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ORIGINAL ARTICLE

MicroRNA-21-5p Regulates CD3+T Lymphocytes Through VCL and LTF in Patients with Immune Thrombocytopenia

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SUMMARY

Background: **Immune thrombocytopenia (ITP) is an autoimmune hemorrhagic disease with distinct clinical manifestations such as extensive skin petechiae, mucosal bleeding, and even visceral hemorrhage. In this study, CD3+T lymphocytes from ITP patients were screened for differentially expressed genes. The expression of miR-21 and miR-155 in T lymphocytes of ITP patients were investigated. The downstream target genes of miR-21 and miR-155 were also searched for the correlation between differentially expressed genes of ITP.**
Methods: **Differential gene screening was performed using the GSE43177 data set in the GEO database, and the expression of miR-21 and miR-155 in T lymphocytes of ITP patients was verified by qPCR. The interaction network of core downstream target genes and ITP differentially expressed genes of miR-21 and miR-155 were constructed with the STRING database, and the associated factors were verified by qPCR.**
Results: **In ITP patients, the expression of CD8+T lymphocytes increased, the expression of CD4+T lymphocytes decreased, and the ratio of CD4+/CD8+T cells decreased. Fourteen genes were differentially expressed in CD3+T lymphocytes, all of which were upregulated, and the expression of S100A8 was increased in ITP patients. The expression of miR-21-5p and miR-155-5p increased in CD3+T lymphocytes of initial ITP patients. The core downstream target gene VCL of miR-21 was associated with the differentially expressed genes such as LTF, LCN2, and DEFA4 in the interaction network. VCL expression was decreased and LTF expression was increased in ITP patients.**
Conclusions: **S100A8 plays an important role in the regulation of CD3+T lymphocytes in ITP patients. MiR-21-5p regulates the differentially expressed gene LTF by inhibiting the core downstream target gene VCL and participates in the immune mechanism of T lymphocytes in ITP patients. MiR-155-5p is also involved in the immunoregulatory mechanism of T lymphocytes in ITP patients.**
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KEY WORDS

immune thrombocytopenia, CD3+T lymphocyte, differentially expressed mRNA, microRNA-21, VCL, LTF

INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune hemorrhagic disease with distinct clinical manifestations such as extensive skin petechiae, mucosal bleeding, and even visceral hemorrhage. The platelet counts in the peripheral blood of ITP patients is decreased with a characteristic normal or increased number of mega-

karyocytes in the bone marrow, accompanied by a disorder of megakaryocyte maturation [1]. The underlying etiology and pathogenesis of ITP are not clearly understood, but hereditary factors, drugs, infection, oxidative stress, and abnormal immune regulation play important roles [2]. Recently, it has been found that cellular immunity participates in ITP onset, which is mainly manifested in the imbalance of T helper cells 1 or 2 (Th1/Th2), Th17/Treg cells, or T lymphocytes such as CD8+ T cells [3].

MicroRNA (miRNA) is a type of small non-coding RNA with a length of 18 - 22 nucleotides, which widely exists in eukaryotes. MiRNA can recognize specific mRNA and inhibit the synthesis of target proteins through a nucleic acid complementary sequence, thereby regulating the expression of corresponding genes at the post transcription level [4]. In recent years, more and more attention has been paid to the regulatory role of miRNA in the pathogenesis of ITP. In particular, immune factors such as miRNA-21 (miR-21) and miRNA-155 (miR-155) have become classic miRNA molecules widely associated with a variety of autoimmune diseases [5]. MiR-21 plays an important role in autoimmune diseases by acting on T lymphocytes, especially Th17 cells [6]. Similarly, miR-155 is an important miRNA molecule involved in the pathogenesis of ITP and plays a regulatory role in CD8+T cells [7]. However, it is not clear whether miR-21 and miRNA-155 are overexpressed in CD3+T lymphocytes of ITP patients. Gene chip has a high-throughput characteristic that can effectively detect the level of gene expression in samples, thus playing an important role in functional genomics research. Recently, many abnormal gene expressions were found in ITP patients [8]. This study collected and analyzed flow indexes of peripheral blood T lymphocyte subsets in ITP patients and healthy controls. Bioinformatics was used to screen differentially expressed genes in CD3+T lymphocytes of ITP patients, and qPCR was used to verify the partial differential genes. Then, the expression levels of miR-21 and miR-155 in T lymphocytes of ITP patients were explored, and the interaction between the core downstream target factors of miR-21 and miR-155 and the differentially expressed molecules of ITP diseases were analyzed. Therefore, we aim to study the molecular mechanism of miR-21 and miR-155 in ITP and their potential role in providing early clinical diagnosis and treatments of ITP patients.

MATERIALS AND METHODS

Experimental materials

Lymphocyte Separation Medium, human (Solarbio, P8610). Red Blood Cell Lysis Buffer (Solarbio, R1010). CD3 MicroBeads, human (Miltenyi, 130-097-043). MS Columns: (Miltenyi, 130-042-201). autoMACS Running Buffer (Miltenyi, 130-091-221). Trizol (Invitrogen, 15596026). FastKing RT Kit (With gDNase) (TIANGEN KR116). SYBR Green (TIANGEN FP209).

General information and peripheral blood T lymphocyte subsets

Peripheral blood samples of 10 ITP cases and 10 cases of healthy controls were collected. All cases were primary ITP cases. Among them, 3 were males and 7 were females, with an average age of 57.4 years. The baseline platelet count of all patients before treatment was lower than 80×10^9/L. Among the 10 healthy controls, 6 were male and 4 were female, with an average age of 41.25 years. Flow cytometry results were analyzed for T lymphocyte subsets in peripheral blood of both ITP patients and healthy controls.

Source of information and screening of differentially expressed genes

This study obtained a data set of mRNA chip expression spectrum GSE43177 from the Gene Expression Database (GEO) on the NCBI website. The data set was submitted by Jernås M et al., including the mRNA expression profile of CD3+T lymphocytes in peripheral blood of 9 ITP patients (GSM1057953-GSM1057961) and 10 healthy controls (GSM1057943-GSM1057952) [9]. The data set is tested by the GPL570 chip platform, namely, the Affymetrix human genome U133 Plus 2.0 array gene chip, and then uploaded to the GEO database in CEL format after standardization and other standardization processing. All GSM samples in the GSE43177 data set were downloaded, decompressed, and imported into BRB-ArrayTools software. First, the chip data were preprocessed and standardized, and then the corrected p-value (Q value) of each gene was calculated by random variance *t*-test. The screening criteria of differentially expressed genes were Q value < 0.05 and the multiple difference ≥ 2. The differentially expressed genes were plotted by volcano map and heat map, and then the differentially expressed genes in the heat map were hierarchically clustered.

