prediction analysis showed that *TP53* regulates *HRAS* to participate in immune related signaling pathways. Drug prediction analysis identified 792 drugs that interact with the targeted core genes.

Conclusions: This study explored the molecular mechanisms involved in the development of endometriosis and identified potential biomarkers of endometriosis. This data may provide novel targets and research directions for the diagnosis and treatment of endometriosis.

Keywords: Endometriosis; Short Time Series Expression Miner (STEM); protein-protein interaction network (PPI network); enrichment analysis; modules

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Introduction

Endometriosis is a chronic and often undiagnosed disease affecting 5–10% of women of child-bearing age. It is characterized by the growth of extrauterine endometrial tissue, most commonly in the abdominal cavity (1), and can manifest as pelvic pain, heterotopia, dysuria, dysmenorrhea, and infertility (2). Unfortunately, for endometriosis and other diseases with similar symptoms, the overall sensitivity and specificity of transvaginal ultrasonography (TVUS) ranges from 53% to 93% (3). At present, there is no reliable

marker to treat this disease (4).

The pathophysiology of endometriosis is complex and not fully understood (5). Study showed that endometriosis results from a combination of anatomical, hormonal, immune, response, oestrogen, genetic, epigenetic and environmental factors in affected women (6). There is increasing evidence that hormones and immune factors may create a microenvironment conducive to inflammation, thereby promoting the persistence of endometriosis (7). The molecular studies have been supported by clinical observations of disease resolution, symptom relief, and so-called "treatment" of endometriosis response to anti-inflammatory drugs (8). However, due to the lack of reliable diagnostic tools and nonspecific symptoms, diagnosis of endometriosis can be delayed by up to 8–10 years. This results in a heavy economic burden as the chronic and debilitating pain caused by endometriosis may hinder work efficiency, while infertility can lead to serious psychological and economic pressures on the affected women and their partners (9).

The American Society of Reproductive Medicine (ASRM) guidelines classify endometriosis into 4 stages using a scoring system, namely stage I, II, III, and IV, which correspond to minimal, mild, moderate, and severe endometriosis, respectively (10). While the mechanisms of malignant transformation of endometriosis have not been fully elucidated, oxidative stress, inflammation, and changes in the hormone environment are all plausible pathogenic factors (11). Hormone levels, especially estrogen, plays an important regulatory role in the deterioration of endometriosis (12). In addition, some high throughput studies have found a role for certain candidate genes in the pathogenesis of endometriosis (13-15). Currently, there is no cure for endometriosis and existing drugs can only help to reduce the symptoms of the disease, with a recurrence rate of 35–50%. Therefore, it is necessary to develop diagnostic indicators and potential treatments for the management of patients with endometriosis (16).

An increasingly accepted view is that diseases can be considered a disturbance to a genetic network (17). The protein-protein interaction (PPI) with known disease genes have been used to identify new disease genes (18). This current study screened and identified potential biomarkers for endometriosis with different severity and further examined the molecular mechanisms of endometriosis. The following article is presented in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4806/rc).

Methods

Data source and difference analysis

The GSE51981 dataset for endometriosis was obtained from the Gene Expression Omnibus (GEO) database and analyzed. The dataset included 34 control samples, 28 samples from patients with mid endometriosis, and 49 samples from patients with severe disease. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The original data from GSE51981 was standardized with the RMA function of the Affy R package to construct the gene expression profile. The differential expression between the mild and control cases, as well as the severe and mild cases, were constructed using the limma R package. The filter threshold was set to a P value <0.05. The Short Time Series Expression Miner (STEM) (v1.3.11) software was used to cluster the differentially expressed genes (DEGs) to screen out the genes with persistent expression disorder (19). Then, DEGs between early-secretory and proliferation, or between mid-secretory and early-secretory were identified using limma R package.

PPI network and decision curve analysis (DCA)

The PPI network was constructed by mapping the genes with continuous expression disorder into the String database (20). The network was visualized using the Cytoscape (3.6.1) software. The important modules in the PPI network were determined using the Molecular Complex Detection (MCODE) plug-in (21), where K-score =5 was used as the standard of MCODE analysis. The R-package circlize was used to show the differences in the genes from each module. Finally, the top 5 genes were selected as the core genes using the Betweenness algorithm of the CytoHubba plug-in.

Furthermore, DCA curve (22) was used to evaluate the diagnostic roles for core genes in GSE51981.

Enrichment analysis

The Gene Ontology (GO) and Kyoto Encyclopedia of Gene and Genome (KEGG) pathways were identified using the clusterProfiler R package (23). The significance level of enrichment of GO and KEGG pathways was set at P<0.05. In addition, the Gene Set Variation Analysis (GSVA) R software package was used to determine the most relevant pathway of persistent maladjustment genes (24).

Regulator prediction

The data of transcriptional target regulation relationship was obtained from the RAID v2.0 database (25). Under the background of transcription factor pairing data, the P value was set to <0.01. The regulatory gene network was then displayed using the Cytoscape software.

The DGIdb (https://www.dgidb.org/) database was used to predict the relationship between core genes and drugs.

Immune levels in endometriosis

Single-sample Gene Set Enrichment Analysis (ssGSEA) was used to calculate the levels of 24 immune cell types between the mild and control cases, or between the severe and mild in GSE51981. Correlation analysis to further show the core genes correlated with immune cells.

Statistical analysis

Using bioinforcloud platform (http://www.bioinforcloud.org.cn) to analyze the all methods in this study. The P value <0.05 was considered statistical significance.

