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Metabolic Reprogramming Mediates Chronic Stress–Induced Immune Dysregulation in Endometriosis

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Abstract

Evidence indicates that the initiation and progression of endometriosis are intimately tied to persistent psychological strain. The precise manner in which sustained stress contributes to metabolic alterations in women with endometriosis has not been elucidated. Our objective was to expose the mechanistic underpinnings of how sustained stress influences endometriosis evolution and, potentially, to formulate candidate biomarkers to gauge the effect of accelerated, persistent stress on endometriosis invasiveness. Ectopic tissue was excised surgically from ten affected patients, who were then separated into two categories through a psychological evaluation. A human mRNA gene expression microarray was used to assess differences in mRNA expression patterns between those under continuous stress and their counterparts. The reliability of the microarray results was further corroborated by metabolite profiling using liquid chromatography–tandem mass spectrometry (LC-MS/MS) and quantitative reverse transcription polymerase chain reaction (RT–PCR). Microarray analysis of genes that were markedly overexpressed and differentially expressed between the ongoing stress and comparison groups revealed genes largely belonging to metabolic and immunological pathways, including immune response processes, inhibition of T lymphocyte proliferation, leucine breakdown, and L-cysteine processing ($P < 0.05$). LC-MS profiling demonstrated that the distinguishing metabolites chiefly involved arginine and proline processing, D-glutamine and D-glutamate processing, aspartate handling, glycine, serine metabolism, and tyrosine metabolism ($P < 0.05$). A mechanism may exist in which prolonged stress impairs immune defense in endometriosis via metabolic reconfiguration. Extended stress curtails the provision of energy substrates such as arginine and serine, suppresses T lymphocyte activation, and weakens antineoplastic immunity, thus facilitating the displacement and penetration of endometriotic tissue in chronically stressed patients.

Keywords: Chronic stress, Endometriosis, Immune response, Metabolic reprogramming

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Introduction

Endometriosis (EM) is a disorder in which endometrial cells and stroma implant and grow in locations outside the uterine cavity, affecting 10%–15% of females in their childbearing years [1]. The pathological mechanisms encompass amplified cellular attachment, new blood vessel formation, disrupted programmed cell death, and genetic factors, yet a comprehensive understanding remains lacking. From a clinical standpoint, the illness manifests through painful menses, discomfort during intercourse, non-menstrual pelvic aching, and reduced fecundity, all elements that considerably impair the well-being of those diagnosed [2-4]. As a result, women battling endometriosis tend to exist under a persistent psychological burden, involving depressive symptoms, anxious states, and insufficient social backing [5-8]. In particular, individuals enduring deep infiltrative

discomfort originating from endometriosis may suffer exceptionally severe ongoing stress, a condition often mitigated after operative removal of the lesions [9].

A growing body of evidence has substantiated that sustained stress plays a vital role in the progression of endometriosis [10-12]. Still, the particular part that chronic stress plays in the pathogenesis of endometriosis remains to be entirely mapped out.

Ongoing stress triggers a multifaceted cascade that can stimulate the sympathetic nervous system (SNS) and the hypothalamic–pituitary–adrenal (HPA) axis [13, 14]. It can unleash a succession of detrimental secondary consequences throughout the organism [15]. Epinephrine (E) and norepinephrine (NE) reportedly remain persistently elevated in subjects experiencing continuous stress, whereas dopamine (DA) concentrations drop [16, 17]. Persistent stress also engages the GC receptor (GCR), which forms part of the regulatory loop governing inflammatory and immunological activities [18].

Appleyard *et al*. [19] demonstrated that prior exposure to swimming-induced stress before the surgical induction of endometriosis in rodents resulted in a notable increase in the size and number of endometrial implants [20]. Guo *et al*. [11] asserted that sustained stress accelerates endometriosis progression and operates through stimulation of Adrenoceptor Beta 2 (ADRB2) and cyclic adenosine monophosphate response element-binding protein (CREB) signaling pathways, and that targeting ADRB2 signaling might represent a novel therapeutic approach for endometriosis treatment [21]. Much like the connection between sustained psychological burden and cancer advancement implicating the SNS and HPA [15], a study demonstrated that oncology subjects recounting elevated degrees of ongoing stress displayed irregular NE and cortisol concentrations [7]. Nevertheless, the mechanisms of continuous stress in endometriosis progression are not fully understood.

Virtually every living entity can adapt to its surrounding milieu to modulate metabolic operations. Consequently, cells must continually sense environmental shifts and adjust their metabolic requirements accordingly. Different cell populations, however, exhibit distinct metabolic modifications. Standard cells predominantly exploit glucose breakdown through mitochondrial oxidative phosphorylation to synthesize adenosine triphosphate (ATP). Malignant cells, in contrast, adopt an alternative metabolic route, namely glycolysis. Even when oxygen is plentiful, neoplastic cells continue to employ glycolysis, which represents a process that is neither resource-sparing nor highly efficient relative to other pathways—the phenomenon termed aerobic glycolysis (Warburg effect) [22]. The metabolic profile of cancerous cells impacts not only the malignant cells themselves but also adjacent cells, including innate and adaptive immune effectors, endothelial linings, and neoplasm-associated fibroblasts within the immunological network [23]. As neoplastic tissue enlarges, immune elements populating the local microenvironment experience metabolic reconfiguration, triggering phenotypic transformations [23]. The fundamental scientific question this work explores is how the metabolic restructuring program of neoplastic cells shapes anti-tumor immunity; this underlies our investigation into metabolite distinctions, immunological disparities, and their mutual connections.

In the present investigation, we conducted an mRNA human gene expression microarray survey to examine differences in mRNA transcription patterns between chronic stress and control endometriosis patient cohorts. Moreover, using metabolite-centered inquiries based on both LC-MS/MS and quantitative RT–PCR, we validated the reliability of the mRNA human gene expression microarray approach. Our objective was to expose the mechanistic underpinnings of how sustained stress influences endometriosis evolution and, potentially, to formulate candidate biomarkers to gauge the effect of accelerated, persistent stress on endometriosis invasiveness.

