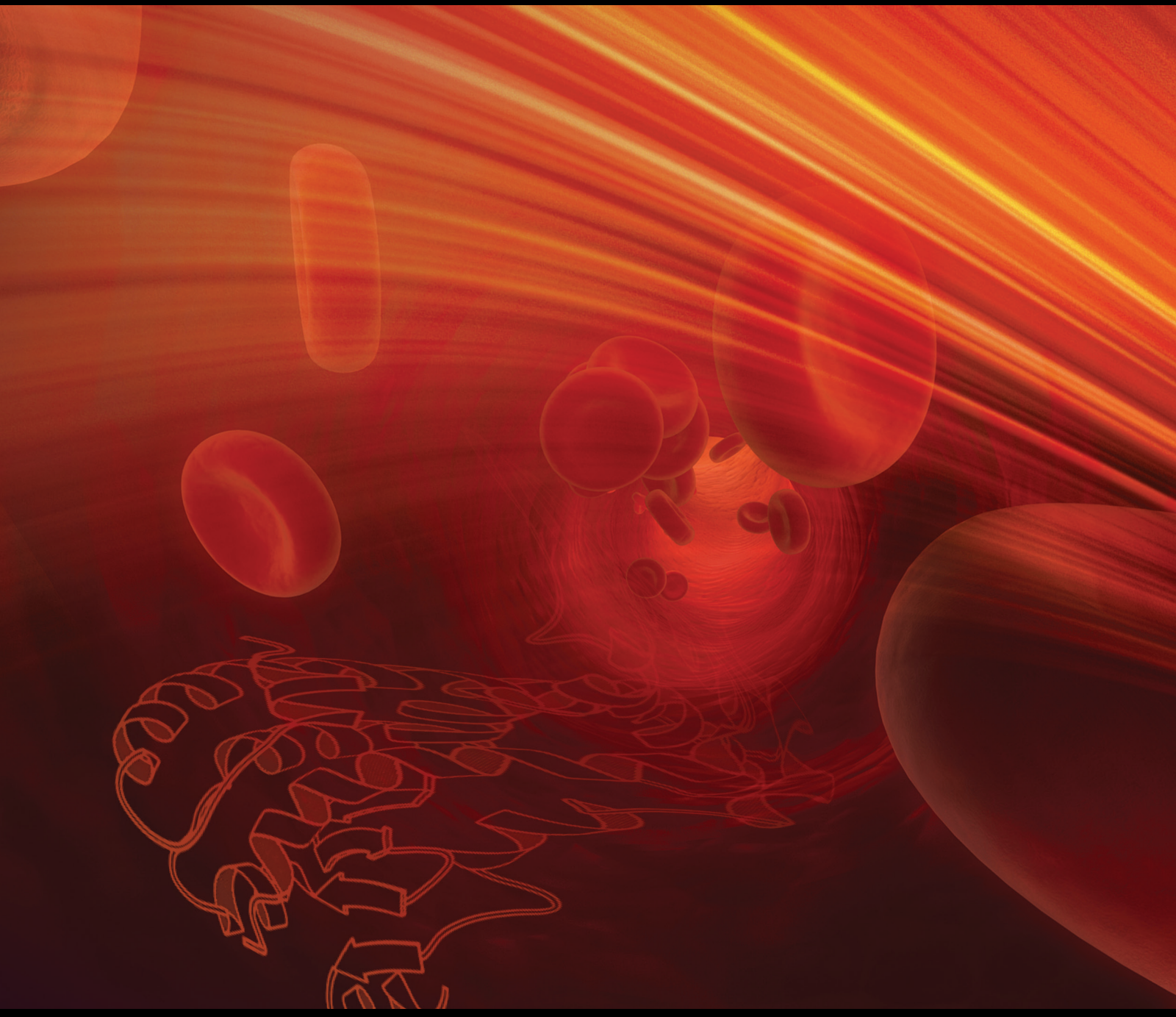
PPAR Research

Weighting PPARs for their Roles in Relevant Diseases

Lead Guest Editor: Hongbao Cao
Guest Editors: Shaolei Teng and Sha Liu



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Research Article

Role of PPARG in Chemosensitivity-Regulating Network for Hypopharyngeal Squamous Cell Carcinoma

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PPARG has been reported to promote chemosensitivity in hypopharyngeal squamous cell carcinoma (HSCC). However, few studies tested its significance in the texture of a complex molecular network regulating chemosensitivity in HSCC. Here, we first employed RNA expression data analysis and literature data mining to uncover candidate genes related to HSCC chemosensitivity. Then, we constructed the molecular network regulating chemosensitivity in HSCC. After that, we employed degree centrality (DC) and weighted centrality (WC) to test the significance of PPARG within the regulating network. Pathway enrichment was done to study the cofunctions of PPARG and the rest of the genes within the network. The findings of our study contribute to the construction of a comprehensive network that regulates HSCC chemosensitivity, consisting of 57 genes, including PPARG. Notably, within this network, PPARG demonstrates a ranking of #5 and #13 based on DC and WC, respectively. Moreover, PPARG is connected to 29 out of the 57 genes and plays roles in multiple functional groups. These top related genes include AKT1, TP53, PTEN, MAPK1, NOTCH1, BECN1, PTGS2, SPP1, and RAC1. PPARG gets enriched in several key functional groups that have been implicated in the regulation of chemosensitivity, including those associated with the response to nutrients, vitamins, and peptides, the cellular response to chemical stress, and the regulation of hormone secretion and growth. Our results emphasize the involvement of PPARG and its interconnectedness with other genes in the regulation of HSCC chemosensitivity.