Results

Persistent dysregulated genes involved in endometriosis

The normal, mild, and severe endometriosis samples in the GSE51981 dataset were examined using principal component analysis (PCA). The outlier level between the disease and the control samples was higher, and that of the mild and severe cases were close (*Figure 1A*). A total of 16,453 DEGs were identified between mild endometriosis and normal samples (table available at https://cdn.amegroups.cn/static/public/10.21037atm-22-4806-1.xlsx, *Figure 1B*), while 7,793 DEGs were found between severe and mild endometriosis (table available at https://cdn.amegroups.cn/static/public/10.21037atm-22-4806-2.xlsx). Among them, 6,768 genes were common in the 2 groups of DEGs (*Figure 1C*). To identify the expression trend of these common genes, 3,472 genes were obtained through STEM clustering and shown to be continuously upregulated or downregulated in the progression from normal to mild and then from mild to severe endometriosis, suggesting that these DEGs are persistent dysfunctional genes in the progression of endometriosis. Four significant clustering modules were further identified (*Figure 1D,1E*), and these may be associated with the continuous deterioration of endometriosis (tables available at https://cdn.amegroups.cn/static/public/10.21037atm-22-4806-1.xlsx and https://cdn.amegroups.cn/static/public/10.21037atm-22-4806-2.xlsx, *Figure 1*).

Identification of key disordered genes

To identify the key factors associated with the continued deterioration of endometriosis, 3,472 genes were mapped into the PPI network (*Figure 2A*). A total of 466 genes and 11,211 interacting pairs were obtained, and 12 modules were identified (*Figure 2B,2C*). Correlation analysis showed that modules 6, 7, and 9 were significantly correlated with normal and disease state, and module genes may be related to the occurrence and development of disease (*Figure 2D*).

The top 5 genes were considered core genes, including *HRAS*, *HSP90AA1*, *TGFB1*, *TP53*, and *UBC*. Among them, *HRAS*, *TGFB1*, and *TP53* were upregulated in the process of endometriosis deterioration, while *HSP90AA1* and *UBC* were downregulated (*Figure 2E*). Their expression trend was consistent with the continuous up-down relationship between the STEM box chart and the heat chart. In addition, using receiver operating characteristic (ROC) curve analyses, the area under the curve (AUC) values for mild and severe endometriosis cases revealed that these genes had good clinical diagnostic ability (*Figure 2F*). Using the DCA analysis to assess the diagnostic performance, we found the higher net benefit in the mild endometriosis (*Figure 3A*) and severe endometriosis (*Figure 3B*), thus with better diagnostic role for endometriosis.

In addition, we found there were 28, 18, and 29 samples of endometriosis in proliferation, early-secretory, and mid-secretory, respectively (*Figure 3C*). The difference analysis results showed that there were10,499 DEGs between early-secretory and proliferation, and 7,086 DEGs between mid-secretory and early-secretory (*Figure 3D*). Among these, we found core genes were also differed significantly (*Figure 3E*).

Molecular mechanism of endometriosis

To identify the molecular mechanisms of these DEGs, the 466 genes were analyzed using GO and KEGG enrichment. A total of 3,197 biological processes (BP), 401 cell components (CC), 508 molecular functions (MF), and 126 KEGG signaling pathways were screened. Among them, module 6 genes were significantly related to oxygen levels, module 7 genes were significantly related to metallic process, and module 9 genes were significantly related to

