Background

Psoriasis is a chronic, inflammatory skin disease that is closely associated with immune dysfunction. In recent years, the incidence of psoriasis has continued to rise, affecting 2%–3% of the global population [1]. As the underlying pathogenesis of psoriasis is yet unclear, at present, there is no cure for the disease in modern medicine. Psoriasis is protracted, recurrent, and difficult to heal; it significantly affects the quality of life of patients and can cause serious emotional and medical complications, endangering human health [2]. As a result of long-term accumulated experience, traditional Chinese medicine (TCM) shows potential in the treatment of psoriasis and offers a broad range of interventions.
Notably, the TCM treatment of psoriasis begins with blood-related factors, which are commonly recognised in the TCM community [3, 4].

The PSORI-CM01 formulation was optimised by Professor Guowei Xuan of Guangdong Provincial Hospital of Chinese Medicine, a TCM master, from the clinically proven prescription “Yinxieling” and intended for the treatment of blood stasis syndrome of psoriasis by promoting blood circulation, removing blood stasis, and cooling and detoxifying of the blood. In 1998, Yinxieling tablets (Z20080123, Patent no. CN 105233150A) were approved by the Guangdong Food and Drug Administration, Guangdong Province, China, for prescription use in hospitals. With over 22 years of clinical application in more than 200,000 people, the Yinxieling formulation has been proven to be safe and to have significant treatment effects [5]. PSORI-CM01 (Patent no. CN 101632827A) was optimised by Professor Chuanjian Lu’s team of Guangdong Provincial Hospital of Chinese Medicine over ten years ago and has a sound basic experimental research foundation and positive clinical trial results [6–8].

With the rapid development of sequencing technology, a large amount of genomic information has been discovered. Gene chip technology is one of the high-throughput detection technologies; it has been widely used in the research of various diseases [9]. In recent years, researchers have used this technique to conduct several studies on the gene expression of psoriasis [10–12]. Most of the studies are using gene chip to study the pathogenesis of diseases, and there are a few studies on the target of drug therapy, especially traditional Chinese medicine.

In order to establish a basis for the clinical development of the PSORI-CM01 formula as a safe and effective modern prescription drug, it is necessary to conduct experimental controlled trials to compare the mechanism of action of PSORI-CM01 and Yinxieling in the treatment of patients with psoriasis. We used gene chip technology to analyze a series of differential expression of genes in clinical samples of PBMCs.

2. Materials and Methods

2.1. Clinical Study. Blood samples from patients with psoriasis vulgaris and the healthy volunteers were obtained from the Department of Dermatology, Guangdong Provincial Hospital of Chinese Medicine, and used in a randomised, double-blind, and double-dummy clinical study [13]. The diagnostic criteria were in line with those previously published for psoriasis vulgaris [14]. Detailed information on the psoriatic patients, including their demographics, and psoriasis area and severity index (PASI) is provided in Table 1. The study was performed in accordance with the Declaration of Helsinki and the relevant clinical trial research regulations of China (Trial registration ChiCTR-TRC-14005185 registered on August 8, 2014). All procedures involving human participants in this study were performed in accordance with the ethical standards of the Guangdong Provincial Hospital of Chinese Medicine, Clinical Research Ethics Committee (ethical approval no. B2012-53-03). Before the collection of samples, all patients enrolled in the study were required to sign an informed consent form, and the researchers explained the purpose and treatments in the study to them (Figure 1).

2.2. Drugs. As described previously [15, 16], ultrahigh liquid chromatography/electrospray ionisation hybrid linear trap quadrupole Orbitrap mass spectrometry (UHPLC-ESI- LTQ/Orbitrap-MS) and high-performance liquid chromatography–photodiode array (HPLC-PDA) were used to examine and verify the batches of PSORI-CM01 and Yinxieling for quality control purposes. PSORI-CM01 granules and matching placebo granules were prepared by the Tianjiang Pharmaceutical Co., Ltd., Jiangyin, Jiangsu Province, China (catalogue no. 1511329). The Yinxieling tablets and the matching placebo tablets were prepared by the Guangzhou Kangyuan Pharmaceutical Science & Technology Co., Ltd., Guangzhou, Guangdong Province, China (catalogue no. 20151003).