1. Introduction

Hypopharyngeal squamous cell carcinoma (HSCC) is one of the worst prognostic malignant tumors [1, 2], characterized by hidden location, strong infiltration, easy submucosal spread, and multicentric growth of the primary lesion. Because there are no apparent symptoms in the early stage, 70-85% of patients with HSCC are diagnosed at stage III or IV, with 5-year overall survival rates around 15-45% [3].

Combined chemotherapy, surgery, and radiation therapy are commonly used in the multidisciplinary treatment of HSCC, and cisplatin is the most widely used platinum-based chemotherapeutic agent for HSCC [4]. However, chemotherapy resistance could severely limit the clinical efficiency and the improvement of survival rate in patients with HSCC [5].

There is a suggestion that PPARG has the potential to enhance the chemosensitivity of HSCC tumor cells [6]. However, significant expression variation of PPARG exists in chemotherapy-sensitive patients, which may be influenced by multiple factors, including the TNM (tumor, node, and metastasis) stage, a predictor of chemosensitivity [7]. Moreover, multiple other chemosensitivity promoters have been shown to be inhibited by HSCC, such as TIPE2 [8, 9] and BECN1 [10, 11]. Zhao et al. showed that TIPE2 might enhance chemosensitivity by downregulating MDR1 transcription in hypopharyngeal carcinoma [9]. Sun et al. found that overexpression of the BECN1 gene could upregulate

chemosensitivity to anticancer drugs by enhancing therapy-induced apoptosis in cervix squamous carcinoma CaSki cells [11]. However, the expression of both TIPE2 and BECN1 was found to be significantly downregulated in HSCC [8, 10]. Identifying the relationship between PPARG and these genetic markers influencing chemosensitivity may help in understanding the role of PPARG in the chemosensitivity of HSCC.

Here, we employed two gene expression datasets and literature-based knowledge data to construct genetic networks regulating chemosensitivity in HSCC and evaluate the significance of PPARG and its cofunctions with other genetic markers. Our results proposed a complex network that could influence the chemosensitivity of HSCC and highlighted the importance of PPARG within the network.

2. Materials and Methods

2.1. Extract CSP-Significant Genes from Gene Expression Datasets. Two microarray expression datasets, including 32 HSCC patients, were used to explore candidate genes that were related to chemotherapy in HSCC, including 19 chemotherapy-sensitive patients (CSP) and 13 chemotherapy-non-sensitive patients (CNSP). All the patients were recruited by the Department of Head and Neck Surgery, Beijing Tongren Hospital. We employed the two datasets in exploring the possible relationship between PPARG and chemosensitivity in HSCC [6, 7]. Both datasets, with the GEO IDs GSE85608 and GSE85607, were submitted to the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/). Written consent forms were obtained from all patients included in the study at the time of data acquisition for use of the datasets in publication. We noted during the previous studies that, besides PPARG, there were multiple other genes that presented significant expression changes and thus may play a role in HSCC chemosensitivity regulation. Here, we extracted and analyzed the genes identified from the CSP and CNSP comparison. These genes, together with literature data mining uncovered chemosensitivity genes, were used to construct the chemosensitivity-regulating network for HSCC. The significance of normalized degree centrality (DC) and weighted centrality (WC), widely employed metrics for assessing the significance of a vertex (gene) in a network, was utilized to evaluate the involvement of PPARG in the chemosensitivity-regulating network for HSCC [12]. The chemosensitivity gene identification using literature data mining was described in the following section.

2.2. Identify Chemosensitivity Genes for HSCC Using Literature Data Mining. Assisted by using Pathway Studio (http://www.pathwaystudio.com), we employed data mining to identify genes promoting chemosensitivity and also inhibited by HSCC, or genes inhibiting chemosensitivity but activated by HSCC. The union of the two gene groups was used as candidate genes that promote chemosensitivity genes in HSCC.