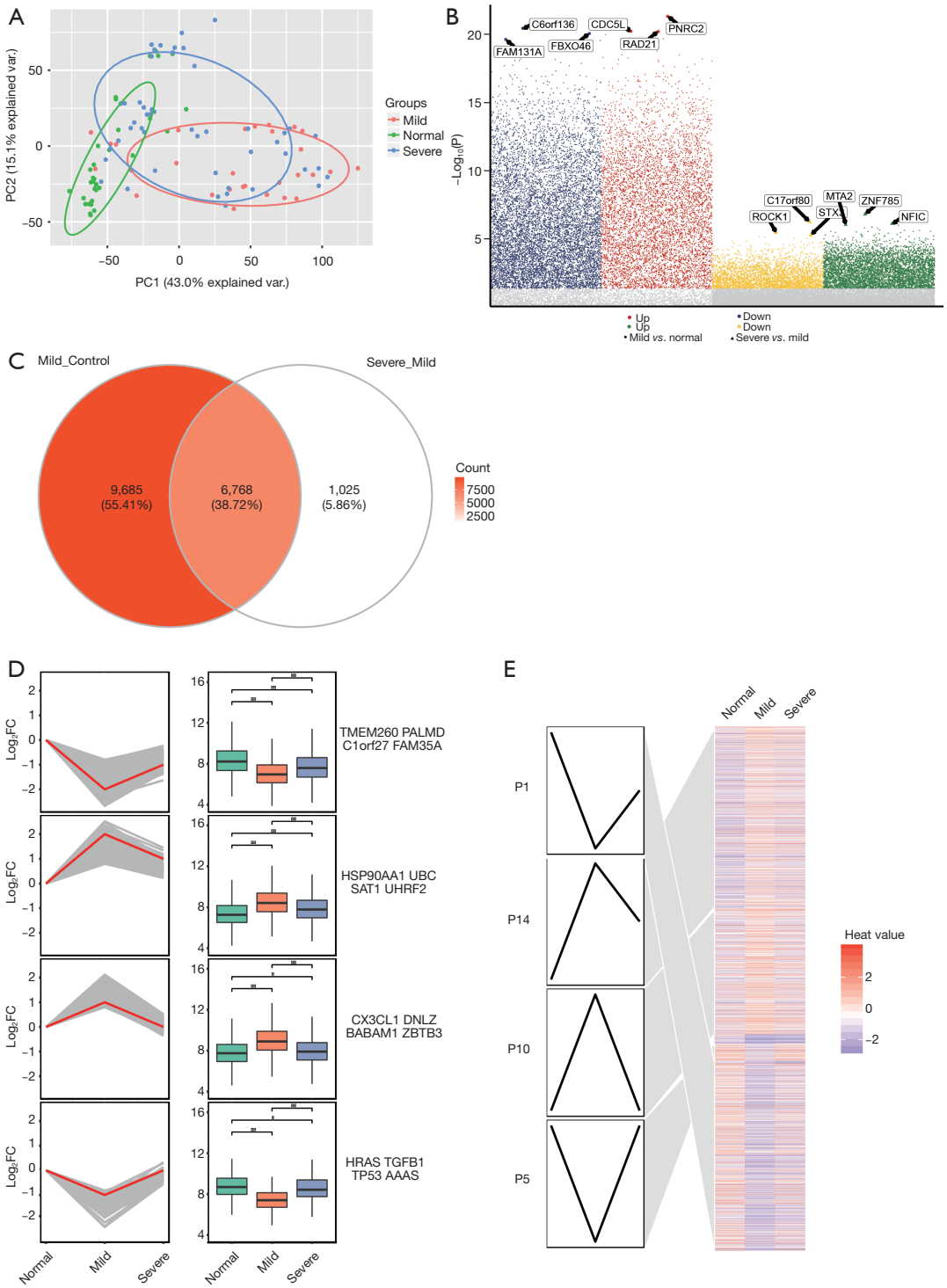


Figure 1 Dysregulated genes that are continuously expressed in endometriosis from normal to mild, and from mild to severe. (A) Principal component analysis for samples of normal, mild, and severe endometriosis. (B) A Manhattan map of the differentially expressed genes for the three groups. (C) The common differentially expressed genes between mild-normal and severe-mild endometriosis. (D) Genes are organized into different clusters based on expression pattern using STEM. Line plots and box plots are used to show fold changes (log_2FC) and absolute expression levels (log_2FPKM), respectively. (E) Line plots and heatmap of different gene clusters. var, variance; PC, principal component; FPKM, fragments per kilobase million; STEM, Short Time Series Expression Miner; FC, fold change.

hormone levels (*Figure 4A*,*4B*).

Through GSVA, we calculated the top changes of KEGG pathways in normal, mild, and severe endometriosis (*Figure 4C*,*4D*). In the upregulated pathway, the changes of olfactory transduction, taste transduction, autoimmune thyroid disease, and nicotine addiction were more obvious. Furthermore, the changes in Fanconi anemia pathway, and ribosome and DNA replication were more obvious (*Figure 4*).

Transcriptional regulators associated with the core disordered genes

To investigate the pathogenesis of endometriosis, the transcription factors that regulate the core genes were predicted. A total of 9 transcription factors were found to regulate the core genes. Among them, *JUND* and *TGFβ1*, *STAT1* and *HSP90AA1*, and *TP53* and *HRAS* were highly correlated, and thus, *JUND*, *STAT1*, and *TP53* were considered key regulators (*Figure 5A*). *TP53* was also one of the core genes. These transcription factors that regulate the core genes were found to be involved in many signaling pathways, including oxidative stress, hormone regulation, and inflammation related signaling pathways (*Figure 5B*), as well as playing a key role in the disease progression of endometriosis. In addition, drug prediction analyses using the DGIdb database identified 792 drugs that target the core genes (table available at https://cdn.amegroups.cn/static/public/10.21037atm-22-4806-3.xlsx, *Figure 5*).