2.3. Group Intervention. Participants in the PSORI-CM01 group received 5.5 g PSORI-CM01 granules twice daily after meals, and the controls received 5.5 g placebo granules three times daily after meals for 12 weeks. The participants in the Yinxieling group received five Yinxieling tablets three times daily after meals, and the controls received five placebo tablets two times daily after meals for 12 weeks. The details of the treatments and procedures have been previously described [13], instruments, reagents, and software. The following reagents, kits, and sets were used in this study: TRI Reagent (Sigma, Germany, catalogue no. T9424), miRNasy Micro Kit (QIAGEN, Germany, catalogue no. 217084), RNase-Free DNase Set (QIAGEN, Germany, catalogue no. 79254), TIANGEN miRcute miRNA cDNA first-strand synthesis kit (TIANGEN, Beijing, China, catalogue no. KR201), and SYBR Green PCR kit (TIANGEN, Beijing, China, catalogue no. FP411-02). An Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) was used for electrophoresis and an Agilent Scanner G2505 C (Agilent Technologies, Santa Clara, CA, USA) for chip scanning. The following software was used: Feature Extraction (version 10.7.1.1, Agilent Technologies) was used for data extraction, Genespring (version 13.1, Agilent Technologies) for data processing, and the Affymertix GeneChip® Clarion D array human chip (Affymertix) for transcriptome gene expression levels.

2.4. Sample Collection and Processing. Ten millilitres of cubital venous blood was collected from each participant and placed in an anticoagulation tube containing heparin. According to the instructions of reagent test kits, peripheral blood mononuclear cells (PBMCs) were separated from the blood samples and stored in a refrigerator at −80°C.

2.5. RNA Extraction and Quality Control. Total RNA extraction from PBMCs was performed using TRI Reagent (Cat# T9424, MilliporeSigma, 2020 Merck KGaA,
Darmstadt, Germany) according to the standard operating procedures provided by the manufacturer. The total RNA samples were further purified using a miRNeasy Micro Kit (Cat# 217084, QIAGEN, GmBH, Germany) and an RNase-Free DNase Set (Cat# 79254, QIAGEN, GmBH, Germany). During each round of RNA extraction, a control total RNA was used as the positive quality control, and RNase-free water was used as the negative quality control. If any degradation of the positive quality control occurred during the process of RNA extraction, the sample would exhibit the same effects. If the negative quality control was contaminated by positive total RNA during the extraction, the sample would also be contaminated by the exogenous total RNA. After the validation of quality control, the integrity of purifying the total RNA was checked using an Agilent Bioanalyzer 2100 electrophoresis analyzer (Agilent Technologies, Santa Clara, CA, USA). Samples which met the RNA integrity number value of ≥6 were loaded onto the chips for analysis.

2.6. Gene Chip Analysis. In vitro reverse transcription and amplification of mRNA in the sampled total RNA were performed using the Affymetrix expression profiling kit (GeneChip® WT PLUS Reagent Kit; Cat# 902280, Affymetrix, Santa Clara, CA, USA), following the standard procedures.
operating procedures specified by the manufacturer; the cRNA produced was labelled using biotin.

The biotin-labelled cRNA was added to the cDNA chip according to the Affymetrix chip hybridisation procedure. Then hybridisation was carried out in a rolling hybridisation oven (Hybridization Oven 645, Cat# 00-0331-220V, Affymetrix, Santa Clara, CA, USA), at 45°C for 16 h following the standard hybridisation process using the supporting kit (GeneChip Hybridization, Wash and Stain Kit, Cat# 900720, Affymetrix, Santa Clara, CA, USA) provided with the expression profile chip. Following hybridisation, the chip was washed using a Fluidics Station 450DX2 (Cat# 00–0335, Affymetrix, Santa Clara, CA, USA) according to the standard operating procedure provided by Affymetrix.

The gene chip was scanned using a GeneChip® Scanner 3000DX2 (Cat# 00–0334, Affymetrix, Santa Clara, CA, USA). The original data were read with Command Console Software 3.1 (Affymetrix, Santa Clara, CA, USA). Data quality control was also performed, and data reports were issued by Command Console Software 3.1. The qualified data was normalised using the gene-level SST-RMA algorithm of the software.