2.3. Construct the Chemosensitivity-Regulation Network for HSCC. For the genes that were identified from the above two steps, we constructed the gene-gene interaction (GGI) network following the instruction: https://supportcontent.elsevier.com/Support%20Hub/Pathway%20Studio/Network%20Builder%20basic%20_Interactive%20NB%20v114.pdf. These unconnected genes were removed from the network without further analysis. The exclusion of these genes was motivated by two primary reasons. Firstly, we posited that genes involved in the regulation of HSCC chemosensitivity should exhibit functional connections. Candidates lacking connections with other regulators may be considered outliers with limited or no significance in HSCC regulation. Therefore, removing such genes helps reduce noise in the potential HSCC regulating network. Secondly, the assessment of significance was based on two network centrality metrics (detailed descriptions are provided in the following section), which are not applicable to genes lacking connections since they would receive a score of zero. Moreover, we add the term "chemosensitivity" and "hypopharyngeal squamous cell carcinoma" to address their significance to chemosensitivity in HSCC.

2.4. Evaluate the Significance of PPARG in the Chemosensitivity-Regulation Network for HSCC. Vertex centrality is used to measure the significance of a vertex in the network. The simplest vertex centrality is degree centrality (DC), defined as the number of edges incident upon a vertex. For a graph with n vertices, the normalized degree centrality for vertex v is defined in

$$\mathrm{DC}(v) = \frac{\mathrm{Degree}(v)}{\sum_i \mathrm{Degree}(v_i)}, \tag{1}$$

where $\mathrm{Degree}(v_i)$ is the degree for vertex v_i, which equals the edges connected to the vertex; $i = 1, \cdots, n$.

In a weighted network, the corresponding vertex strength centrality is defined as the sum of the weights of these edges [12]. Considering that the literature-based relationships were reported by a different number of publications in different publication years, we employ a quality score (QScore) for each relationship (edge) as the weight [13]. QScore has been proposed as an effect metric evaluating literature-based relationships. Then, the normalized weighted degree centrality is defined as

$$\mathrm{WC}(v) = \frac{\mathrm{QScore}(v)}{\sum_i \mathrm{QScore}(v_i)}, \tag{2}$$

where $\mathrm{QScore}(v_i)$ is the sum of the QScores of all the relationships (edges) connecting the vertex v_i; $i = 1, \cdots, n$.

2.5. Enrichment Analysis. To enhance our comprehension of the relationship between PPARG and its role in regulating HSCC chemosensitivity within the network, we carried out a gene set enrichment analysis utilizing Gene Ontology (GO) terms. This analysis involved utilizing the genes that make up the HSCC chemosensitivity-regulating network as input. The findings encompass the identification of highly enriched pathways and an evaluation of gene cofunctions, which are determined by shared GO terms.

Table 1: Genes show significant expression change in CSP vs. CNSP comparison.

(a) 51 genes from GSE85607

SPP1	SH3GL3	NGF	ABCA4	MPP4	Hs.552282	CORO6	TMEM45A	ADPRHL1
BST1	PTPRD	EHBP1	STC2	FEM1B	GADD45G	NET1	DST	LRP8
C6orf52	*KATNAL1*	*NT5M*	*CTRC*	*ADCY8*	*GRK4*	*POMZP3*	*Hs.21820*	*PRR5L*
CROT	*FAM169A*	*ITPRIPL1*	*RAI2*	*PAX8*	*Hs.533844*	*SELENBP1*	*ACOX2*	*Hs.565170*
KIR2DL5B	*CD34*	*DRC7*	*OR1V1*	*IGFBP4*	*CCDC184*	*PRND*	*GPER1*	*PAX8-AS1*
GPER1	*GGT6*	*RBP1*	*SNCAIP*	*NDUFA4L2*	*KREMEN2*			

(b) 21 genes from GSE85608

SFRP1	PPP1R3C	CYP2J2	C11orf44	LOC339535	RIMS2	C16orf73	UPK1A	B3GNT3
SPIN2B	MYOM3	ACOT11	SPTLC3	*LILRA5*	*FCN3*	*SLCO3A1*	*CCL3L1*	*GCLC*
PRPH	*LOC392437*	*TSPAN18*						

3. Results

3.1. Significant Genes from CSP vs. CNSP Expression Comparison. For dataset GSE85607, 51 significant genes (LFC > 1 or < -1; $p < 0.01$) were identified from the comparison of the CSP and CNSP groups. The number of significant genes from dataset GSE85608 was 21, as shown in Table 1. The upregulated genes were highlighted in italics, and downregulated ones were not. Interestingly, no overlap was identified between the two groups of significant genes, suggesting the diversity of genetic markers influencing the chemosensitivity of HSCC. Although a significant expression change in CSP patients does not guarantee a gene regulating the chemosensitivity, it indicates that the gene could be a candidate worthy of further evaluation for its significance in the chemosensitivity of HSCC. In summary, the analysis of RNA expression data revealed a set of 31 genes that have the potential to act as inhibitors of HSCC chemosensitivity (downregulated in the CSP group), as well as 41 genes that show potential as promoters (upregulated in the CSP group).