Materials and Methods

Patients

Individuals whose endometriosis was confirmed by intraoperative pathological examination were enrolled from the Fudan University Affiliated Obstetrics and Gynecology Hospital. Psychological evaluation instruments, specifically the Patient Health Questionnaire-9 (PHQ-9) and the Generalized Anxiety Disorder-7 (GAD-7) scales, were administered to assess participants' psychological condition. Our assessment of each participant's psychological status was finalized before the surgical procedure, and every patient was informed regarding the purpose of the evaluation. Participants completed the questionnaires under the supervision of trained instructors. We assigned patients exhibiting mild or more pronounced levels (defined as a score of 5 or higher) of both depression and anxiety to the persistent stress arm, and those presenting with neither anxiety nor depression to the reference arm. Tissue samples were obtained during the operation, transferred into sterile containers, and promptly frozen at −80 °C pending subsequent analyses.

Transcriptomic profiling: RNA harvesting, library construction, RNA sequencing, and differential expression assessment

The Agilent SurePrint G3 Human Gene Expression v3 Microarray (8x60K, Design ID: 072363) was employed for this investigation, and bioinformatic analysis of the entire set of 10 specimens was performed.

Whole RNA from the endometriotic lesions was recovered using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the supplier's recommended procedure. RNA quantity was determined using the NanoDrop ND-2000 instrument (Thermo Scientific, New York, NY, USA), and RNA integrity was assessed using the Agilent Bioanalyzer 2100 system (Agilent Technologies, USA). The procedures for sample labeling, chip hybridization, and washing steps were performed exactly as stipulated in the manufacturer's documentation. To summarize, whole RNA was reverse-transcribed to generate double-stranded cDNA, after which complementary RNA (cRNA) was produced and conjugated with Cyanine-3-CTP dye. The dye-coupled cRNA products were then hybridized onto the microarray slide. After the wash steps, the chips were imaged using the Agilent Scanner G2505C (Agilent Technologies, Santa Clara, CA, USA).

Feature Extraction software (version 10.7.1.1, Agilent Technologies, Santa Clara, CA, USA) served to interpret the array images and extract raw signal intensities. GeneSpring (version 13.1, Agilent Technologies, Santa Clara, CA, USA) was used to process the raw datasets initially. As a first step, the quantile normalization method was enforced on the raw measurements. Probes that received a "Detected" flag in no fewer than 100% of the values across any single experimental condition were retained and advanced to the subsequent tiers of exploration. Thereafter, p-values were derived from repeated variance assessments and t-tests, enabling the identification of genes whose expression differed significantly. The criteria for classifying transcripts as either heightened or diminished were a fold change ≥ 1.5 paired with $P \leq 0.05$. GO term enrichment evaluation and pathway assignment were subsequently undertaken to elucidate the functional significance of these differentially regulated RNA species. As a final step, hierarchical clustering was performed to visualize how the expression patterns of the discriminatory genes clustered across the sample collection.

Metabolomic evaluations: tissue metabolite recovery and metabolite profiling pipeline

A panel of 30 endometriotic tissue specimens (comprising 15 from the persistent stress arm and 15 from the comparator arm) was assembled for metabolite measurement via LC-MS/MS-driven detection. LC-MS/MS was conducted on a UHPLC platform (1290, Agilent Technologies, Santa Clara, CA, USA) equipped with a UPLC BEH Amide separation column (1.7 μm, 2.1 100 mm, Waters, Milford, MA, USA) and coupled to a Triple TOF 6600 mass analyzer (Q-TOF, AB Sciex). The mobile solvents were constituted by a water-based solution containing 25 mM NH4OAc combined with 25 mM NH4OH (pH = 9.75) (solvent A) and acetonitrile (solvent B). The elution timetable was arranged according to the following profile: 0 min, 5% A; 0.5 min, 5% A; 7 min, 35% A; 8 min, 60% A; 9 min, 60% A; 9.1 min, 5% A; and 12 min, 5% A, with a continuous flow setting of 0.3 mL/min. A 2 μL aliquot was introduced per injection. During LC/MS acquisitions, MS/MS fragmentation data were collected using information-dependent acquisition logic on the triple TOF device. In this operational mode, the controlling software (Analyst TF 1.7, AB Sciex, Framingham, MA, USA) continuously monitored the full-scan survey MS signal while simultaneously capturing and triggering MS/MS spectral acquisition according to predefined inclusion rules. In every iterative scan, six precursor species exceeding an intensity cutoff of 100 were chosen and dissociated under a collision energy (CE) setting of 35 V (15 MS/MS events, with a 50 msec dwell period for each fragment ion). The ESI source conditions were fixed as follows: ion source gases 1 and 2 at 60 psi each, curtain gas at 30 Psi, source block heated to 550 °C, and ion spray voltage floating (ISVF) set to 5500 V and −4500 V for positive and negative ionization, respectively.

Quantitative real-time polymerase chain reaction

Total RNA was purified from cryopreserved tissue pieces and converted into complementary DNA using the PrimerScript RT Kit (Takara, Shiga, Japan) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed with SYBR Premix Ex Taq (Takara, Japan) on a 7500 RT-PCR detection system (Applied Biosystems, Foster City, CA, USA). Each sample was run in triplicate. Target mRNA abundance values were standardized to the 18S reference transcript and calculated using the $2^{-\Delta\Delta Ct}$ relative quantification method.

Statistical procedures

The experimental data are expressed as the mean ± standard deviation. For contrasts involving more than two group means, one-way ANOVA was applied, while pairwise group contrasts were evaluated using the shortest effective range procedure. The SPSS 20.0 statistical suite (IBM SPSS Statistics, Inc., Chicago, IL, USA) was used for the computational analyses. Graphical outputs were produced using GraphPad Prism 6.0 software (San Diego, CA, USA), and between-cohort comparisons relied on Student's t-tests. Discrepancies between cohorts were considered statistically significant when p-values were below 0.05.