2.7. Validation by Quantitative Real-Time Polymerase Chain Reaction. The RNA isolated from the PBMC samples of different groups was reverse transcribed directly into cDNA using a SuperScript IV Reverse Transcriptase (Thermo Fisher, Dalian, China) according to the manufacturer’s instructions. The PCR amplification conditions were as follows: initial denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s, and 60°C for 30 s, then annealing at 95°C for 5 s and 60°C for 1 min. The expression levels of genes GPVI, TNFSF4, CXCR3A, CXCR3B, CXCL4L1, and TUBBI were examined by quantitative real-time polymerase chain reaction (qRT-PCR) using an SYBR Green PCR kit (TaKaRa, Dalian, China). The results were expressed as means ± standard errors of the means. The Student’s t-test was used for the statistical analyses. P values <0.05 were considered significant.

2.8. Data Analysis. Preliminary gene-level differential expression analysis was performed using Transcriptome Analysis Console Software (Affymetrix, Santa Clara, CA, USA). Genes with P value <0.05 and fold change ≥2 were screened as differentially expressed genes and enriched by the DAVID Gene Functional Classification Tool (v6.8 http://david.abcc.ncifcrf.gov). The pathways with a P value <0.05 were selected for further analysis. The FDR-BH adjusted P values obtained by t-test and fold change values were used for differential gene screening. The screening criteria were a fold change value of up- or downregulation >2 and an FDR-BH P value <0.05. Paired-sample t-tests were performed to compare the intragroup difference before and after treatment within groups A and B, whereas two-sample independent t-tests were performed to compare the intergroup differences. The FDR-BH values were calculated after sorting the P values in ascending order. The screened differentially expressed genes were clustered by unsupervised hierarchical cluster analysis using the online OmicShare Tools (https://www.omicshare.com/tools/) and displayed as volcanic and heat maps. To determine the biological functions or signalling pathways affected by the differentially expressed genes, the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed using the online string and metascape tools (https://string-db.org/ and https://metascape.org/gp/index.html#main/step1, resp.).

3. Results

3.1. Quality Control of Gene Chip Data. Combined with information from the clinical samples, the results of the Agilent microarray analysis of the 51 samples (21 pairs of samples from patients with blood stasis syndrome of psoriasis, including 12 samples before and after treatment with prescription PSORI-CM01 and nine samples before and after treatment with Yinxieling) and 9 samples before and after placebo treatment in the controls showed that the quality of the original data was well controlled (Figure 2).

3.2. Screened Differentially Expressed Genes between Groups. As shown in Table 2, comparing the genes with an FDR_BH <0.05 and fold change >2, between patients in the PSORI-CM01 group before treatment and the controls, a total of 178 differentially expressed genes were found, including 147 upregulated and 31 downregulated, whereas comparing the genes between patients in the PSORI-CM01 group after treatment and the controls, a total of 908 differentially expressed genes were found, of which 758 were upregulated and 150 were downregulated. However, comparing the genes before and after treatment within the PSORI-CM01 group, a total of 668 differentially expressed genes were found, of which 445 were upregulated and 223 were downregulated. Contrarily, comparing the genes between patients in the Yinxieling group before treatment and the controls, a total of 276 differentially expressed genes were found, of which 445 were upregulated and 223 were downregulated. Whereas comparing the genes between patients in the Yinxieling group after treatment and the controls, a total of 732 differentially expressed genes were found, including 475 upregulated and 257 downregulated. However, comparing the genes before and after treatment within the Yinxieling group, a total of 657 differentially expressed genes were found, of which 168 were upregulated and 489 were downregulated.

3.3. Differences in Gene Expression Profiles between Patients in the PSORI-CM01 Group, Yinxieling Group, and the Controls. The differences in gene expression in the PSORI-CM01 group before treatment versus the control, in the PSORI-CM01 group after treatment versus the control, and in the PSORI-CM01 group before treatment versus PSORI-CM01 group after treatment were compared and displayed in volcanic maps and heat maps (Figure 3). The differences in gene expression in the Yinxieling group before treatment versus the control, the Yinxieling group after treatment versus the control, and the Yinxieling group before
treatment versus the Yinxieling group after treatment were compared and displayed in volcanic maps and heat maps (Figure 4). In the volcano plot, red is the upregulation of genes and green is the downregulation. The larger the fold change value, the larger the variable indicating the amount of difference in gene expression between the two groups. In the heatmap plot, each small square represents each gene, and its color represents the expression level of the gene. The greater the expression level, the darker the color (red for upregulation, green for downregulation). Each row represents the expression level of each gene in different samples, and each column represents the expression level of all genes in each sample.