3.2. Uncover HSCC Chemosensitivity-Related Genes. To explore the relationship between PPARG and other HSCC chemosensitivity-related genetic markers, we first used literature data mining that uncovered 523 genetic markers promoting chemosensitivity in different diseases. These 523 promoters were supported by over 2,900 scientific references (see Supplementary Table 1). However, only a few have been reported to have a direct role in promoting chemosensitivity in HSCC or hypopharyngeal cancer, including ING4, TP53, PPARG, and PTEN. Moreover, out of these 523 chemosensitivity promoters, three got inhibited in HSCC, as shown in Figure 1 (highlighted in red). Therefore, we identified seven literature-based molecules as chemosensitivity promoters in HSCC.

We also identified 593 genetic markers inhibiting chemosensitivity, which were supported by over 3,100 references (see Supplementary Table 2). However, only four genes have been reported to inhibit the chemosensitivity in HSCC or hypopharyngeal carcinoma, including PTGS2, PHF20, ABCC1, and MCL1. In addition, out of these 593 chemosensitivity inhibitors, ten were got promoted in HSCC, as shown in Figure 1 (highlighted in green). Thus, we identified 14 literature-based molecules as chemosensitivity inhibitors in HSCC.

3.3. Construct HSCC Chemosensitivity-Regulating Genetic Network. For the 48 chemosensitivity promoters and 45 inhibitors identified as mentioned above, a GGI analysis has been conducted by using Pathway Studio, as shown in Figure 2. In total, 36 genes presented no connection with any other genes, leaving 57 genes to compose the chemosensitivity-regulating network (Figure 2), including 24 inhibitors and 33 promoters. To address the significance of these molecules to HSCC and chemosensitivity, we added these two items into the GGI network for later evaluation purposes. In total, there were 467 edges within the network, supported by over 13,000 references (see Supplementary Table 3).

3.4. Significance Weight of PPARG. By using degree centrality (DC), PPARG ranked no. 5 out of 57 molecules with a DC = 5.15. The average DC of all 57 molecules is 1.65 ± 2.11. Our results indicate that PPARG is a hub molecular within the HSCC chemosensitivity-regulating network.

By using weighted centrality, PPARG ranked no. 13 out of 57 molecules with a WC = 1.98. The average WC of all 57 molecules is 1.65 ± 3.32. These results indicate that the connections of PPARG to other vertex within the HSCC chemosensitivity-regulating network are also well supported by literature data (Figure 3).

3.5. Enrichment Analysis. Enrichment analysis showed that the 57 molecules were significantly enriched within 35 Gene Ontology (GO) terms. PPARG has been enriched within six out of the top ten GO terms (FDR corrected p value < 6.83 $e - 4$), as shown in Figure 4(a). Most of these pathways have been implicated with chemosensitivity in different diseases, supporting the importance of PPARG in chemosensitivity regulation.

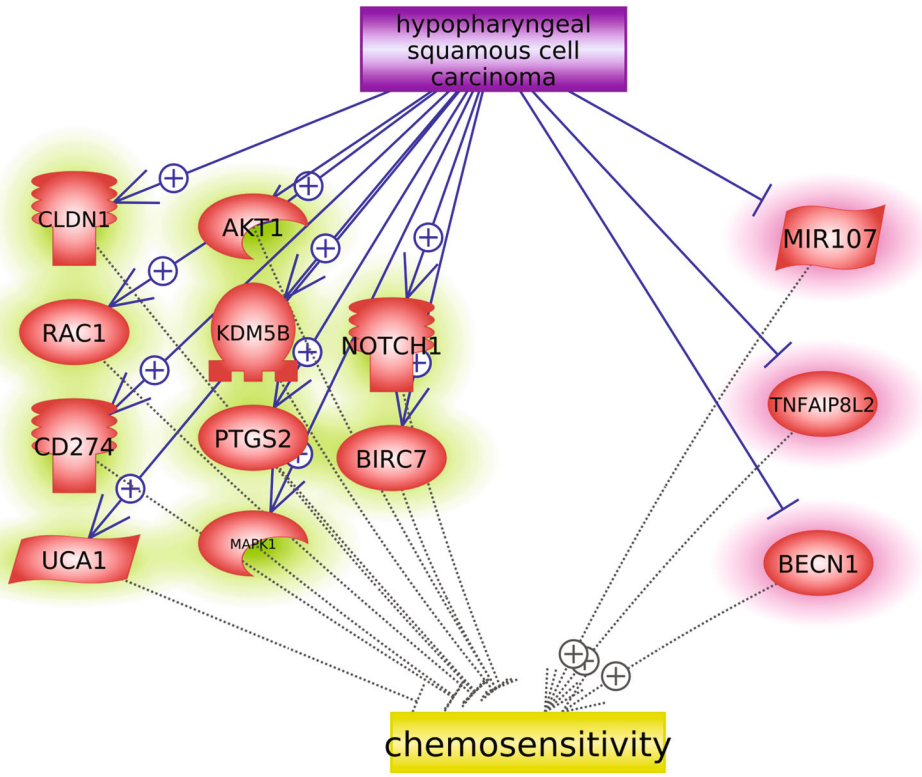

Figure 1: HSCC-driven molecules influencing chemosensitivity.