Results and Discussion

Characteristics of the study population

Thirty subjects were enrolled in this investigation and subsequently assigned to either a persistent stress cohort or a reference cohort according to their scores on psychological assessment instruments. Pathological

evaluation confirmed the diagnosis of endometriosis in all participants in the present study. The depressive and anxious symptomatology of the individuals with endometriosis was examined by means of the Patient Health Questionnaire-9 (PHQ-9) and the Generalized Anxiety Disorder-7 (GAD-7) psychological scales. Age and disease stage, treated as potential confounders, were excluded from consideration **(Figures 1a and 1b)**.

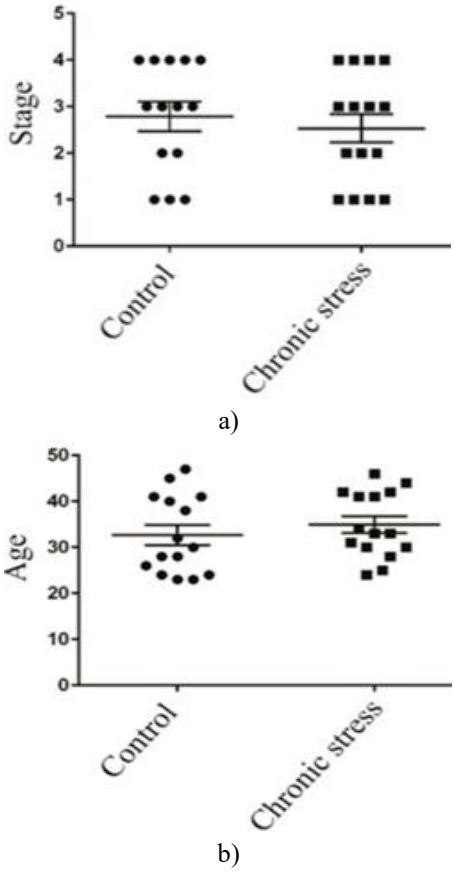


Figure 1. Characteristics of the study population: (a) Scatter plot comparison of age distribution between the reference group and the persistent stress group among patients subjected to metabolome profiling; (b) scatter plot comparison of stage distribution between the reference group and the persistent stress group among patients subjected to metabolome profiling.

Divergent gene expression patterns in endometriosis patients with or without persistent stress

The Agilent SurePrint G3 Human Gene Expression v3 microarray (8 × 60K, Design ID: 072363) was used to generate mRNA expression signatures. Five tissue specimens from each experimental arm were sent for transcriptomic analysis. The data indicated a considerable number of genes whose regulation differed between endometriosis patients under persistent stress and those free of such stress. Hierarchical clustering revealed that endometriotic lesions harvested from stressed subjects display a gene expression profile clearly distinguishable from that of unstressed subjects. The accompanying dendrogram also shows that many differentially expressed transcripts are either up- or downregulated in the lesional tissue of endometriosis patients experiencing stress **(Figures 2a and 2b)**.

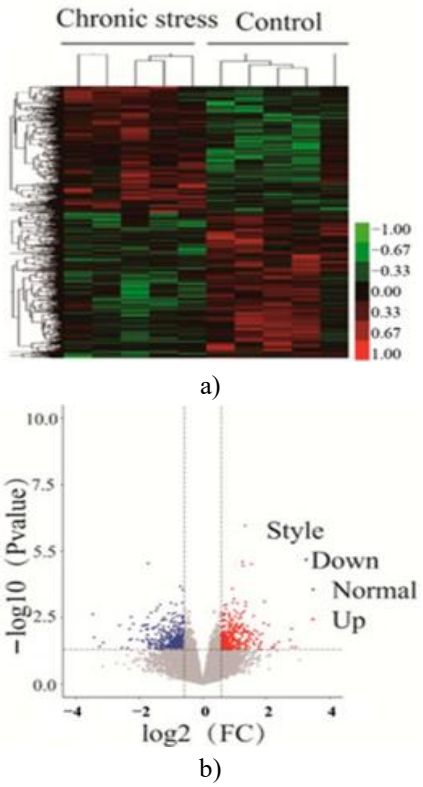
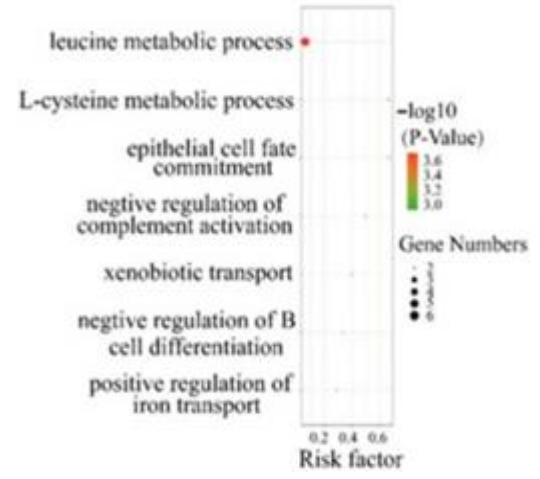


Figure 2. (a) Heat map illustrating mRNA signatures within endometriotic tissues of the persistent stress group relative to matched control endometriotic tissues—red coloration: heightened genes; green coloration: diminished genes. mRNA expression values are arranged via hierarchical clustering along the vertical axis, while tissue specimens are arranged via hierarchical clustering along the horizontal axis (fold change: 2; $P < 0.05$). Alphanumeric labels correspond to five persistent stress endometriotic tissue samples and five matched control endometriotic tissue samples, respectively. (b) The overlapping sector in the volcano plot designates genes that were concordantly modulated, as per the selection criteria of a ≥ 2-fold alteration ($P < 0.05$), when comparing persistent-stress endometriotic tissue with the control.

Collectively, 34,757 distinct expressed gene products were detected across the endometriotic specimens. From this pool, we identified 1381 mRNAs that showed differential expression between the persistent stress and control cohorts. Among them, 689 mRNA species were up-regulated, while 692 were down-regulated in the persistent stress group specimens.