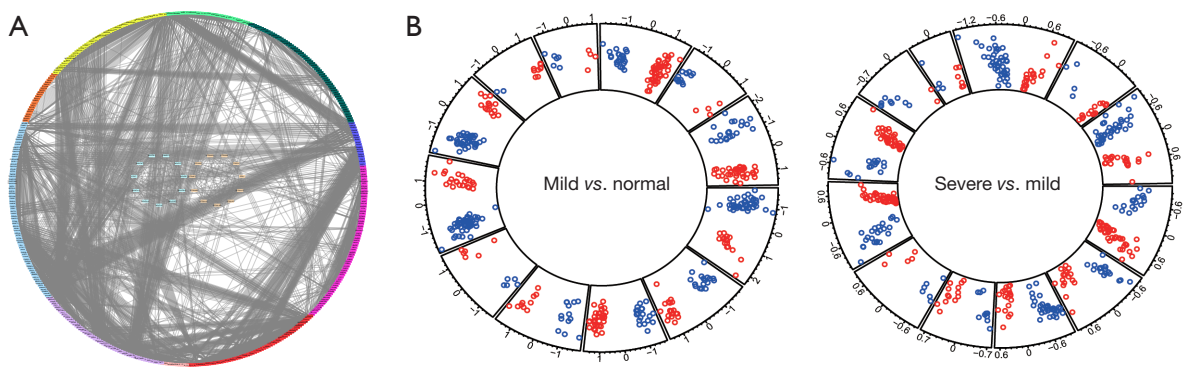
Immune analysis

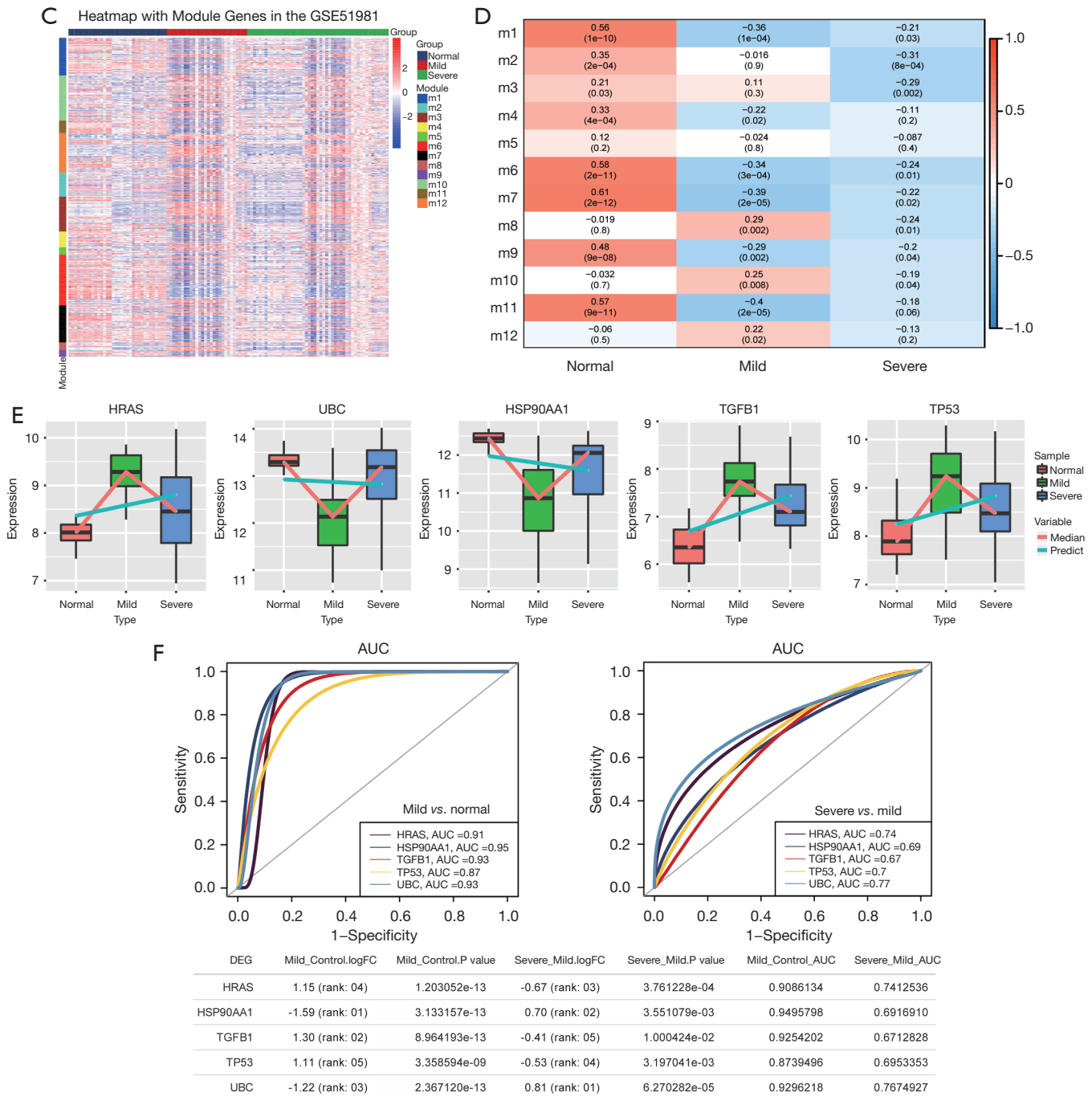
Through ssGSEA, we found that Th2, T helper cells, and central memory T cells (Tcm) were significantly decreased in mild compared with normal (*Figure 6A*), while significantly elevated in severe compared with mild (*Figure 6B*). The follicular helper T cells (Tfh) and immature dendritic cell (iDC) were significantly elevated in mild compared with normal, and significantly decreased in severe compared with mild. Correlation results showed that T helper cells and natural killer (NK) cells had the biggest correlation with core genes in both mild (*Figure 6C*) and severe (*Figure 6D*) endometriosis. Interestingly, there was a completely opposite correlation between NK and core genes, and between T helper cells and core genes.

Discussion

The implantation and growth of endometrial cells is the main cause of endometriosis. Endometriosis exerts a significant social and psychological burden on women in many respects, including quality of life, intimacy, childbearing, education and work, and emotional health (26). Furthermore, endometriosis is often related to cardiovascular and autoimmune diseases (27). Recent advances in the field of endometriosis have identified novel biomarkers that may be developed as targets for the treatment of endometriosis, including immunomodulators and inhibitors, which have been tested on animal models (28). This current study identified and examined the molecular targets and mechanisms involved in the progression of endometriosis. This data will contribute to the diagnosis, treatment, and management of patients with endometriosis.