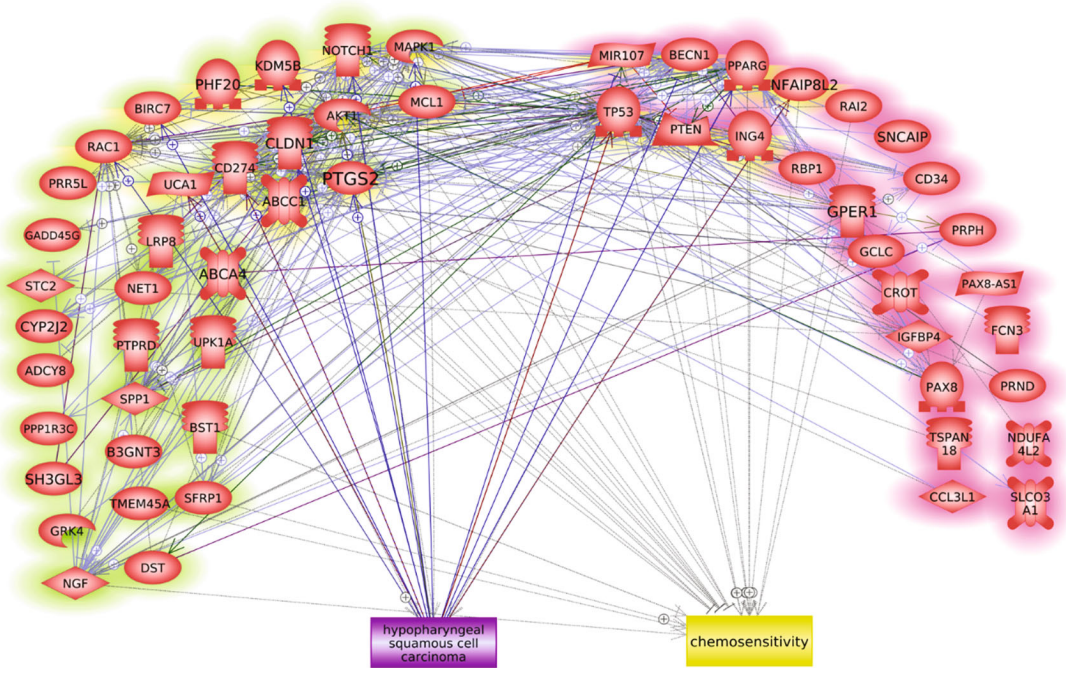

Figure 2: HSCC chemosensitivity-regulating network. Molecules highlighted in green are inhibitors of chemosensitivity; those in red are chemosensitivity promoters.

It is also worth mentioning that PPARG was connecting with 29 out of the 57 molecules in different GO terms influencing chemosensitivities, as shown in Figure 4(b). The numbers within the map indicate the number of GO terms the two corresponding molecules function in.

4. Discussion

In this study, we identified 93 candidate molecules regulating HSCC chemosensitivity using HSCC RNA expression data and literature data mining. Out of these 93 molecules,