Functional enrichment analysis and pathway enrichment analysis

Gene Ontology (GO) is a standardized classification system of gene function, including cell components (CC), molecular functions (MF), and biological processes (BP) [10]. Pathway enrichment analysis is based on mapping the signal pathway in the Kyoto encyclopedia of genes and genomes (KEGG) database to the genome to identify the main metabolic and cell transduction pathways involved in differential genes [11]. This study used DAVID and KOBAS databases for GO functional enrichment analysis and pathway enrichment analysis of the screened differential genes. Q value < 0.05 was considered statistically significant.

The mRNA expression of DEFA1, S100A8, miR-21, and miR-155 in peripheral blood CD3+T cells were detected by qPCR

Peripheral blood samples of 10 ITP cases and 10 healthy controls were collected. Peripheral blood mononuclear cells were extracted, magnetic beads sorted CD3+T cells, then total RNA was extracted by Trizol, and the total RNA of each group was reverse transcribed. Bio-Rad CFX fluorescence quantitative PCR instrument was used to conduct real-time quantitative PCR reactions. The relative expression of each gene was calculated by $2^{-\triangle\triangle CT}$.

Interaction network analysis between miR-21-5p and miR-155-5p core target gene proteins and differentially expressed gene proteins

Three online target mRNA prediction software programs (TargetScan, miRDB, and mirTarBase) were used to predict the downstream target gene of miR-21-5p and miR-155-5p. Then, the target mRNA predicted by the three software programs were combined to form a core target gene set for further analysis. The PPI network of differentially expressed mRNA was constructed and imported into Cytoscape for visualization. The node connectivity (degree) of each mRNA was calculated, and the interaction between the downstream target mRNA of miR-21-5p and miR-155-5p and the differentially expressed mRNA was analyzed.

The mRNA expressions of VCL, LTF, LCN2, and DEFA4 in peripheral blood CD3+T cells were detected by qPCR

Peripheral blood samples of 10 ITP cases and 10 healthy controls were collected. The mRNA expression of DEFA4 after vinculin (VCL), LTF, and LCN2 were detected. The peripheral blood of 10 patients who recovered from ITP was further collected, and the mRNA of VCL was detected as the ITP remission group. Peripheral blood mononuclear cells were extracted, magnetic beads sorted CD3+T cells, and then total RNA was extracted by Trizol, and the total RNA of each group was reverse transcribed. Bio-Rad CFX fluorescence quantitative PCR instrument was used to conduct real-time quantitative PCR reactions. The relative expression of each gene was calculated by $2^{-\triangle\triangle CT}$.

The list of primers for miRNA and mRNA in the qPCR experiment

The reverse transcription primer sequence of miR-21-5p:	**GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAACA**
The reverse transcription primer sequence of miR-21-3p:	**GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACAGCC**
The reverse transcription primer sequence of miR-155-5p:	**GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACCCC**
The reverse transcription primer sequence of miR-155-3p:	**GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGTTAA**
The reverse transcription primer sequence of U6:	**AACGCTTCACGAATTTGCGT**
PCR primer sequence of miR-21-5p:	**F: GCGCGTAGCTTATCAGACTGA R: AGTGCAGGGTCCGAGGTATT**
PCR primer sequence of miR-21-3p:	**F: GCGCAACACCAGTCGATG R: AGTGCAGGGTCCGAGGTATT**
PCR primer sequence of miR-155-5p:	**F: CGCGTTAATGCTAATCGTGATA R: AGTGCAGGGTCCGAGGTATT**
PCR primer sequence of miR-155-3p:	**F: GCGCGCTCCTACATATTAGCA R: AGTGCAGGGTCCGAGGTATT**
PCR primer sequence of U6:	**F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT**
PCR primer sequence of DEFA1:	**F: TCCCTTGCATGGGACGAAAG R: GGTTCCATAGCGACGTTCTCC**
PCR primer sequence of S100A8:	**F: ATGCCGTCTACAGGGATGAC R: ACTGAGGACACTCGGTCTCTA**
PCR primer sequence of VCL:	**F: CTCGTCCGGGTTGGAAAAGAG R: AGTAAGGGTCTGACTGAAGCAT**
PCR primer sequence of LTF:	**F: AGTCTACGGGACCGAAAGACA R: CAGACCTTGCAGTTCGTTCAG**
PCR primer sequence of LCN2:	**F: GACAACCAATTCCAGGGGAAG R: GCATACATCTTTTGCGGGTCT**
PCR primer sequence of DEFA4:	**F: CCTTTGCATGGGATAAAAGCTCT R: ACACCACCAATGAGGCAGTTC**
PCR primer sequence of β-actin:	**F: CATGTACGTTGCTATCCAGGC R: CTCCTTAATGTCACGCACGAT**

Table 1. Analysis of T lymphocyte subsets in each group.

	Healthy control group (n = 10)	ITP patients (n = 10)
CD3+CD8+	23.83 ± 7.11	29.32 ± 10.99 *
CD3+CD4+	43.43 ± 9.63	39.53 ± 9.39 *
CD3+CD4+CD8+	0.46 ± 0.44	0.53 ± 0.83
CD16+CD56+	12.74 ± 5.87	14.76 ± 6.97
CD19+	9.20 ± 5.43	13.15 ± 16.09
CD4+/CD8+	2.09 ± 1.01	1.63 ± 0.91 *

*** p < 0.05.**

Table 2. List of differentially expressed mRNA in ITP patients.

mRNA Name	Probe number	Fold change	Q value
DEFA1	205033_s_at	4.51	0.0266338
S100A8	214370_at	3.01	0.0033589
S100A8	202917_s_at	2.87	0.0040219
DEFA4	207269_at	2.84	0.0222109
LTF	202018_s_at	2.78	0.0091624
IGH	217022_s_at	2.64	0.0108928
S100A9	203535_at	2.59	0.0035414
LCN2	212531_at	2.54	0.0190266
CEACAM8	206676_at	2.52	0.0217029
IGHG1	211430_s_at	2.32	0.0082189
IGK	221651_x_at	2.29	0.0218875
IGK	221671_x_at	2.25	0.0241334
IGK	224795_x_at	2.25	0.0212497
IGLC1	214677_x_at	2.14	0.0349175
CYAT1	215121_x_at	2.11	0.0322241
S100A12	205863_at	2.07	0.0148792
IGLC1	209138_x_at	2.06	0.044968
S100P	204351_at	2.03	0.0087279

RESULTS

Flow cytometry analysis of T lymphocyte subsets in each group

As shown in Table 1, the expression of CD3+CD8+T lymphocytes increased, the expression of CD3+CD4+T lymphocytes decreased, and the ratio of CD4+/CD8+T cells decreased in ITP patients compared to the healthy control group. These results indicate that the expression of CD3+T lymphocytes and immune function are abnormal in ITP patients.