The continuous disordered expression of certain genes is likely to be involved in the development of endometriosis and may be potential therapeutic targets. This study screened and identified a series of persistent maladjustment genes. Among them, *HRAS*, *HSP90AA1*, *TGFB1*, *TP53*, and *UBC* were identified as core factors. Previous studies have





DEG	Mild_Control.logFC	Mild_Control.P value	Severe_Mild.logFC	Severe_Mild.P value	Mild_Control_AUC	Severe_Mild_AUC
HRAS	1.15 (rank: 04)	1.203052e-13	-0.67 (rank: 03)	3.761228e-04	0.9086134	0.7412536
HSP90AA1	-1.59 (rank: 01)	3.133157e-13	0.70 (rank: 02)	3.551079e-03	0.9495798	0.6916910
TGFB1	1.30 (rank: 02)	8.964193e-13	-0.41 (rank: 05)	1.000424e-02	0.9254202	0.6712828
TP53	1.11 (rank: 05)	3.358594e-09	-0.53 (rank: 04)	3.197041e-03	0.8739496	0.6953353
UBC	-1.22 (rank: 03)	2.367120e-13	0.81 (rank: 01)	6.270282e-05	0.9296218	0.7674927

Figure 2 The PPI network of the continuously expressed maladjusted genes. (A) PPI networks of the continuously expressed maladjusted genes. (B) The differentially expressed genes of the two groups are displayed in the modules. (C) A heatmap of the differentially expressed genes in modules. (D) The results of correlation analysis between modules and phenotypes. (E) The expression trend of the core genes in the process of endometriosis. (F) The AUC value of the core genes in the different endometriosis processes. AUC, area under the curve; DEG, differentially expressed gene; FC, fold change; PPI, protein-protein interaction.

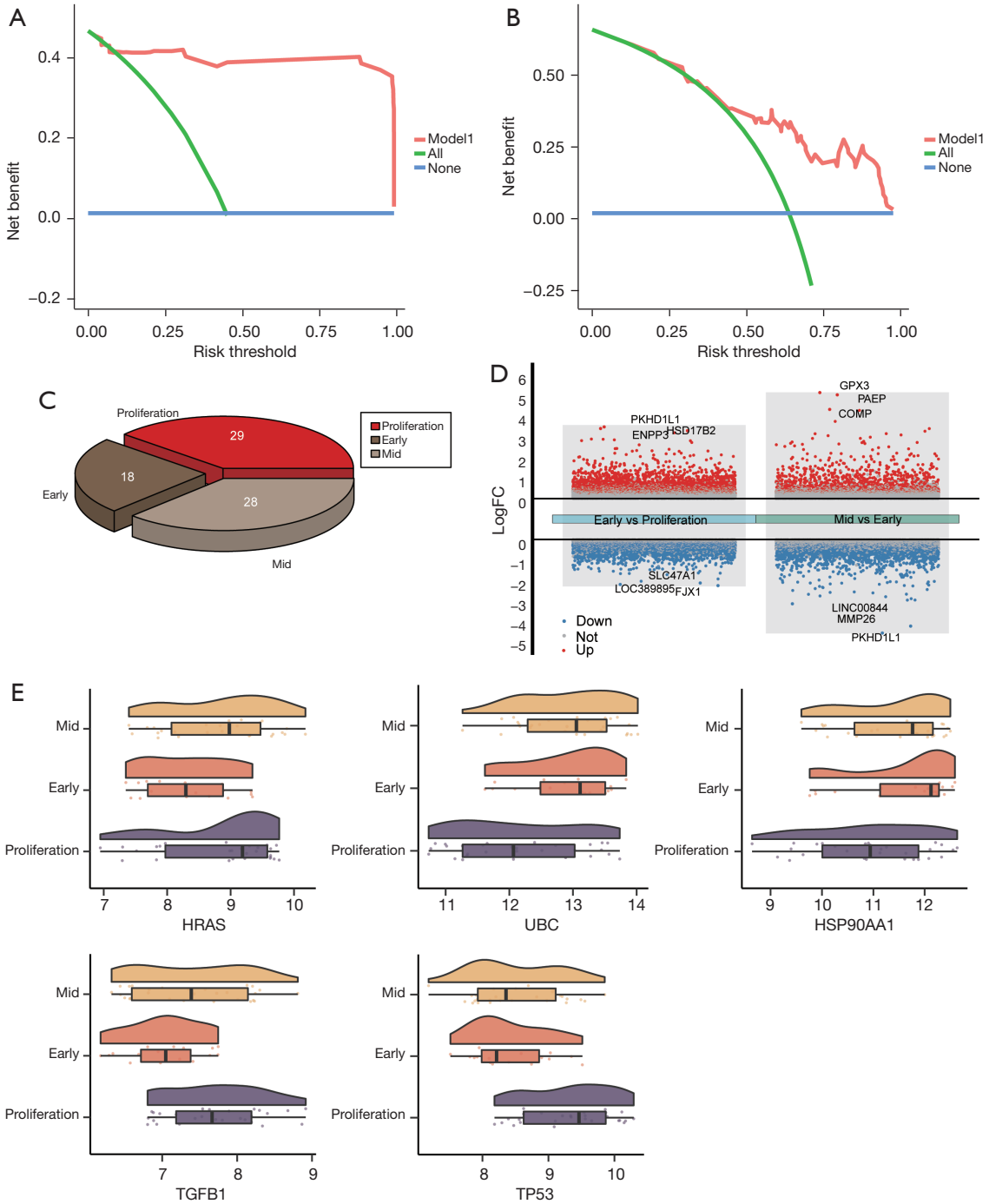


Figure 3 Evaluation of core genes. (A) Decision curve analysis for detectable mild endometriosis risk. (B) Decision curve analysis for detectable severe endometriosis risk. (C) Sample in different stages of the menstrual cycle of endometriosis. (D) Differentially expressed genes between early-secretory and proliferation and between mid-secretory and early-secretory. Red is upregulation and blue is downregulation. Genes with the largest fold change are labeled. (E) The expression of core genes in different stages of the menstrual cycle. FC, fold change.