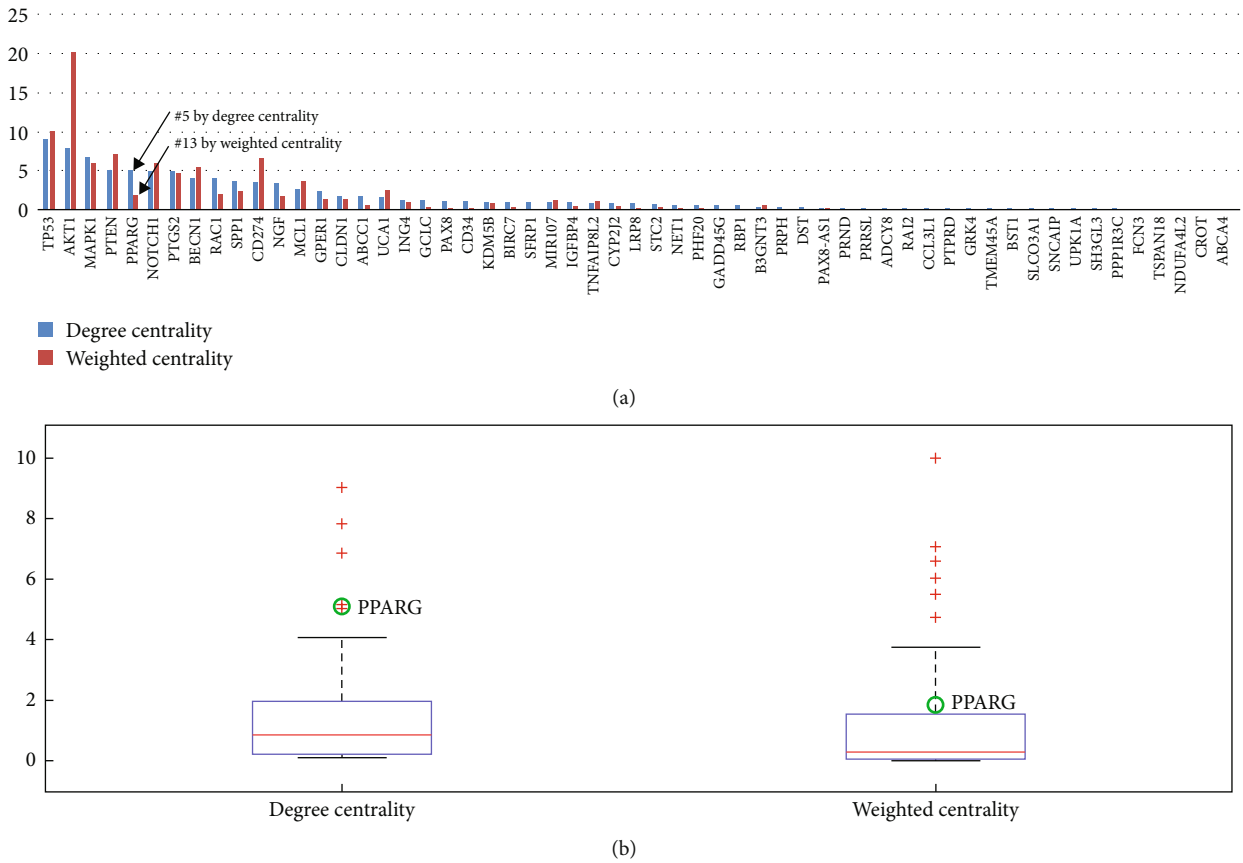

Figure 3: Weight of PPARG in the HSCC chemosensitivity-regulating network. (a) Bar plot of the weight of each molecule. (b) Boxplot depicting the results of centrality weighting using two different types.

57 presented complex connections with each other, including PPARG, composing a genetic network regulating HSCC chemosensitivity. The centrality score analysis suggested that PPARG is a hub gene within the network, and the relations (edges) connecting PPARG were well supported by literature data. Moreover, PPARG cofunctions with 29 out of the 57 genes in different GO terms, which may add to the understanding of the roles of PPARG in chemosensitivity regulation.

The four genes that outweigh PPARG in terms of degree centrality are TP53, AKT1, MAPK1, and PTEN. Two of these genes were implicated as inhibitors of chemosensitivity (MAPK1 and AKT1) that got activated in HSCC. Back in 2007, Shimada et al. showed that MAPK1 activation reduces the chemosensitivity in human prostate cancer cells [14]. Multiple other studies later pointed out that MAPK1 inhibition could induce apoptosis and enhances chemosensitivity in tumor cells [15, 16]. Similarly, the downregulation of AKT1 has been shown to enhance the chemosensitivity of multiple tumor cells, including non-small-cell lung cancer, esophageal squamous cell carcinoma, and head and neck squamous cell carcinoma [17–19]. The levels of AKT1 and MAPK1 could increase significantly with the progression of the clinical stage of HSCC [20], which is consistent with the stage-associated chemotherapy resistance in HSCC [7].

The other two genes that outweigh PPARG in terms of degree centrality are TP53 and PTEN. These two genes are chemosensitivity promoters that get inhibited in HSCC. PTEN has been found to enhance the chemosensitivity of multiple cancer cells, including hypopharyngeal cancer [21], endometrial carcinoma cells [22], ovarian cancer [23], and bladder cancer cells [23]. As a well-known tumor suppressor, P53 promotes DNA damage and apoptosis and plays a key role in the chemosensitivity of many tumor types, including hypopharyngeal cancer [24–26]. Recently, Sun et al. revealed that low expression of TP53 was associated with the advanced stage of HSCC [27]. TP53 is an activator of PTEN by binding to a site within the PTEN promoter region [28]. Therefore, the suppression of TP53 expression by HSCC may also influence the expression of PTEN.

To note, PPARG was linked to all these four genes with a higher degree centrality score. It has been shown that PPARG can induce the expression of TP53 and PTEN by binding directly to the promoter region of these two genes [29, 30] and therefore contribute to the chemosensitivity promotion in HSCC. PPARG activation has also been shown to inhibit AKT1 [31] and PDGF-BB-mediated phospho-MAPK1 activity by blocking its nuclear translocation [32]. This could also add to its mechanism in chemosensitivity promotion. Moreover, in the pathway enrichment analysis, TP53, AKT1, PTEN, and MAPK1 cofunction with PPARG in 10, 7, 6, and 2 GO terms, respectively (Figure 4(b)). These results provide further support to the connection between PPARG and chemosensitivity regulation in HSCC.