Screening of differential genes

According to the expression profile of CD3+T lymphocytes in peripheral blood of ITP patients and healthy volunteers, there were 54,675 probes and 1,915 differential mRNA on the GPL570 chip. Furthermore, in the database, the limited Q value was less than 0.05, and the multiple of differences was more than 2-fold. Fourteen differentially expressed mRNA corresponding to 18 probes were screened, of which 14 mRNA were upregulated. As shown in Table 2, The two upregulated genes DEFA1 and S100A8, showed the most significant difference. As shown in Figure 1.1, the volcanic map

Table 3. Typical target mRNA information of miR-21-5p and miR-155-5p.

Typical downstream target mRNA of miR-21-5p		Typical downstream target mRNA of miR-155-5p	
COL4A1	PCBP2	BRWD3	KDM3A
JPH1	PDCD4	CSNK1G2	KPNA1
KLF3	PIK3R1	DMTF1	RCN2
KRIT1	PPP1R3B	ETS1	RREB1
MBNL1	RTN4	FBXO11	SMARCA4
MTMR12	TESK2	HIF1A	SOCS5
NFIB	TET1	HIVEP2	STRN3
PCBP1	VCL	IRF2BP2	TBC1D15

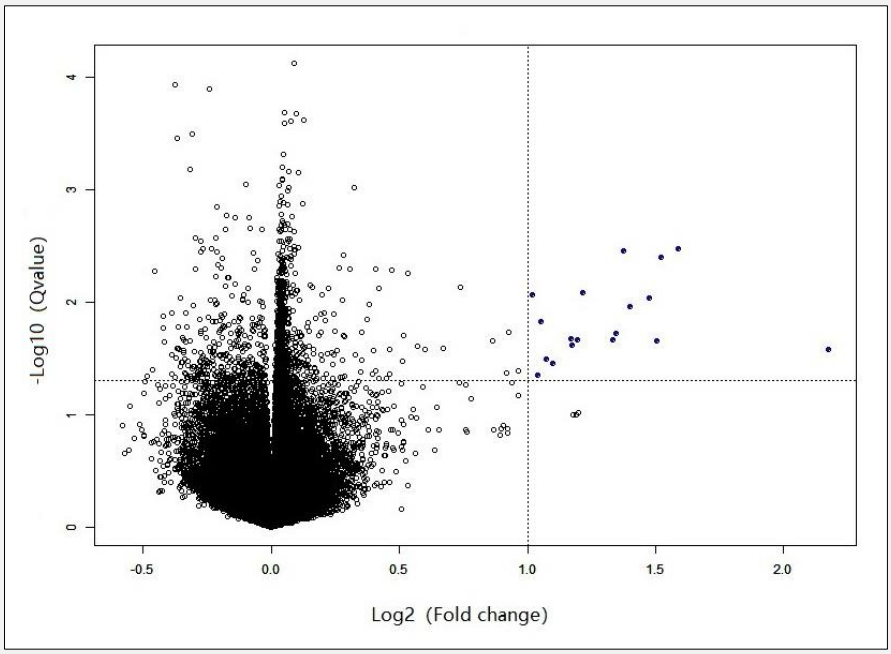

Figure 1.1. Volcano map of ITP patients.

Volcano maps are used to show the distribution of gene expression differences. The abscissa represents Log2 Fold change; the more the deviation from the center, the greater the multiple differences. The ordinate is -log10 Q-value; the larger the value, the more significant the difference is. All the points in the figure represent 1,915 differentially expressed genes. The blue dots in the upper right corner represent 14 of the 18 probes with screened p-values and corrected p-values < 0.05 and a multiple of difference ≥ 2.

shows 18 blue spots in the upper right corner which are differentially expressed positive genes. Similarly, the heat map is shown in Figure 1.2.

Functional enrichment analysis and pathway enrichment analysis of differential mRNA in ITP patients

After annotating the differential genes by the GO classification system, 96 GO pathways were significantly enriched. The top 10 pathways with the most significant enrichment level were selected in each of the three

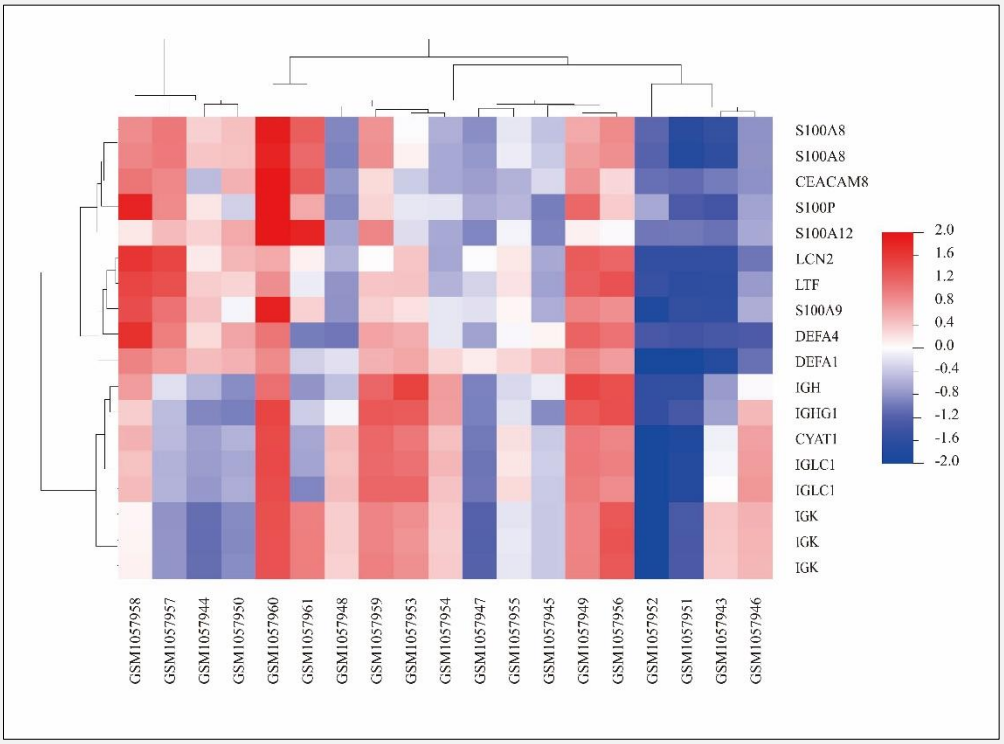

Figure 1.2. Cluster diagram of ITP patients.