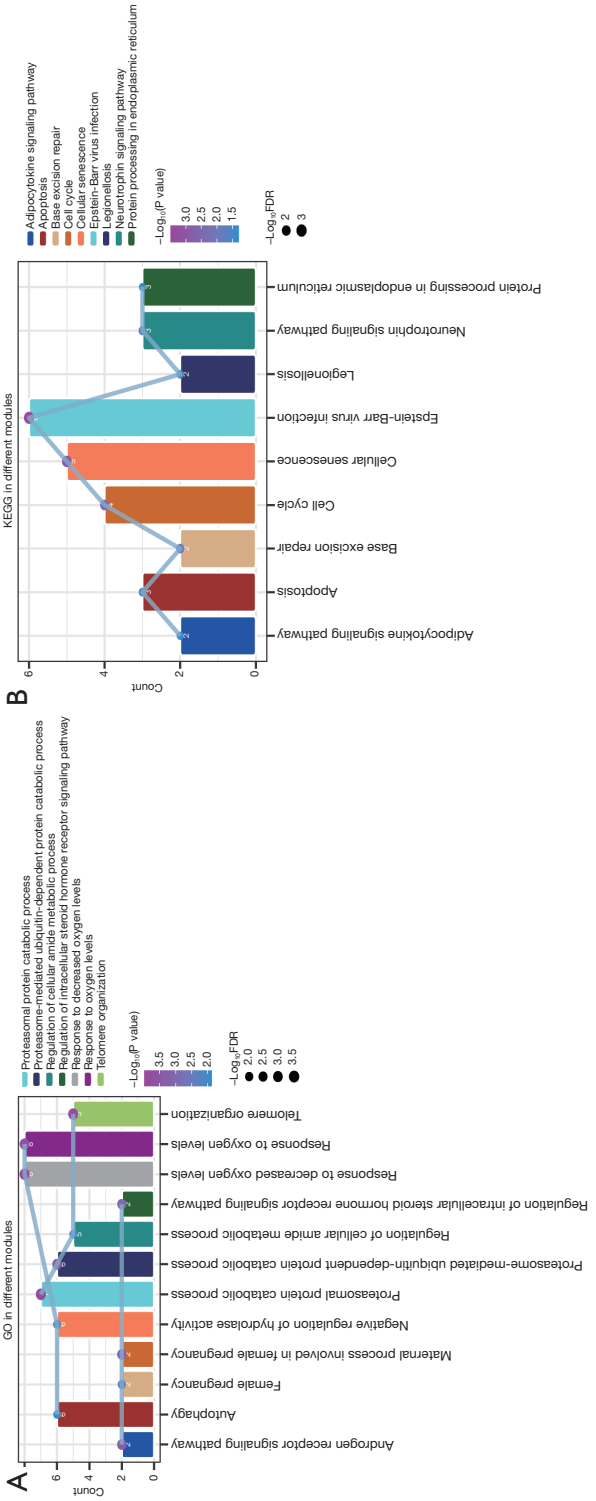


Figure 4 The biological function and signaling pathways associated with the persistent maladjustment genes. (A) The biological processes associated with the dysfunctional genes. (B) The KEGG pathways associated with the dysfunctional genes. (C) The signaling pathways showing an upward trend in normal, mild, and severe endometriosis according to the GSVA results. (D) The signaling pathways with a downward trend in normal, mild, and severe endometriosis according to the GSVA results. KEGG, Kyoto Encyclopedia of Gene and Genome; GSVA, Gene Set Variation Analysis; GO, Gene Ontology; FDR, false discovery rate.