Functional categorization through Gene Ontology (GO) enrichment of differentially expressed genes

To acquire broader mechanistic insights into the biological roles of the differentially expressed transcripts, we conducted GO enrichment analyses by interrogating each differentially expressed gene identified between the persistent stress and reference cohorts. GO evaluation of the prominently up-regulated, differentially expressed gene set between the two groups brought to light a predominant enrichment of genes linked to immunological and metabolic pathways, such as the immune reaction pathway, the inflammatory reaction, the immune apparatus pathway, modulation of immune reaction, suppression of T lymphocyte proliferation, the leucine processing pathway, and the L-cysteine processing pathway ($P < 0.05$) **(Figure 3)**. The down-regulated differentially expressed gene cluster was chiefly associated with immune-related processes, whereas the up-regulated differentially expressed gene cluster was predominantly associated with metabolism-related processes.



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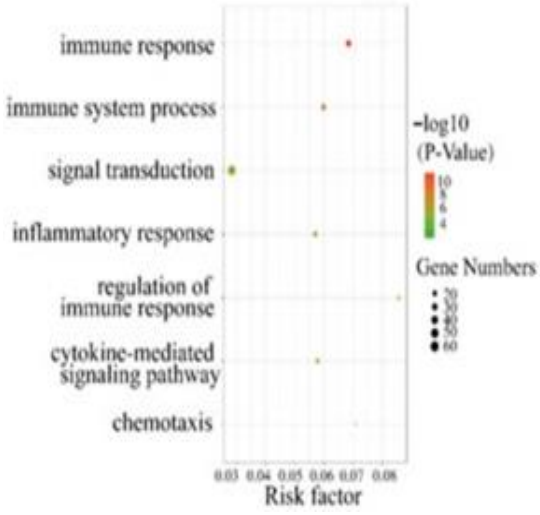
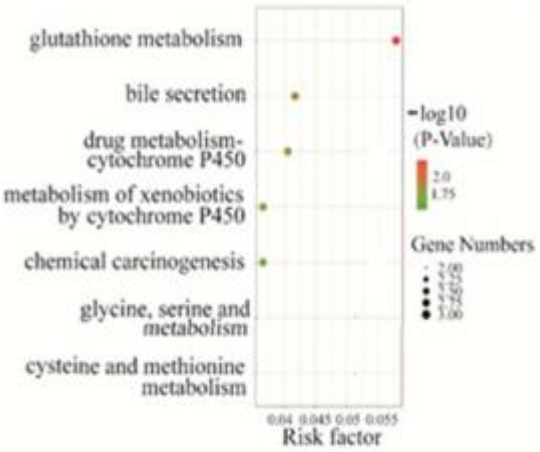


b)

Figure 3. Gene ontology (GO) categorization of differentially expressed (DE) genes. The y-axis represents the GO designation, and the x-axis represents the −Log10 (p-value) attributed to the significantly enriched GO designations. Red circular markers signify corrected p-values below 0.05. (a) The seven most significant GO designations (biological process category) are tied to the detected up-regulated DE gene set. (b) The seven most significant GO designations (biological process category) are tied to the detected down-regulated DE gene set.

Pathway-level characterization of differentially expressed genes

We consulted the KEGG database to identify the principal signaling pathways involving the differentially expressed genes identified in this study. Our findings demonstrated that 14 pathways were meaningfully enriched among the cataloged DEGs. Beyond that, pathway-level inspection pointed to the involvement of these differentially expressed gene products in complement and coagulation cascades; cell adhesion molecules (CAMs); cytokine–cytokine receptor interplay; phagosomes; the NF-kappa B signaling route (NF-κB); cysteine and methionine processing; glycine, serine, and threonine processing; together with D-Arginine and D-ornithine processing **(Figures 4a and 4b)**.



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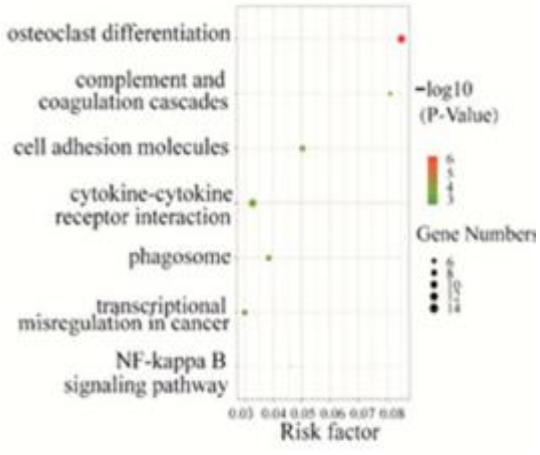

b)

Figure 4. Pathway-level characterization of differentially expressed gene sets. The y-axis indicates

the pathway category, and the x-axis indicates the −Log10 (p-value) corresponding to the significantly enriched pathway designations. Red bars signify corrected p-values below 0.05. (a) The seven most significant pathways are tied to the detected down-regulated differentially expressed gene set. (b) The seven most significant pathways are tied to the detected up-regulated differentially expressed gene set.

Pathway–act network construction and inspection

To acquire a richer understanding of how pathways interact and to isolate those routes likely occupying commanding positions, we assembled a pathway–act network grounded in the direct or systemic associations documented within the KEGG resource **(Figure 5)**. As depicted in **Figure 6**, a subset of differentially expressed genes active in essential routes in both the persistent stress arm and the reference arm was identified, encompassing metabolic circuits such as glutathione, cysteine and methionine, and glycine, serine, and threonine handling, each of which was shown to be transcriptionally enhanced. Beyond this, the FoxO signaling circuit, the Jak-STAT signaling circuit, and the NF-kappa B signaling circuit situated within the interaction web were also forecasted to exert considerable influence.