Each small square in the figure represents each mRNA, and the color represents the expression amount of this mRNA. Red means upregulated, and blue represents downregulated; dark colors indicate high expression. Each row represents the expression amount of each differential mRNA in different samples, and each column represents the expression amount of all differential mRNA in each sample. The dendrogram above the heat map represents the cluster analysis results of different samples in different experimental groups. The dendrogram on the left of the heat map represents the cluster analysis results of different genes in each sample.

types, as shown in Figure 2.1. Pathway enrichment analysis revealed that the related genes in T lymphocytes of ITP patients were mainly enriched in the IL-17 signaling pathway, Staphylococcus aureus infection, and NOD-like receptor signaling pathway, as shown in Figure 2.2.

QPCR results of DEFA1 and S100A8 in initial ITP patients and healthy controls

QPCR showed that the mRNA expression of DEFA1 were not statistically significant in initial ITP patients compared with the healthy controls. The mRNA expression of S100A8 increased, and the difference was statistically significant. As shown in Figures 3.1 and 3.2.

QPCR results of miR-21 and miR-155 in initial ITP patients and healthy controls

The qPCR experiment showed that the mRNA expression of miR-21-5p and miR-155-5p in ITP patients in the initial ITP group was increased compared with the healthy controls, and the difference was statistically significant. However, the mRNA expression of miR-21-3p and miR-155-3p were not statistically significant, as shown in Figures 4.1, 4.2, 4.3, and 4.4.

Prediction of downstream target genes of miR-21-5p and miR-155-5p

By crossing the predicted miR-21-5p and miR-155-5p target mRNA in 3 prediction software programs, 16 core downstream targeting mRNA of miR-21 and 16 core downstream targeting mRNA of miR-155 were obtained. Details are shown in Table 3.

Analysis of interaction network between core target mRNA and differential mRNA of miR-21-5p and miR-155-5p

There are 46 mRNA comprising of the core target mRNA of miR-21-5p and miR-155-5p and the differentially expressed mRNA. PPI analysis results of 30 mRNA were summarized, as shown in Figure 5. A total

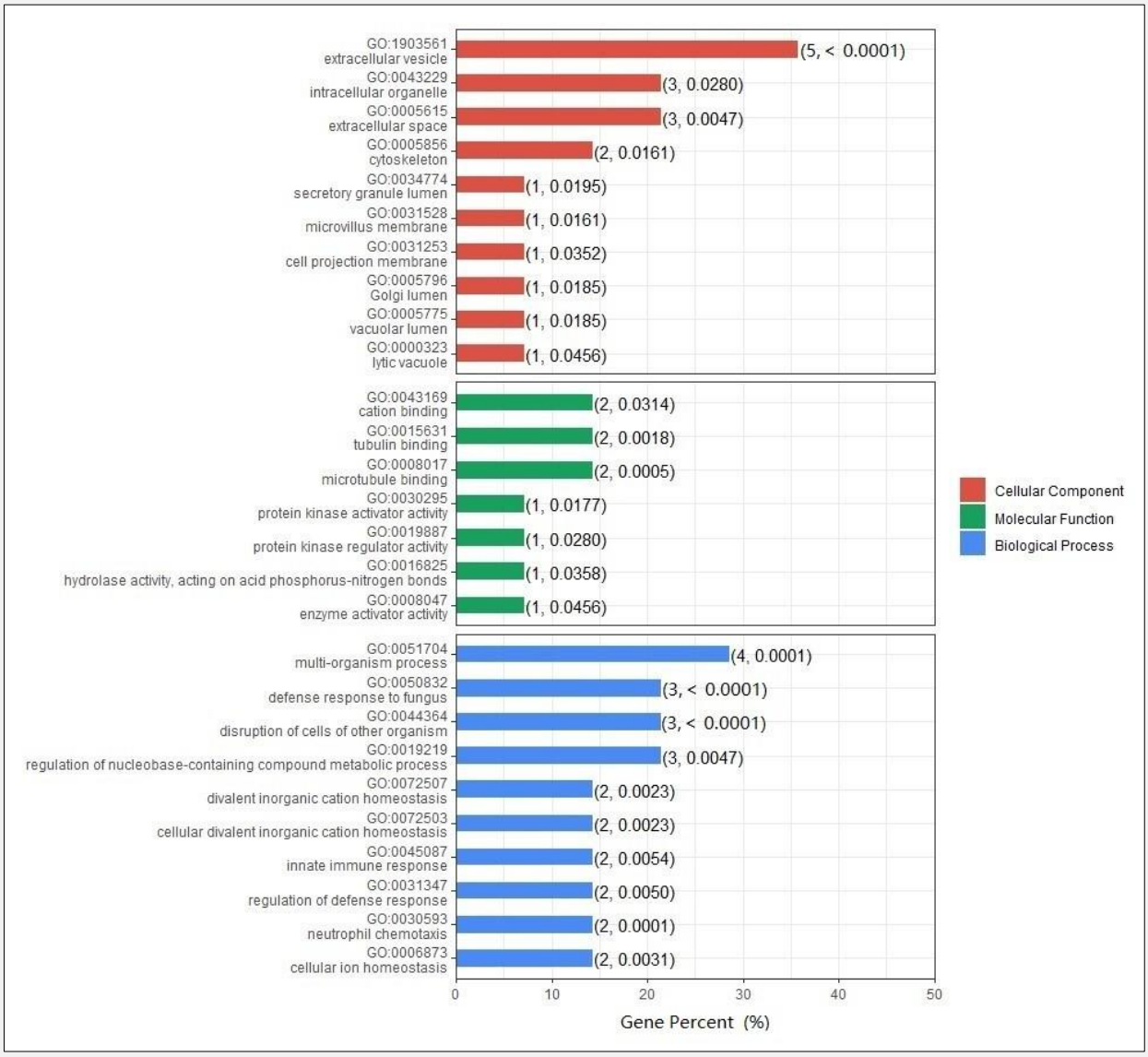

Figure 2.1. Functional enrichment analysis of differential genes in ITP patients.

The abscissa represents the percentage of the number of genes, and the ordinate is the classification of GO. The red part represents CC, the green part represents MF, and the blue part represents BP. The top 10 pathways with the most significant enrichment level were selected for the three GO classifications inordinate, and each classified GO pathway is arranged from large to small by the number of genes.

of 21 nodes and 28 edges were involved in the PPI network, among which there were 4 core mRNA whose node connectivity (degree) was greater than or equal to 5, namely LTF, LCN2, S100A12, and DEFA4. All 4 were differentially expressed mRNA. The most representative core mRNA had a degree of LTF of 7. The degree of LCN2 was 6, while the degree of S100A12 and DEFA4 was 5. As demonstrated, VCL, the core downstream target mRNA of miR-21-5p, is related to LTF, LCN2, and DEFA4, all of which are differentially expressed mRNA.