3.4. Venn Analysis of Differentially Expressed Genes. Results of the comparison of differentially expressed genes using Venn analysis showed that there were 113 differentially expressed genes between the PSORI-CM01 group before treatment (PSORI-CM01 group 0 weeks) and the healthy control group. After 12 weeks of treatment, there were 892 differentially expressed genes between the PSORI-CM01 group (PSORI-CM01 group 12 weeks) and the healthy control group. The intersection after the two comparisons can be seen as a total of 35 common differentially expressed genes that existed in both differential gene comparisons at the same time (Figure 5(a)). In comparison, there were 113 differentially expressed genes between the Yinxieling group before treatment (Yinxieling group 0 weeks) and the healthy controls, 724 differentially expressed genes between the Yinxieling group after treatment (Yinxieling group 12 weeks) and the healthy controls, and 39 common differentially expressed genes both before and after treatment in the Yinxieling group (Figure 5(b)). Twenty-three genes were differentially expressed in both groups after treatment, compared to the healthy controls, and they overlapped completely (Figure 5(c)). Seventy-eight genes were not differentially expressed in the PSORI-CM01 group, and 74 were not differentially expressed in the Yinxieling group after treatment, compared with the healthy controls. Among these genes, 72 were common to two groups, which were the genes on which the two drugs acted jointly. Additionally, compared with the healthy controls, six genes, PTX3, ALAS2, Skerber, Toby, HBB, and HBA1 were not differentially expressed in the PSORI-CM01 group but were in the control group.

Table 2: Analysis of differentially expressed genes between groups.

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Differential genes</th>
<th>Upregulated genes</th>
<th>Downregulated genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSORI-CM01 group 0 weeks, versus healthy control group</td>
<td>12 vs 9</td>
<td>178</td>
<td>147</td>
</tr>
<tr>
<td>PSORI-CM01 group 12 weeks, versus healthy control group</td>
<td>12 vs 9</td>
<td>908</td>
<td>758</td>
</tr>
<tr>
<td>PSORI-CM01 group 0 weeks, versus PSORI-CM01 group 12 weeks</td>
<td>12 vs 12</td>
<td>668</td>
<td>445</td>
</tr>
<tr>
<td>Yin Xie Ling group 0 weeks, versus healthy control group</td>
<td>9 vs 9</td>
<td>276</td>
<td>246</td>
</tr>
<tr>
<td>Yin Xie Ling group 12 weeks, versus healthy control group</td>
<td>9 vs 9</td>
<td>732</td>
<td>475</td>
</tr>
<tr>
<td>Yin Xie Ling group 0 weeks, versus Yin Xie Ling group 12 weeks</td>
<td>9 vs 9</td>
<td>657</td>
<td>168</td>
</tr>
</tbody>
</table>
Yinxieling group; compared with the healthy controls, two genes (doyzor and mokari) were not differentially expressed in the Yinxieling group but were in PSORI-CM01 group (Figure 5(d)).

3.5. Pathway Enrichment Analysis for Differentially Expressed Genes. KEGG enrichment analysis was performed using DAVID online tools, to determine the biological functions or signalling pathways affected by the differentially expressed genes. The results showed that the top ten major signalling pathways targeted by PSORI-CM01 treatment included haemostasis, PI3K-Akt signalling pathway, G alpha (s) signalling events, neutrophil chemotaxis, formation of fibrin clot (Clotting Cascade), platelet activation, response to mycotoxin, factors involved in megakaryocyte development and platelet production, Rho GTPase effectors, and leukocyte differentiation (Figures 6(a) and 6(b)). However, the top ten major signalling pathways targeted by Yinxieling

Figure 3: Unsupervised hierarchical cluster analysis of differentially expressed genes before and after PSORI-CM01 treatment. (a) Volcanic map of PSORI-CM01 group before treatment versus the control group; (b) volcanic map of PSORI-CM01 group after treatment versus the control group; (c) volcanic map of PSORI-CM01 group after treatment versus PSORI-CM01 group before treatment; (d) heat map of PSORI-CM01 group before treatment versus the control group; (e) heat map of PSORI-CM01 group after treatment versus the control group; and (f) heat map of PSORI-CM01 group after treatment versus PSORI-CM01 group before treatment.
treatment included haemostasis; RUNX1 regulates genes involved in megakaryocyte differentiation and platelet function, platelet aggregation, formation of fibrin clot (Clotting Cascade), cell surface interactions at the vascular wall, positive regulation of cell migration, negative regulation of cell activation; and Rho GTPases activate PAKs, megakaryocyte differentiation, and cell projection assembly (Figures 6(c) and 6(d)). In addition, results of Venn analysis on the signalling pathways in two treatment groups showed that haemostasis and Rho GTPases were the common signalling pathways of drug action shared by the two groups (Figure 6(e)).