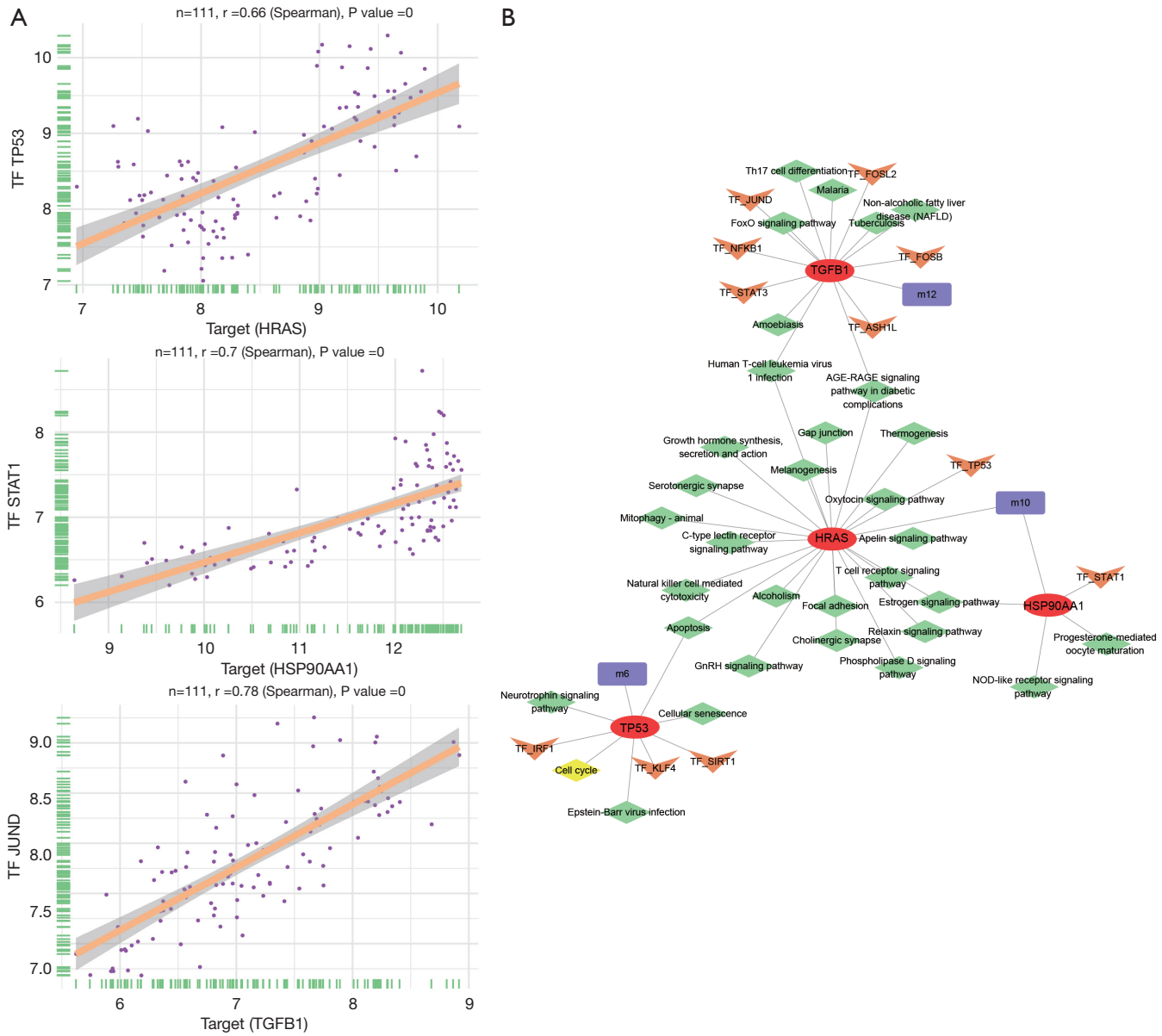


Figure 5 Transcription regulators of the core genes. (A) Transcription regulators that show a high correlation with the core genes. (B) Regulatory factors that regulate the network of core genes involved in signaling pathways. TF, transcription factor.

shown that *HRAS* is significantly related to the occurrence and development of endometriosis (29). The *HRAS* proto-oncogene and GTPase (HRAS) can regulate the level of ferritin, thus increasing the pool of unstable iron and the proliferation index in cells (30). In addition, *HRAS* is related to the chemical family of polychlorinated biphenyls (PCBs), and PCB exposure is closely related to the development of endometriosis (31). Interestingly, the expression of the *HSP90AA1* gene differs significantly between severe and mild endometriosis (32). *TGF*-β plays an important role in the pathogenesis of peritoneal endometriosis (32). Furthermore, it is differentially expressed in patients with endometriosis, and may promote invasion, differentiation, angiogenesis, and immune escape (33). The expression of *TGF-β1* and its signal targets in the eutopic endometrium of patients with endometriosis is different to that in healthy women (34). Compared with normal endometrium, tumor protein p53 (*TP53*) is more frequently detected