QPCR results of VCL, LTF, LCN2, and DEFA4 in ITP patients and healthy controls

Compared with the healthy control group, the expression of VCL mRNA in the initial ITP group was decreased. Compared with the initial ITP group, the mRNA expression of VCL in the ITP remission group

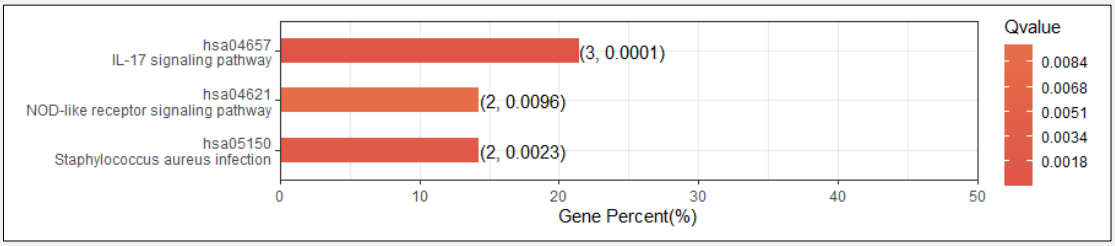

Figure 2.2. Enrichment analysis of differential gene pathways in ITP patients.

The abscissa represents the percentage of the number of genes, and the ordinate is the screened three eligible pathways, each of which is arranged from large to small according to the number of differential genes. The color depth of the histogram indicates the Q value of the pathway. The larger the Q value, the lighter the color of the histogram, and the smaller the Q value, the darker the color of the histogram.

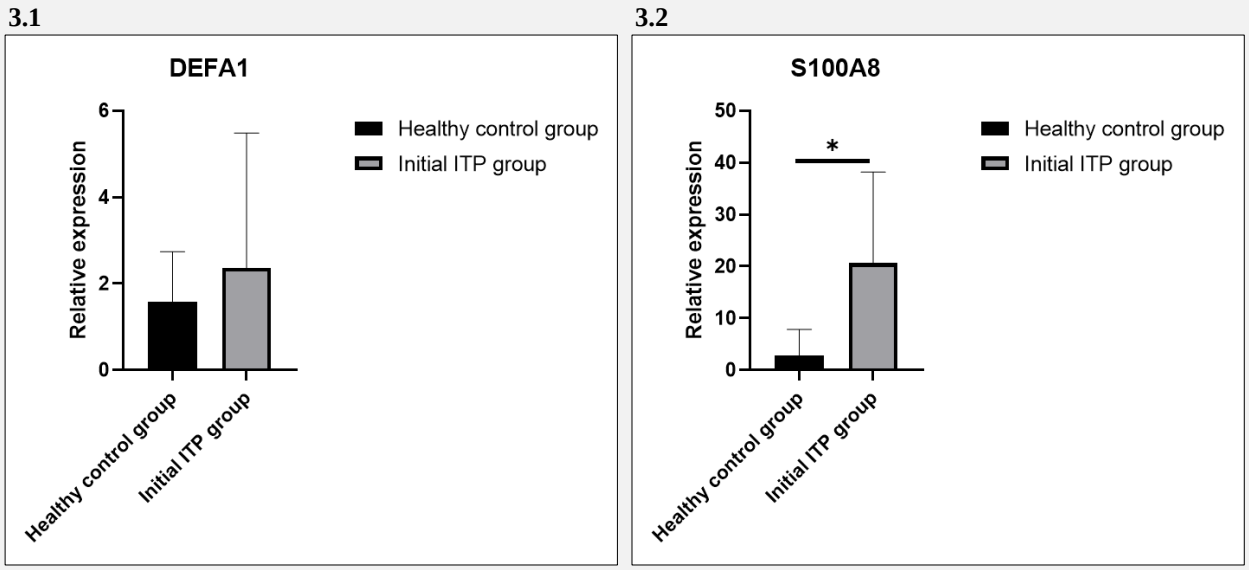

Figure 3.1. qPCR results of DEFA1 in initial ITP patients and healthy controls. The mRNA expression of the healthy control group was 1.59 ± 1.16, and that of the initial ITP group was 2.36 ± 3.13 ($p > 0.05$).
Figure 3.2. qPCR results of S100A8 in initial ITP patients and healthy controls. The mRNA expression of the healthy control group was 2.85 ± 5.01, and that of the initial ITP group was 20.64 ± 17.54 ($p < 0.05$).

was increased, and the difference was statistically significant. Compared with the healthy control group, the expression of LTF in the initial ITP group were significantly higher than the healthy control group. However, there was no significant difference in the expression of LCN2 and DEFA4 in the initial ITP group, as shown in Figures 6.1, 6.2, 6.3, 6.4.

DISCUSSION

ITP is a commonly diagnosed acquired autoimmune hemorrhagic disease. The incidence of ITP is 2 - 4/ 100,000, and it is increasing over time, posing a serious impact on patients [12]. Regarding the etiology and pathogenesis of ITP, the traditional view is that thrombocytopenia is mainly due to the combination of anti-