3.6. Verification of Selected Genes. Using qRT-PCR, we verified the six differentially expressed genes obtained from the microarray analysis. As shown in Figure 7, the expressions of GPVI, TNFSF4, CXCR3A, CXCR3B, CXCL4L1,
and TUBB1 were in accordance with the results of the microarray analysis.

4. Discussion

Psoriasis is a chronic proliferative skin disease with immune abnormalities that is determined by multiple genes and induced by various environmental factors. Because it is inherited, difficult to treat, and easy to relapse, there is no modern medical treatment or radical cure for it. Most current allopathic therapies have significant toxic or side effects or both and thus cannot be used for long-term treatment. TCM has accumulated significant experience in the treatment of psoriasis. TCM syndrome differentiation and treatment can relieve symptoms, delay progression, reduce recurrence, and improve quality of life, and it has few side effects, which make it suitable for long-term treatment.

Results of differentially expressed genes from Venn analysis between the groups showed that, compared with the controls, 78 genes in the PSORI-CM01 group and 74 in the Yinxieling group were not differentially expressed after treatment, of which 72 genes were common between the PSORI-CM01 and Yinxieling groups before treatment that showed no significant difference after treatment compared with the control group.

Figure 5: Venn analysis of differentially expressed genes between groups. (a) Differentially expressed genes between the PSORI-CM01 and the control groups before and after treatment; (b) differentially expressed genes between the Yinxieling and the control groups before and after treatment; (c) differentially expressed genes before and after treatment, common between PSORI-CM01 and Yinxieling groups, compared with the control group; and (d) differentially expressed genes, common between the PSORI-CM01 and Yinxieling groups before treatment.
Figure 6: Continued.
mediate the antiangiogenic effect and chemotactic activity of platelet factor 4 variant (CXCL4L1) [22]. Nomura et al. used microarray technology to compare the differences of gene expression levels between psoriasis patients and atopic dermatitis patients; it was found that there were 62 differentially expressed genes in the lesions of patients with psoriasis, among which the gene encoding CXC chemokine was significantly upregulated [23]. In addition, abnormal expression of TUBB1 is closely related to thrombocytopenia in congenital and acquired platelet diseases and affects platelet function by altering microvascular formation [24]. Therefore, we speculated that the genes mentioned above might be involved in the pathological mechanism and treatment process of psoriasis. When compared with the controls, six genes, PTX3, ALAS2, Skerber, Toby, HBB, and HBA1 were not differentially expressed in the PSORI-CM01 group but were in the Yinxieling group. Pathway enrichment analysis demonstrated that these genes are primarily correlated with the metabolism of glycine, serine, and threonine.

The result of Venn analysis on the signalling pathways in two drugs groups showed that haemostasis and Rho GTPases were the common pathways targeted by the two treatments. Studies of haemostasis on patients with psoriasis show that there are varying degrees of abnormalities in blood coagulation and fibrinolysis [25–28]. A recent study demonstrated that psoriasis is linked to increased cardiovascular risks. Since chronic skin-specific inflammation may promote atherosclerosis, myocardial infarction or stroke may also be correlated with haemostatic disorders; there were dysfunctions of fibrinolysis and thrombosis in patients with psoriasis [29]. Rho GTPases are a major branch of the Ras superfamily and play an important role in the signal transduction pathway of cells [30]. Since the overexpression of the Rho protein is related to the initiation and development of tumours, Rho GTPases, which are involved in cytoskeleton remodelling, cell movement, gene transcription regulation, and cell cycle regulation, can play a role in the proliferation and apoptosis of tumours [31]. Psoriasis is an end-point disease similar to epithelial-mesenchymal transition (EMT), which is a process that transforms epithelial cells into fibroblast-like cells, and the extracellular-signal-regulated kinase, Rho, and glycogen synthase kinase-3 can promote the EMT process in psoriatic keratinocytes [32].