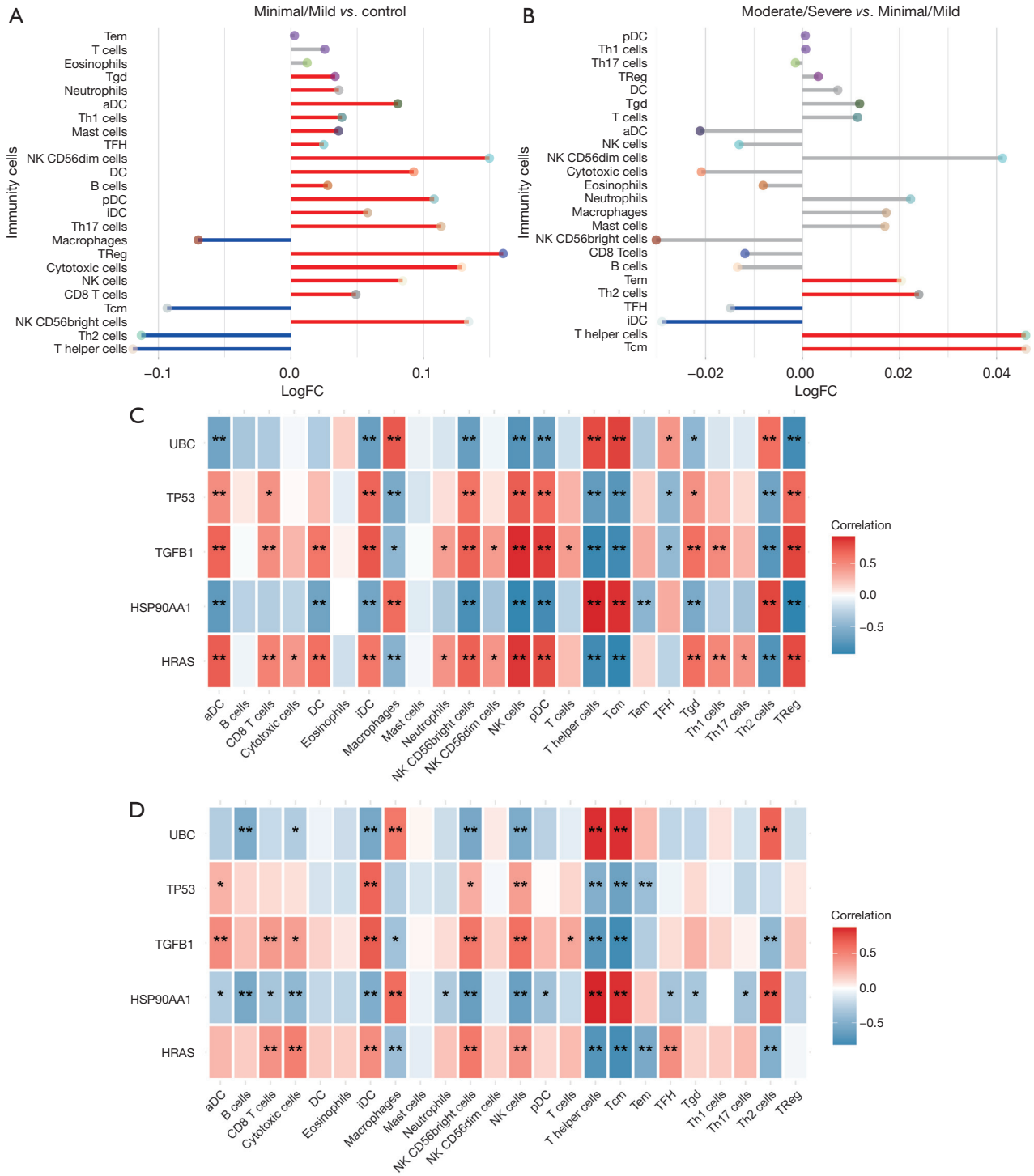


Figure 6 Immune levels in endometriosis. (A) Differential levels of immune cells between mild endometriosis and normal samples. Red is upregulation and blue is downregulation in mild endometriosis. (B) Differential levels of immune cells between severe and mild endometriosis samples. Red is upregulation and blue is downregulation in severe endometriosis. Gray lines represent no statistical significance. (C) Correlations between immune cells and core genes in mild endometriosis. (D) Correlations between immune cells and core genes in severe endometriosis. *, P<0.05, **, P<0.01. Tem, effector memory T cell; Tgd, gamma delta T cell; aDC, activated dendritic cell; TFH, follicular helper T cell; NK, natural killer; DC, dendritic cell; pDC, plasmacytoid dendritic cell; iDC, immature dendritic cell; TReg, regulatory T cell; Tcm, central memory T cell; FC, fold change.

in endometriosis tissues (35). *TP53* is believed to be a transcriptional regulator of HRAS and may play a more important role in the development of endometriosis. Therefore, these core genes that are dysregulated in endometriosis are more likely to be potential therapeutic targets. This may pave the way for improved early diagnosis and more effective treatment of endometriosis by using the unique and persistent biomarkers identified herein.

There is evidence that the destruction of female hormones, local inflammation, and the immune process are involved in the occurrence and development of endometriosis (36). In this study, we identified the effects of oxidative stress, hormones, and immune inflammation on endometriosis. Hypoxia plays an important role in the development of endometriosis (37). The invasive mechanism of endometriosis may be related to oxidative stress-related proliferative activity (4). In addition, the tissue niche provides a chronic inflammatory environment for the development of endometriosis, especially in the abdominal cavity, ovary, and uterus (38). Inflammation may lead to increased expression of mitogen activated protein kinase (MAPK) and other signal pathway components in endometriosis (39). Many changes occur in the immune system during endometriosis, including inhibition of T cell cytotoxicity to endometrial cells, reduction of NK cell activity, and increase of activated macrophages and pro-inflammatory cytokines (40). The occurrence and maintenance of endometriosis can be divided into two stages: the initial stage of interleukin 6 (IL-6)-mediated early immunity, and the hormone dominated stage of estrogen signal (41). Indeed, these factors may have a comprehensive impact on the occurrence and development of endometriosis.

The markers of endometriosis have a strong interaction with inflammatory components and the hormone environment, which has a negative impact on its expression and function, so as to promote the sustainable development of endometriosis. We then found high levels of immune cells in the endometriosis. NK cells and dendritic cells, may play specific roles in angiogenesis, growth and invasion of endometriosis cells (42). Podgaec *et al.*, showed that the inflammatory symptoms of endometriosis may involve a shift towards Th2 immune response components (43). The dysregulation of immune cells showed no progressive trend in the course of endometriosis. Although the reason for this phenomenon is unknown, it is suggested that core genes improve the immune response of endometriosis through multiple pathways. This may provide a new ideas and directions for the diagnosis and treatment of endometriosis.

However, this study also has limitations. First, the data used in this study were obtained from public databases, and more clinical information on patients may be missing. Second, for the significant analytical results we obtained, further *in vivo* and *in vitro* experimental validation is warranted. In addition, we should expand the sample size in future studies to enhance the clinical applicability of the results. The method of screening modules and core genes through the PPI network was universal and may lack individualization. Further research using a large number of clinical samples are needed to verify our results.

Conclusions

Endometriosis is a debilitating disease that affects the quality of life of adults and adolescents. Delays in diagnosis are common and can lead to a decline in reproductive potential and fertility. Recognizing disease progression and deterioration may help to improve therapeutic treatments and outcomes. These potential biomarkers may reduce the cost of surgical intervention through early diagnosis of cases, so as to improve the clinical management of the disease.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4806/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4806/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work

have been appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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