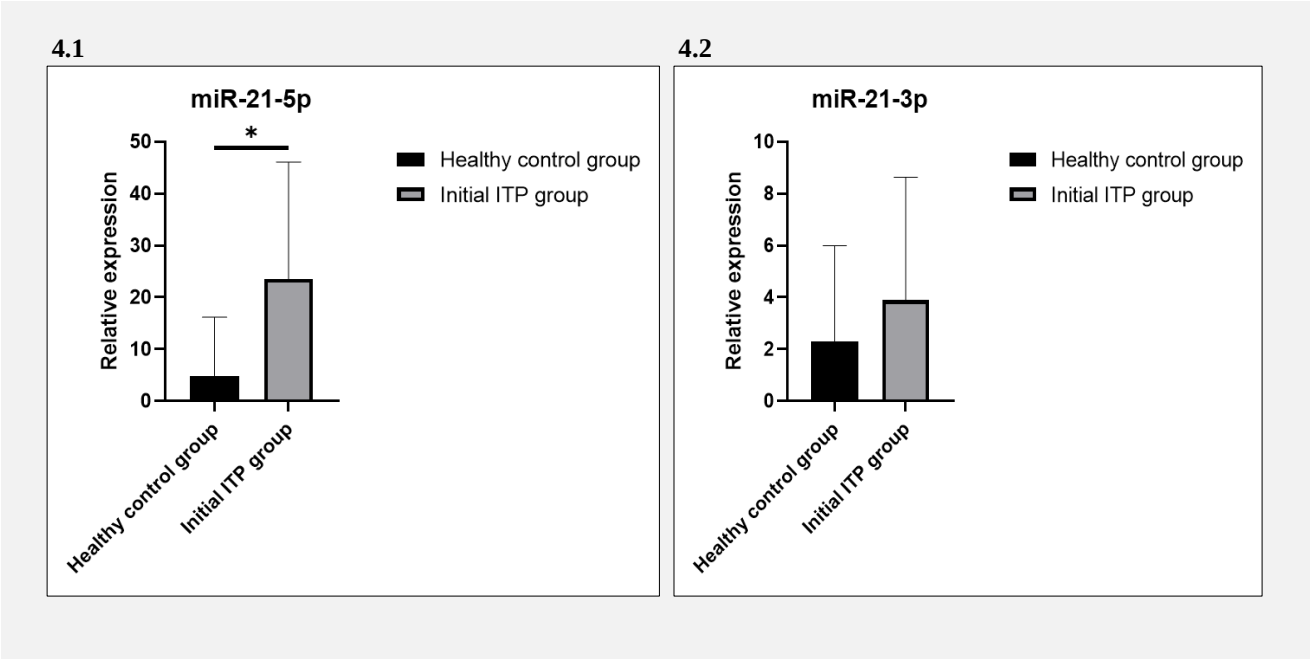

Figure 4.1. qPCR results of miR-21-5p in initial ITP patients and healthy controls. The mRNA expression of the healthy control group was 4.88 ± 11.31, and that of the initial ITP group was 23.58 ± 22.51 ($p < 0.05$).
Figure 4.2. qPCR results of miR-21-3p in initial ITP patients and healthy controls. The mRNA expression of the healthy control group was 2.30 ± 3.70, and that of the initial ITP group was 3.89 ± 4.75 ($p > 0.05$).

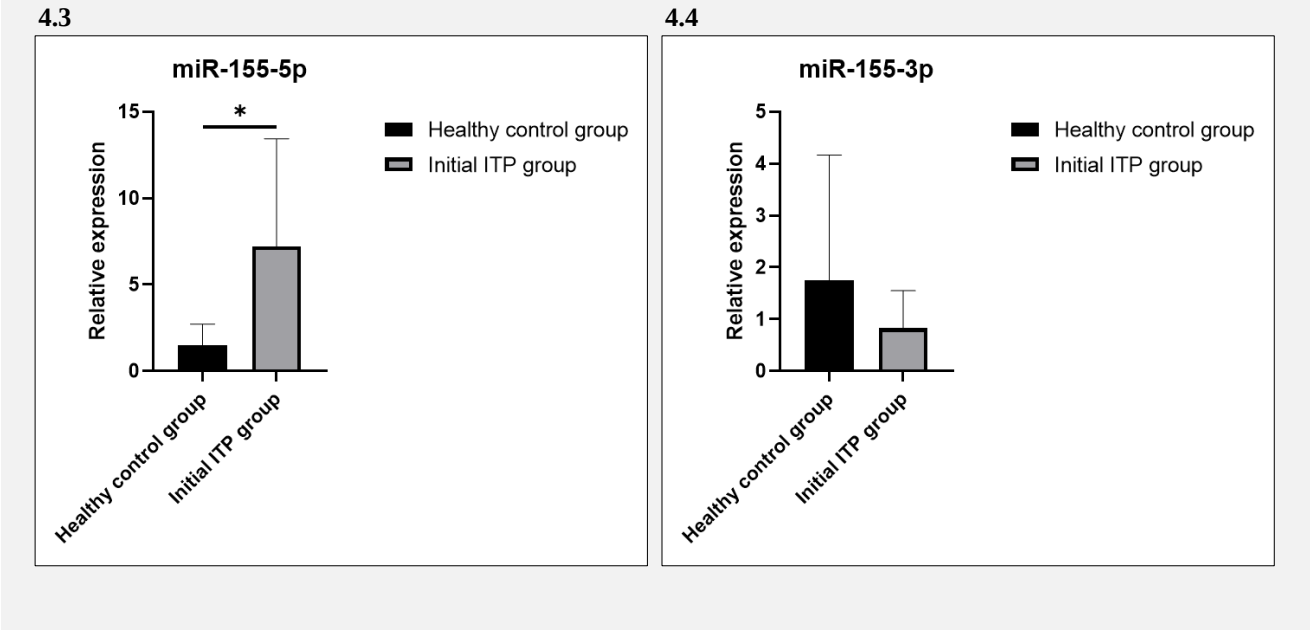

Figure 4.3. qPCR results of miR-155-5p in initial ITP patients and healthy controls. The mRNA expression of the healthy control group was 1.50 ± 1.21, and that of initial ITP group was 7.21 ± 6.23 ($p < 0.05$).
Figure 4.4. qPCR results of miR-155-3p in initial ITP patients and healthy controls. The mRNA expression of the healthy control group was 1.75 ± 2.41, and that of initial ITP group was 0.83 ± 0.72 ($p > 0.05$).

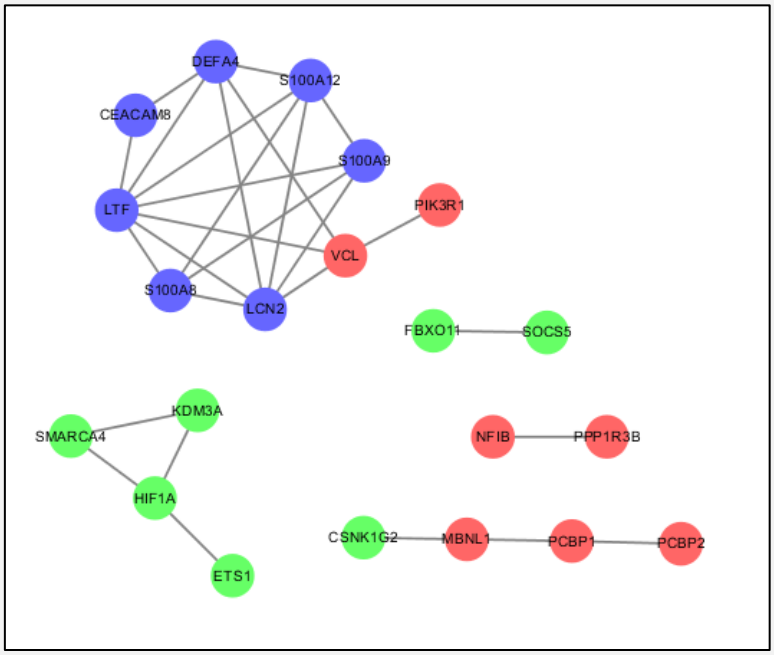

Figure 5. Interaction network of target mRNA and differential mRNA of miR-21-5p and miR-155-5p.

In the figure, the circles represent the mRNA factor, the red circle represents the miR-21-5p target mRNA, the green circle represents the miR-155-5p target mRNA, and the blue circle represents the differential mRNA. The line between circles of different colors represents the relationship and function between proteins.