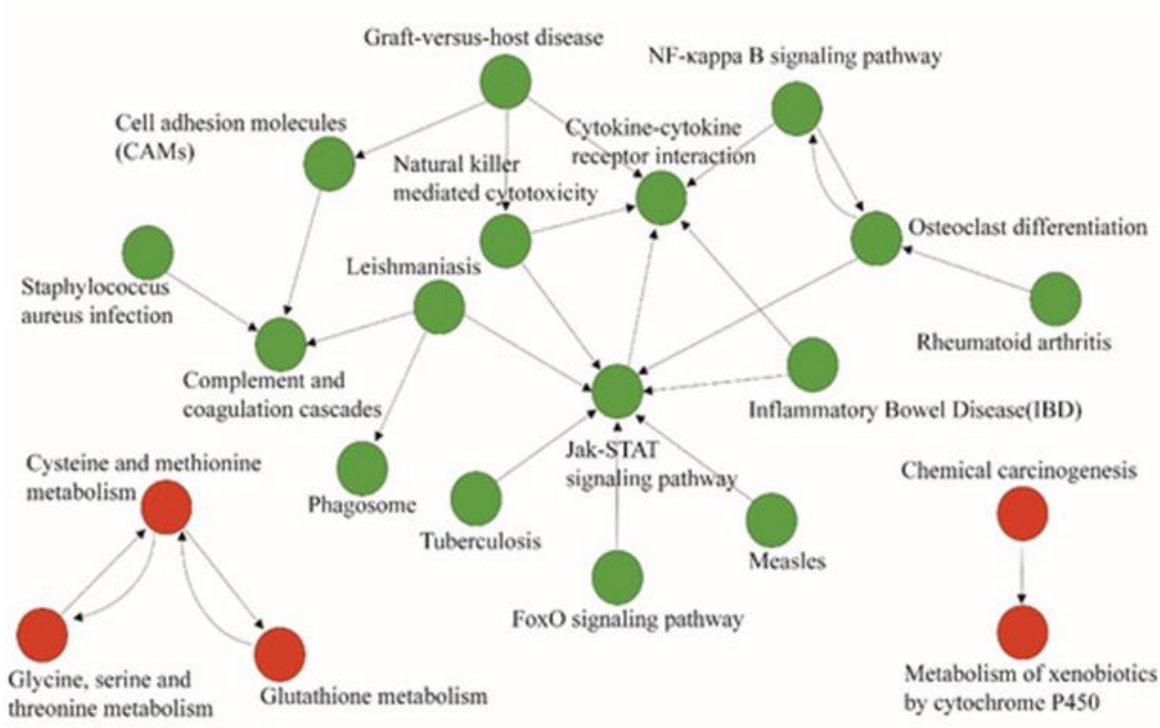

Figure 5. Pathway–act network construction and inspection. The pathway–act network was compiled based on interactions with routes cataloged in the KEGG database. Circular markers correspond to pathways, while directional links between markers represent interaction targets spanning distinct pathways. Markers colored red signal routes that were transcriptionally enhanced, whereas markers colored green signal routes that were transcriptionally suppressed.

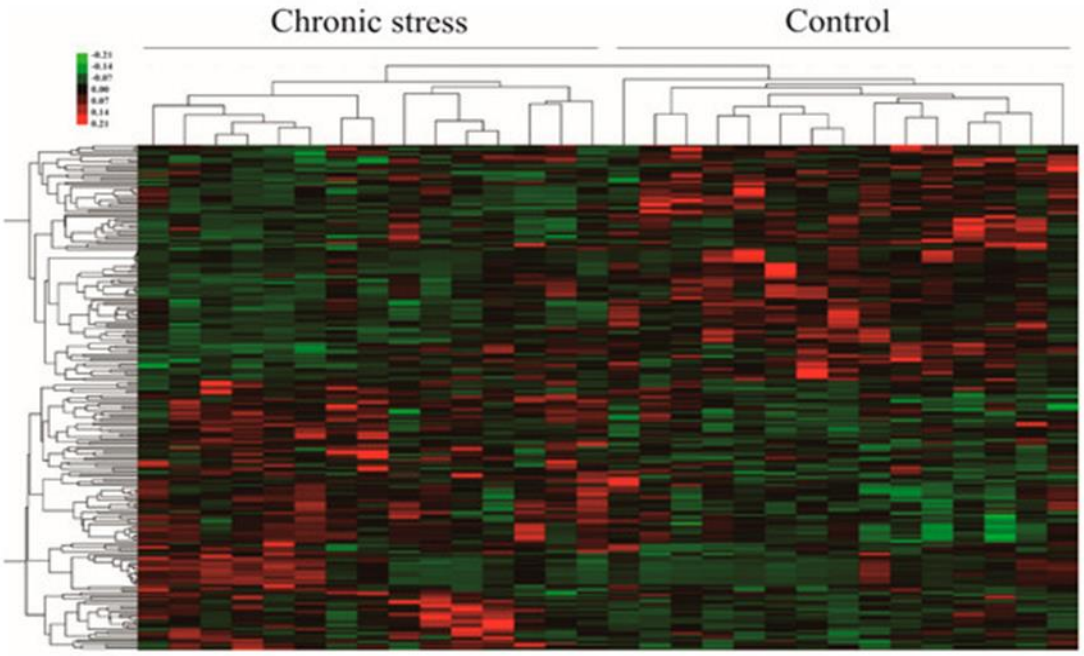

Figure 6. Heat map representation of metabolic patterns across endometriotic lesions taken from the persistent stress arm versus the matched control arm endometriotic lesions—red: metabolites present at higher levels; green: metabolites present at lower levels. Metabolite concentrations are ordered by hierarchical clustering along the vertical dimension, and the tissue samples are ordered by hierarchical clustering along the horizontal dimension (fold change: 2; $P < 0.05$). Alphanumeric designations correspond to 15 persistent stress endometriotic lesions and 15 matched control endometriotic lesions, respectively.