Some researchers have used gene chip technology to study psoriasis, mainly including the pathogenesis mechanism of psoriasis. Bowcock et al. collected samples from 15 patients with psoriasis and found that there were 177 gene expression differences between lesions and nonlesions [33]. Another research used gene chip technology and real-time quantitative PCR to detect the expression of G-protein coupled receptors in lesions and nonlesions of psoriatic patients [12]. Our study is based on the clinical efficacy of

![Figure 6: Pathway enrichment analysis for differentially expressed genes between groups. (a) Analysis of regulated differential pathways between PSORI-CM01 and the control group; (b) analysis of differentially expressed genes between the PSORI-CM01 and the control groups before treatment that showed no significant difference after treatment; (c) analysis of regulated differential pathways between the Yinxieling and the control group; (d) analysis of differentially expressed genes between the Yinxieling and the control groups before treatment that showed no significant difference after treatment; and (e) Venn analysis of differentially expressed pathways between the PSORI-CM01 and Yinxieling groups.](image-url)
traditional Chinese medicine prescription before and after treatment to explore the differences of gene expression in PBMCs by utilizing gene chip technology.

There are some limitations in this article, such as the small size of the original sample due to the limitation of the clinically available sample size. This study followed a rigorous screening method and strictly controls the sample quality, so the final sample size that meets the conditions is low. In the future, our research can extend the clinical data collection time and continue to increase the sample size for

**Figure 7:** The gene validation of GPVI, TNFSF4, CXCR3A, CXCR3B, CXCL4L1, and TUBB1 by qRT-PCR (*P < 0.05, **P < 0.01 vs. PSORI-CM01 group 0 weeks, #P < 0.05, ##P < 0.01 vs. Yinxieling group 0 weeks).
related research. We will continue to study microRNA gene chip and perform correlation analysis results between the gene chip and microRNA gene chip.

5. Conclusions

In conclusion, through the comparison of gene expression profiles in PBMC between patients with psoriasis vulgaris before and after treatment with PSORI-CM01 or Yinxieling formulations and the controls and through pathway enrichment analysis of differentially expressed genes, we found that the two drugs had positive effects on psoriasis vulgaris primarily by the regulation of pathways related to platelet activation, aggregation, and blood coagulation. This study clarified the mechanisms of the two Chinese herbal compound prescriptions used in the treatment of psoriasis and laid a foundation for the future study.

Abbreviations

PBMCs: Peripheral blood mononuclear cells
PASI: Psoriasis area and severity index
KEGG: Kyoto Encyclopedia of Genes and Genomes
GPIIb/IIa: Glycoprotein IIb/IIa
ICAM-1: Intercellular adhesion molecule 1
TNFSF4: Tumour necrosis factor superfamily 4
CXCR3: CXC chemokine receptor 3
CXCL4L1: Platelet factor 4 variant
EMT: Epithelial-mesenchymal transition.

Data Availability

The datasets used and/or analyzed during the current study would be available from the corresponding author on reasonable request.

Ethical Approval

The study was performed in accordance with the Declaration of Helsinki and the relevant clinical trial research regulations of China (trial registration no. ChiCTR-TRC-14005185; registration date: 8 August 2014). All the study protocols were in accordance with the ethical standards of Guangdong Provincial Hospital of Chinese Medicine Clinical Research Ethics Committee (ethical approval no. B2012-53-03).

Consent

Each patient enrolled in the study was required to sign an informed consent form. All participants were informed of the purpose of the study and the treatments before their blood samples were collected.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors’ Contributions

All the authors have participated extensively in the study and had proofread the final manuscript. Chuanjian Lu, Ling Han, and Hengjun Gao conceived and designed the research. Yue Lu and Qao Qi conducted the experiments. Yuhong Yan, Danni Yao, Hao Deng, and Jingwen Deng collected patients. Qubo Chen helped sample preservation and transportation. Yue Lu, Yao Qi, and Li Li analyzed the data. Yue Lu wrote the manuscript. Chuanjian Lu, Ling Han, and Hengjun Gao approved and reviewed the final manuscript. All authors read and approved the final manuscript.

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References


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