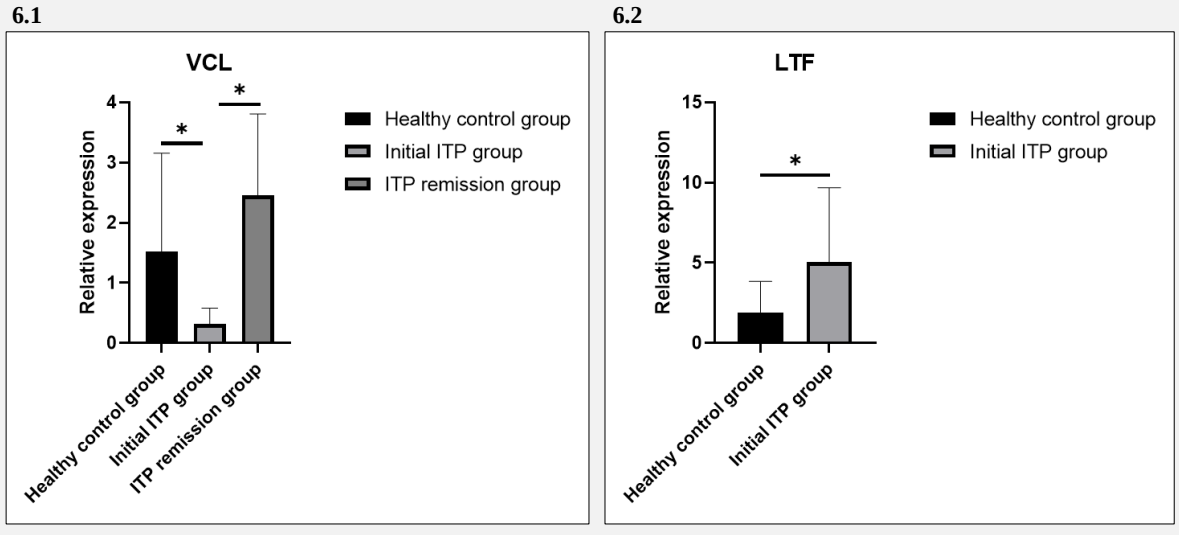

Figure 6.1. qPCR results of VCL in initial ITP group, ITP remission group, and healthy control group. The mRNA expression of the healthy control group was 1.52 ± 1.63, and that of the initial ITP group was 0.32 ± 0.26. The mRNA expression in the ITP remission group was 2.46 ± 1.35 ($p < 0.05$).
Figure 6.2. qPCR results of LTF in initial ITP patients and healthy controls. The mRNA expression of the healthy control group was 1.92 ± 1.92, and that of the initial ITP group was 5.04 ± 4.65 ($p < 0.05$).

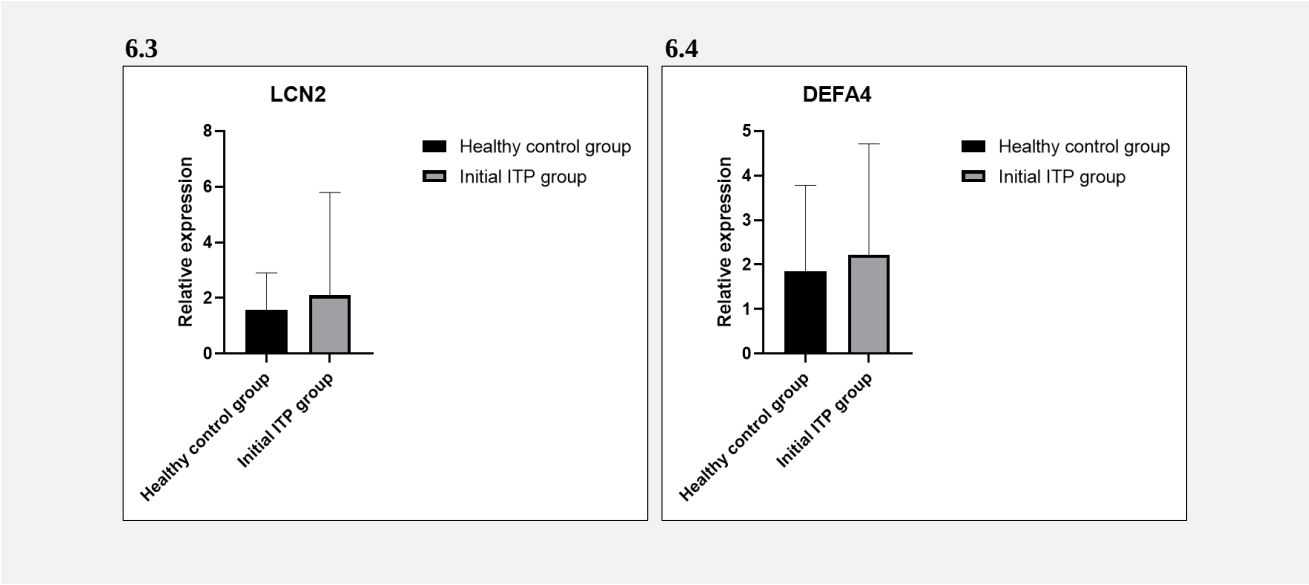

Figure 6.3. qPCR results of LCN2 in initial ITP patients and healthy controls. The mRNA expression of the healthy control group was 1.57 ± 1.33, and that of the initial ITP group was 2.10 ± 3.69 (p > 0.05).
Figure 6.4. qPCR results of DEFA4 in initial ITP patients and healthy controls. The mRNA expression of the healthy control group was 1.84 ± 1.94, and that of the initial ITP group was 2.23 ± 2.49 (p > 0.05).

platelet antibodies and surface antigens of megakaryocytes in bone marrow, leading to megakaryocyte damage and ineffective thrombopoiesis. The increase of platelet destruction is mainly due to the combination of antiplatelet autoantibody and platelet surface antigen, destroyed by macrophages in the liver and spleen, unable to play the normal physiological function [13]. In recent years, studies have found that abnormal cellular immunity also plays an equally important role in the pathogenesis of ITP. Specifically, cellular immunity leads to excessive activation of Th1 cells, decreased activity of Th2 cells, imbalance of the proportion of Th1/Th2 cells, increased secretion of interleukin factors such as IL-2 and IFN-gamma, abnormal differentiation of Th17 cells, and increased secretion of interleukin factors such as IL-17. Moreover, B-lymphocytes are overactivated and produce antiplatelet antibodies, while the abnormal function of T-lymphocyte subsets can lead to the accelerated destruction of B-lymphocytes. In addition, cytotoxic T cells (CTL), dendritic cells, and natural killer cells are all involved in the dysfunction of the immune system in ITP [14,15]. In this study, we found that T lymphocyte subsets of ITP patients and healthy control group had overexpression of CD8+T lymphocytes and reduced expression of CD4+T lymphocytes and the ratio of CD4+/CD8+ in the newly treated ITP patients. We also found an abnormal number and function of CD3+T lymphocytes in ITP patients.