Distinctions in metabolic patterns and accompanying routes between endometriosis patients with and without persistent stress

All 30 biological specimens were dispatched for metabolite profiling. A total of 3629 known metabolites were detected, and their concentrations were measured throughout the endometriotic lesions. We resolved which metabolites diverged between women who have endometriosis who were experiencing persistent stress and those who were not. Of the 3629 metabolites screened, 235 displayed significant variation between the two study arms ($P < 0.05$). To illustrate how these 235 altered metabolites interrelate, hierarchical clustering was employed to arrange the compounds based on their relative abundances across the sample set **(Figure 6)**. Concurrently, route mapping showed that these distinguishing metabolites were mostly engaged in glutathione handling; D-glutamine and D-glutamate handling; arginine and proline handling; taurine and hypotaurine handling; alanine, aspartate, and glutamate handling; glycine, serine, and threonine handling; arachidonic acid handling; tyrosine handling; and purine handling ($P < 0.05$) **(Figure 7)**. The outcomes documented here exhibited near-complete concordance with the shifts observed among divergent routes previously identified through gene expression signature analysis of metabolic variation pathways.

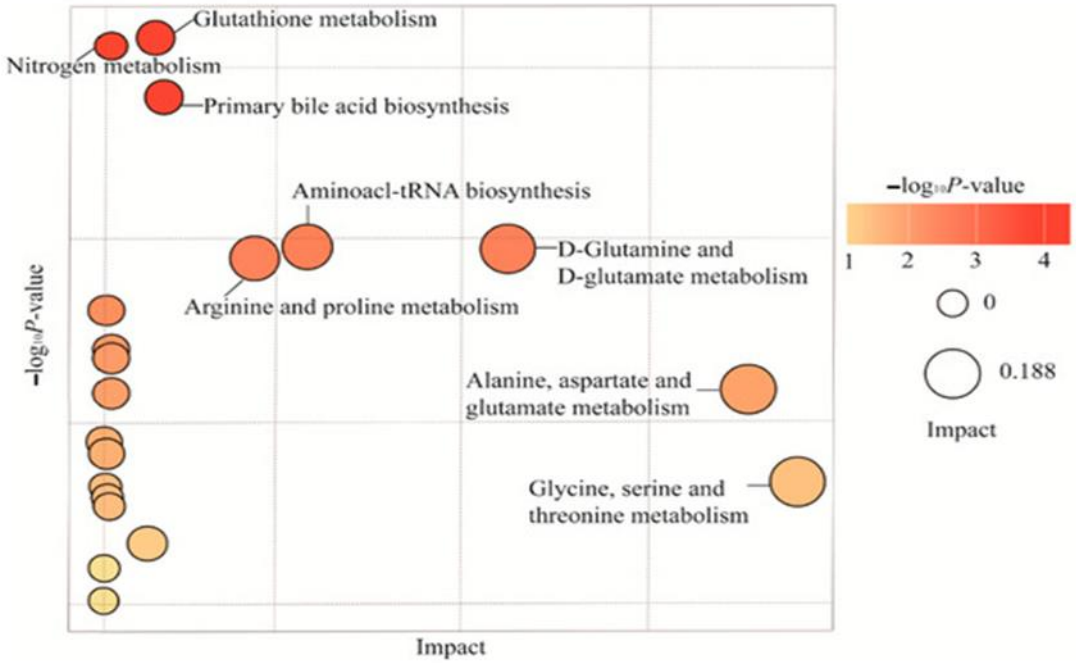

Figure 7. Bubble graphs outlining the divergent metabolic routes detected inside endometriotic lesions from the persistent stress arm compared to the matched control arm.

Verification through quantitative real-time PCR of gene transcripts connected to inflammatory and metabolic circuitry

To substantiate the gene expression signature outputs by means of quantitative real-time PCR, 10 mRNA targets sourced from 10 endometriotic specimens (5 belonging to each experimental arm) were chosen for expression signature evaluation. The quantitative RT-PCR data revealed that the miRNA signals corresponding to IL-1B, CXCR4, GABARAPL1, IL-7R, STAT4, CD14, CCR5, GATM, and GSTM2, together with the mRNA signals corresponding to CXCR4, GABARAPL1, IL-7R, STAT4, CD14, and CCR5, underwent statistically meaningful reductions ($P < 0.05$ for all comparisons) within the persistent stress arm specimens relative to those collected from the reference arm. By contrast, the mRNA signals for GATM and GSTM2 displayed statistically meaningful elevations ($P < 0.05$ for all comparisons) within the persistent stress arm specimens compared to those from the reference arm **(Figure 8)**. The quantitative RT-PCR findings were fully consistent with those from expression signature profiling, thereby reinforcing the reliability of the expression signature data.

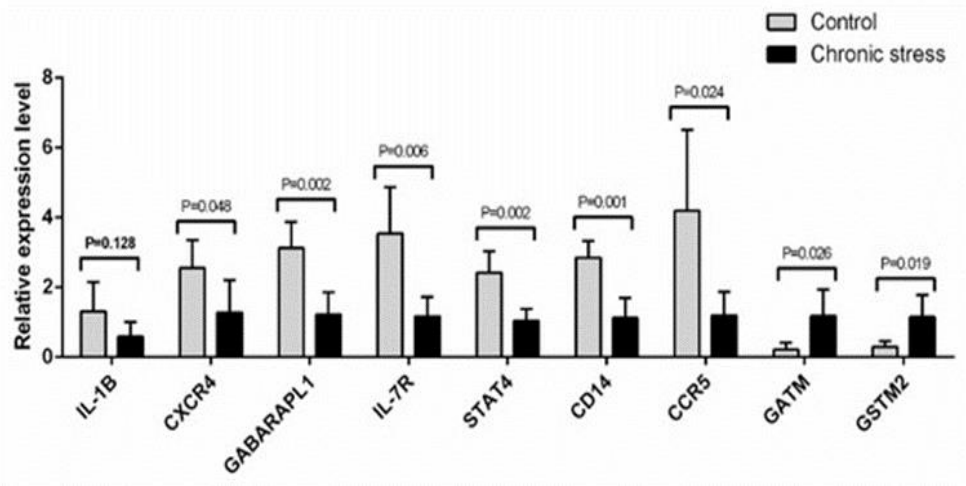

Figure 8. Confirmation of expression signature data via quantitative real-time PCR. Ten mRNA transcripts (IL-1B, CXCR4, GABARAPL1, IL-7R, STAT4, CD14, CCR5, GATM, and GSTM2) were chosen and measured with qRT-PCR to authenticate their abundance levels. The comparative abundance of every target mRNA was normalized. Values presented in the column graphs are shown as means ± standard deviation (SD).