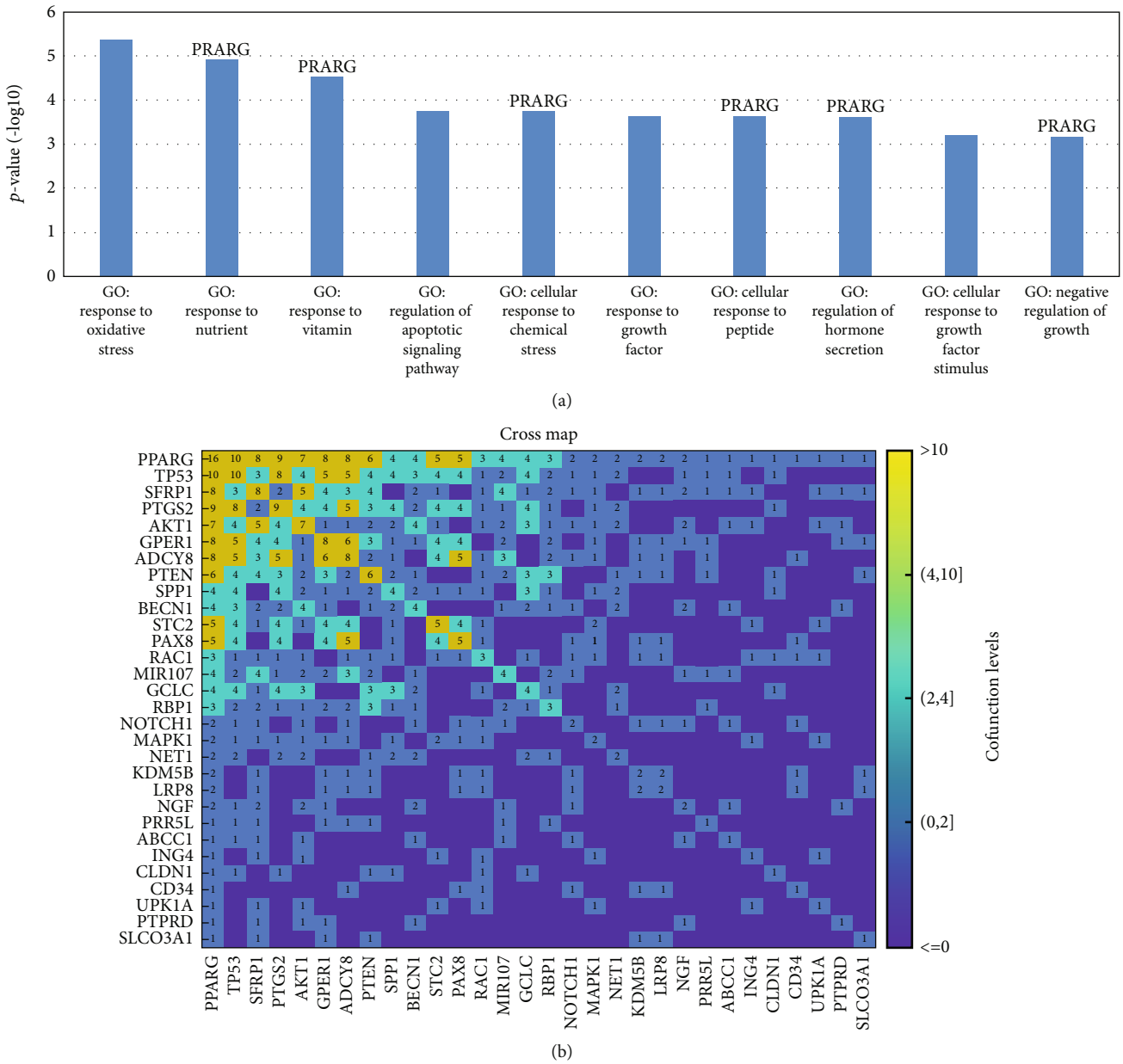

Figure 4: PPARG cofunctions with 29 genes in different GO terms implicated with chemosensitivity. (a) The top 10 GO terms are enriched by the 57 molecules regulating HSCC chemosensitivity. The pathways with "PPARG" on the top represent PPARG gets enriched within the GO term. (b) The cross-map shows the cofunction of PPARG with other molecules. The numbers represent the number of GO terms involving the two corresponding molecules.