In the current study, the expression profile of T lymphocytes from adult ITP patients in the GEO database was used for subsequent analysis using bioinformatics. The results showed that 14 genes in the database were differentially expressed, and all of them were upregulated. Especially the two genes DEFA1 and S100A8 showed more significant differences in T lymphocytes of ITP patients. DEFA1 is a nuclear protein transcription factor that binds to specific DNA target sequences in the nucleus. Studies have found that DEFA1 can establish and maintain the process of peripheral immune tolerance in the human body and regulate the innate immune response in peripheral lymphoid organs [16]. Factors such as S100A8, S100A9, and S100A12 belong to the S100 family of calcium-binding proteins, which neutrophils and monocytes can actively release in the inflammatory response. They stimulate leukocyte recruitment by recognizing TLR4 and NLRP3 and induce the synthesis and secretion of downstream factors [17]. After verification by qPCR in this experiment, it was found that the mRNA expression of DEFA1 was not statistically significant in initial ITP patients compared with the control group. The mRNA expression of S100A8 increased. Recent studies have found that proteins such as S100A8 can be used as biomarkers to monitor the activity of diseases such as inflammatory bowel disease and rheumatoid arthritis [18]. Another study found that the expression levels of S100A8 and S100A9 proteins were positively correlated with the pathogenesis of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus in animal models [19].

GO is mainly used to describe the functional distribution of differential genes. Through GO analysis, we can determine whether the cellular components, molecular functions, or biological processes involved by differential genes have changed [20]. The functional enrichment results of this study showed that CC mainly includes ex-

tracellular space, microvillus membrane, cytoskeleton, and Golgi lumen. MF mainly includes tubulin binding, protein kinase regulator activity, and hydrolase activity. BP mainly includes defense response to fungus, neutrophil chemotaxis, regulation of nuclease metabolism, and innate immune response. KEGG is a biological database constructed by scientists at Kyoto University in Japan, systematically analyzing gene function and genomic information [21]. This study found that the differentially expressed genes in T lymphocytes of ITP patients were mainly enriched in the IL-17 signaling pathway, Staphylococcus aureus infection, and NOD-like receptor signaling pathway.

MiRNA is a class of non-coding short-chain RNA molecules with post-transcriptional regulation, whose functions involve various biological processes. Recent studies have found that various miRNA can participate in the pathogenesis of acute and chronic ITP. Furthermore, research shows that miRNA molecules such as miR-142, miRNA-146, miR-150, and miR-181 can play an important regulatory role in ITP, and these miRNA is also considered specific immunoregulatory miRNA [22]. MiR-21 and miR-155 are equally important immunoregulatory miRNA that can be regulated in a variety of autoimmune diseases. MiR-21 mainly affects the proliferation, differentiation, and apoptosis of various types of T lymphocytes in specific immune responses and can also participate in innate immune responses through toll-like receptor signaling pathways or affect the polarization of macrophages [23]. The study found that the expression of miR-21 was increased in peripheral blood CD4+T cells of SLE patients [24]. Moreover, miR-21 was overexpressed in CD3+T cells in the colonic tissues of ulcerative colitis patients [25].

Further studies revealed that the regulation of miR-21 was associated with genomic DNA hypomethylation. MiR-21 can also lead to hypomethylation of genomic DNA in CD4+T cells by targeting inhibition of DNA methylation transferase 1 (DNMT1) [26]. MiR-155, another immune regulator, also plays an important regulatory role in autoimmune diseases such as ITP [27]. Therefore, we studied whether miR-21 and miR-155 were highly expressed in CD3+T lymphocytes of ITP patients. The results showed that the mRNA expression of miR-21-5p and miR-155-5p in ITP patients were increased, and the difference was statistically significant. Three different prediction software programs were used to screen different downstream target genes of miRNA. After the intersection of the prediction results of the three target gene prediction software programs, we found that there were 16 core downstream target genes of miR-21-5p and miR-155-5p. Next, 32 core downstream target genes of miR-21-5p and miR-155-5p and 14 ITP differential genes showed that the LTF and LCN2 factors had the highest node connectivity, and the target gene VCL factor of miR-21-5p was associated with LTF, LCN2, and DEFA4. It was inferred that miR-21-5p could regulate the difference factors LTF, LCN2, and DEFA4 through the downstream target VCL. Thereby participating in the immune mechanism of T lymphocytes in patients with ITP. VCL is one of the core downstream target genes of miR-21-5p. This protein is not only connected to microfilaments in cells but also can be connected to adhesion molecules such as cell surface integrin and adhesin in vitro [28]. Studies have found that VCL, as an adhesion factor, plays a regulatory role in B lymphocytes [29]. Another study found a correlation between VCL and the incidence of inflammatory bowel disease [30]. Lactoferrin (LTF) is a glycoprotein with many biological functions, including iron metabolism, anti-inflammation, and immune regulation. LTF can not only constitute the body's innate immune system but also activate downstream signaling pathways to affect the adaptive immune system [31]. Lipid carrier protein 2 (LCN2) is mainly secreted by adipocytes and can participate in iron transport and the regulation of innate immune response, and the production of reactive oxygen species [32]. Alpha defensin 4 (DEFA4) belongs to the family of antimicrobial peptides. The study found that each gene of the DEFA family (DEFA1, DEFA3, and DEFA4, etc.) can be expressed in neutrophils and macrophages and play a regulatory role in the innate immune response process [33]. The results of PCR showed that the expression of VCL decreased in the initial ITP group but increased in the remission group. In addition, the expression of LTF, the important regulatory factor related to VCL, was upregulated in initial ITP group. It can be preliminarily explained that miR-21-5p inhibits VCL, and the VCL and subsequent LTF factors have a certain correlation.

In conclusion, we found that the number and function of CD3+T lymphocytes in ITP disease were abnormal. Comparing and analyzing the gene chip data sets of peripheral blood T lymphocytes between adult ITP patients and healthy controls, we found 14 differentially expressed genes in CD3+T lymphocytes of ITP patients, all of which were upregulated genes. Moreover, S100A8 can play a role in CD3+T lymphocytes in ITP patients. MiR-21 and miR-155 are important immune regulatory factors, and to our knowledge, this is the first study found that miR-21-5p and miR-155-5p can play an immunomodulatory role in CD3+T lymphocytes of ITP patients. In addition, miR-21-5p can be associated with LTF through the core target factor VCL to play a role in CD3+T lymphocytes of ITP patients. We believe these findings will provide a theoretical basis and clinical ideas for developing molecular interventions and targeted drug therapy in ITP.

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Declaration of Interest:
The author declares no conflict of interest.

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