To delve into the mechanistic underpinnings of how prolonged stress accelerates endometriosis, we cataloged mRNA transcripts in lesion specimens from 10 patients, some experiencing and others not experiencing long-term psychological strain, using a human gene expression microarray. Our analysis uncovered 1381 mRNAs whose abundance diverged when the two study arms were juxtaposed. Gene Ontology annotation and pathway mapping were subsequently undertaken to infer the likely biological contributions of these differentially abundant transcripts; the outcomes revealed that gene products exhibiting diminished expression chiefly aggregated within immunity-associated functions, whereas those showing elevated expression predominantly gathered within metabolism-associated functions. In addition, we inferred the downstream effectors of the differentially expressed gene products by constructing a pathway–act interaction map. Informed by these findings, we projected that metabolic circuits covering the handling of arginine, glycine, serine, and threonine would emerge among those displaying heightened activity. Alongside these, the FoxO, Jak-STAT, and NF-kappa B signaling hubs, positioned within this interaction architecture, were also forecasted to assume considerable importance. Metabolic profiling, coupled with qRT-PCR, was then used to corroborate the inferences drawn from the computational analyses.

Discomfort and reduced fecundity stand as the two dominant sources of hardship that fuel depressive and anxious moods among women coping with endometriosis [24, 25], and such hardships commonly escape the individual's capacity to manage them and stretch across protracted timeframes. As a result, they stimulate the SNS, which in turn drives the catecholamine–HPA endocrine axis, thereby shaping glucocorticoid output [15]. Within the endometriosis setting, glucocorticoid receptor levels are reportedly up-regulated, lending support to proposals for therapeutic glucocorticoid antagonism [26, 27]. Even so, the exact routes through which glucocorticoids might impart any influence on the course of endometriosis continue to elude clarification. The roles of other neuroendocrine factors released under persistent stress in shaping the endometriosis trajectory are likewise poorly defined, even though their involvement in tumorigenic progression is far better mapped [28, 29].

The mRNA human gene expression microarray evaluation identified discrepancies across both immunity- and metabolism-related circuitries, evident in distinct gene cohorts, when comparing patients subjected to sustained strain with those spared it. Yamauchi *et al*. [30] noted that triggering NF-κB can amplify the synthesis of intercellular adhesion molecules (ICAM-1), MCP-1, and E-selectin; render the endothelium more permeable; and ease tethering to the extracellular matrix (ECM)—events that jointly ignite an inflammatory reaction within the endothelial lining. NF-κB promoted excessive IL-8 production, a process that facilitates neovessel formation within the ectopic endometrial stroma.

Nuclear factor-kappa B (NF-κB) belongs to a family of nuclear transcriptional regulators distributed widely among numerous cell lineages throughout the organism. It maintains close ties to immunological and inflammatory signaling cascades, as well as to scheduled cell death, tumor establishment, and metastatic spread [31]. The involvement of NF-κB in EM may coordinate inflammatory reactions, cellular adherence, tissue penetration, and neovascularization. Wickiewicz *et al*. [32] documented that the IL-6 gene's promoter region harbors a docking motif for NF-κB, and that NF-κB can initiate IL-6 synthesis, thereby substantially boosting IL-6 concentrations inside the peritoneal fluid of EM patients.

Li *et al*. [33] showed that the concerted transcriptional effect exerted by NF-κB, p50, p65, VEGF, and COX-2 within ectopic endometrial stromal cells was markedly enhanced, and that the engaged NF-κB axis could heighten the production of VEGF. This molecule promotes neovascularization, degrades the adjacent matrix, and facilitates the dissemination and re-establishment of displaced endometrial tissue. In the context of our investigation, prolonged psychological strain appears to elevate NF-κB expression in ectopic endometrial lesions, creating a milieu that promotes the adherence, engraftment, and vascularization of the aberrantly located endometrium.

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is a cytokine-triggered intracellular signaling cascade composed of three components: a receptor tyrosine kinase, the tyrosine kinase JAK, and the transcriptional effector STAT [34]. This signaling pathway is involved in cellular proliferation, differentiation, programmed cell death, and immune regulation. When the JAK/STAT axis becomes dysregulated, genes orchestrating cell division and survival, among them cyclin D1, survivin, bcl-2, and bcl-xl, are up-regulated, a shift that propels pathological progression [35]. George *et al*. [36] observed that STAT3 phosphorylation levels during the secretory stage of the normal endometrium were considerably greater than those seen during the proliferative stage. Additionally, STAT3 in the ectopic endometrial deposits of EM patients was chronically overactive, and its phosphorylation intensity did not oscillate with the menstrual cycle, suggesting that the pathological overactivation of STAT3 in EM patients may stem from an impaired decidualization capacity. STAT3 participates in the balancing act between immunological evasion and destruction; once activated, it can promote the generation of immunosuppressive factors, such as IL-10 and TGF-β, while curbing the synthesis of immune-stimulatory molecules, such as IL-12, CD80, and

CD86 [37]. Okamoto *et al*. [38], using gene expression chip-based profiling, demonstrated that STAT3 inhibitors can suppress cell proliferation and VEGF secretion while inducing apoptotic death, suggesting that STAT3 blockade is a potential drug candidate for EM management. In parallel, our study established that this signaling circuit was notably muted in individuals who were not experiencing extended psychological strain.

An expansive collection of published work also addresses how enduring engagement of the stress response influences immunological phenomena linked to endometriosis pathogenesis [24, 39, 40]. Studies have suggested that the compromised functionality of natural killer cells, T lymphocytes, macrophages, mast cells, and NKT cells can pave the way for the implantation of ectopic endometrium; correspondingly, the disordered expression of adhesion-related proteins such as integrin ICAM-1, E-cadherin, and analogous factors may partake in the anchoring and irregular attachment of endometriotic foci. Due to the malfunctioning immune system, recruited immune cells release IL, TNF-α, VEGF, and a host of cytokines, further intensifying the disruption of immune regulation [41-44]. Prolonged psychological stress has a propensity to blunt both the innate and the adaptive branches of the immune defense, curtailing processes such as antigen presentation, cytotoxic T cell function, NK cell expansion, and the elaboration of pro-inflammatory cytokines through pathways involving adrenergic and glucocorticoid-mediated mechanisms [45]. Our data indicate that several mRNA species and signaling pathways associated with immunological competence—namely IL-1B, CXCR4, IL-7R, STAT4, CD14, and CCR5, alongside the FoxO, Jak-STAT, and NF-kappa B signaling hubs—show significant associations with endometriosis.

Research has demonstrated that metabolic processes can influence the trajectory of endometriosis. Concentrations of TGF-1 and lactate are markedly elevated in people with endometriosis relative to healthy women, and notably, disease lesions exhibit strong expression of glycolytic-associated transcripts such as HIf-1α, Glut1, Pdk1, and Ldha; correspondingly, the glycolytic activity within ectopic endometrium shifts toward the Warburg phenotype [46, 47]. Our investigation likewise established that sustained psychological strain remodels the microenvironment surrounding endometrial cells and alters their capacity for engraftment and tissue penetration by modulating numerous metabolic circuits in endometriosis lesions.

Neoplastic cells can adopt alternative metabolic strategies to generate ATP and macromolecular building blocks for their own requirements, depending on the availability and concentration of extrinsic nutrients, including glucose, glutamine, serine, arginine, and fatty acids [22]. Under conditions of glucose or glutamine scarcity, cancer cells can engage oncogenes such as cMyc by controlling the abundance of metabolic enzymes PHGDHP, SAT1, and PSPH within the serine biosynthetic route; they can also exploit residual glutamine or glucose to sustain serine production via endogenous synthesis and preserve redox equilibrium, thus enabling tumor cell survival amidst nutritional deprivation [48]. The metabolic configurations of tumor cells are intricate and plastic, and they select the most favorable metabolic mode for persistence based on the surrounding milieu. Tellingly, such metabolic configurations are also found within immune cell populations.

The immune apparatus comprises diverse immune cell lineages: cells that reside in a quiescent or resting condition within the body, and cells that become rapidly mobilized and responsive when the organism encounters infection, inflammation, or other foreign insults [49]. Accordingly, distinct metabolic wiring patterns can shape the specialization and effector capabilities of various immune cells, thereby influencing tumor initiation and advancement inside the tumor microenvironment [50].

Investigations in tumor immunology indicate that lactate production can compromise immune cells' function, thereby fostering neoplastic progression. Early-stage clinical studies showed that tumor burden in patients correlates with significantly elevated serum lactate concentrations. Subsequent inquiry has demonstrated that lactic acid, rather than the lactate ion, enters CTLs (cytotoxic T lymphocytes) and acidifies their intracellular compartment. This event does not block CTL expansion, cytokine release, or cytotoxicity [51-53]. Within the tumor microenvironment and in the presence of tumor-conditioned macrophages, elevated expression of VEGF and ARG1 occurs through HIF-1α upregulation, and lactic acid-mediated stabilization of HIF-1α under normoxic conditions drives the activation of VEGF and ARG1. This ultimately steers tumor-associated macrophages (TAMs) toward an M2 polarization state, and the ARG1 released by TAM2 cells facilitates tumor growth [54].

Amino acids and their metabolic derivatives produced by neoplastic tissue also affect immune cells and their activities. Evidence suggests that the arginine taken up by tumor cells within the tumor microenvironment is supplied mainly by tumor-associated myeloid cells (including macrophages, monocytes, myeloid progenitors, and neutrophils, among others) [55]. These immune effectors assist tumor cells in withstanding an arginine-depleted microenvironment. Furthermore, large-scale data analytics in recent studies have revealed that T cell activation involves the consumption of considerable amounts of arginine and the generation of downstream metabolic products, and that exogenous glycine can augment intracellular arginine pools and their downstream

derivatives by engaging transcription factor (BAZ1B, PSIP1, and TSN) binding protein complexes through a metabolite-driven shift from glycolysis to OXPHOS, thereby enhancing T cell viability and the abundance of memory cells and bolstering the anti-tumor immune response [56].

Research has demonstrated that metabolic processes can influence the trajectory of endometriosis, with TGF-1 and lactate concentrations in endometriosis patients significantly higher than those in healthy females. In particular, disease lesions exhibit prominent expression of glycolytic genes, including HIF-1α, Glut1, Pdk1, and Ldha. Along with the expression of glycolytic genes, the ectopic endometrium's glycolytic metabolism transitioned toward the Warburg effect [46].

Our investigation likewise established that sustained psychological strain remodels the microenvironment surrounding endometrial cells. It also modulates their capacity for engraftment and tissue penetration by perturbing numerous metabolic circuits and immunological pathways within the lesions of endometriosis patients.

Conclusion

To summarize, our work illustrates that prolonged stress hastens the progression of endometriosis in affected individuals. It further shows that amino acids such as arginine and serine accumulated to a greater extent in the lesions of the persistent stress cohort than in those of the reference cohort. Concurrently, the activity of multiple immune-associated pathways was diminished, suggesting that ongoing psychological strain may impede endometriosis's immune defense through metabolic reconfiguration; the precise mechanism warrants further investigation. Considering the current accessibility of supportive interventions like stress management strategies designed to attenuate the promotional effect of stress on endometriosis advancement, it appears that persistent stress constitutes a modifiable risk determinant for endometriosis.

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