Most of the top enriched pathways have been implicated with chemosensitivity. For instance, oxidative stress and its effectors have been found to play critical roles in carcinogenesis and chemoresistance [33]. LINC01615 maintains cell survival in adaptation to nutrient starvation through the pentose phosphate pathway and modulates chemosensitivity in colorectal cancer [34]. Vitamin D has been found to enhance cisplatin chemotherapy and is suggested to be supplied during chemotherapy [35]. Through enhancing apoptosis, multiple molecules (e.g., Bcl-xL DNAzymes) promote radiosensitivity and chemosensitivity in cancer cells [36]. Recently, Jaidee et al. showed that the inhibition of fibroblast growth factor receptor 2 enhances chemosensitivity to gemcitabine in cholangiocarcinoma [37]. These results not only supported the validity of the 57 candidate chemosensitivity-related genes that we identified through the process of this study but also suggested the potential mechanisms of how these genes, including PPARG, influence the chemosensitivity in cancer cells.

Ranked by weighted centrality score, besides the four genes (AKT1, TP53, PTEN, and MAPK1) we mentioned above, there were also eight other genes that outweigh PPARG, including CD274, NOTCH1, BECN1, PTGS2, MCL1, UCA1, SPP1, and RAC1. As the weight was relationship QScore, which measures the strength of literature support for the relationship [13], a high weighted centrality

indicates a large number of recent studies supporting the relations the gene presented within the chemosensitive regulation network. To note, PPARG got enriched in the same GO terms with most of these genes (9 out of 12; for NOTCH1, BECN1, PTGS2, SPP1, and RAC1, the number of shared GO terms is 2, 4, 9, 4, and 3, respectively), suggesting the functional connection between PPARG and these well-studied genes.

There are several limitations in this study that require attention in future research. Firstly, while the network-based significance ranking highlights the importance of PPARG in HSCC's chemosensitivity compared to other regulators, it does not provide a detailed understanding of PPARG's specific role in HSCC chemosensitivity. Secondly, although gene set enrichment analysis reveals that PPARG interacts with various molecules and participates in multiple functional pathways related to chemosensitivity, this pathway-based information remains somewhat vague when it comes to specifying how PPARG interacts with other regulators and their respective contributions to HSCC chemosensitivity. Therefore, further studies using more extensive data are needed to investigate the precise role of PPARG and its interactions with other regulators involved in HSCC chemosensitivity.

5. Conclusion

The outcomes of our study indicate that PPARG serves as a hub gene within a significant genetic network that regulates chemosensitivity in patients with HSCC. The construction of a comprehensive network comprising 57 genes, along with the identification of enriched GO terms, contributes to a deeper understanding of the roles played by PPARG and other genes in the regulation of HSCC chemosensitivity.

Data Availability

The data of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

All the authors declare no conflict of interest.

Acknowledgments

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Supplementary Materials

Supplementary 1. Supplementary Table 1: the reference information supporting the 523 chemosensitivity promoters.

Supplementary 2. Supplementary Table 2: the reference information supporting the 593 chemosensitivity inhibitors.

Supplementary 3. Supplementary Table 3: the reference information supporting the HSCC chemosensitivity-regulating network.

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Research Article

A Promotion Role of MIR31 in the Process of Vocal Fold Wound Healing

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The role of MIR31 in the wound healing process, specifically in vocal fold wound healing (VFWH), remains uncertain despite its potential to facilitate the process. In this study, we first constructed a literature-based pathway to examine both the positive and negative effects of MIR31 on wound healing. We then conducted animal experiments on 20 rats to investigate MIR31 expression at different time points (1, 4, and 8 weeks) after vocal fold injury. Co-expression analysis and pathway analysis were performed to explore the potential function of MIR31 in VFWH. The literature-based pathway suggested that MIR31 could both impede and promote the wound healing process by regulating 14 and 47 wound healing upstream regulators, respectively. However, the rat experiment indicated that MIR31 expression significantly increased after vocal fold injury ($p < 5.65 \times 10^{-5}$) but decreased in the late stage of VFWH compared with the early and middle stages ($p < 5.40 \times 10^{-3}$. Strong co-expression was observed between MIR31 and 17 VFWH-significant genes (Pearson correlation coefficient $\in (0.63, 0.83)$), primarily involved in collagen production. Overall, our findings suggest that MIR31 plays a critical role in VFWH, particularly in collagen synthesis and other biological processes, which warrant further investigation.

1. Introduction

The structure of the extracellular matrix (ECM) is essential for the functionality of the vocal fold to produce sounds [1]. Vocal fold injury could lead to abnormal massive hyperplasia of the fibrous tissue and ECM disorder in the lamina propria. Vocal fold injury caused fibrous scars that could bring persistent pararthria and irreversible changes [2–4]. So far, it is still a challenging clinical topic to treat vocal fold scars effectively.

Vocal fold wound healing (VFWH) could take from two weeks to several months [5, 6]. Multiple genes and proteins have been shown to play roles in the wound healing process [7]. For instance, Mothers Against Decapentaplegic Homolog 1 (SMAD1) has been suggested as a therapeutic molecular target in skin wound healing that plays an active role in wound repair and regenerative medicine [7, 8]. Spallotta et al.'s study showed that histone deacetylase 2 (HDAC2) inhibition could significantly enhance skin wound repair [8].

MIR31 is a short non-coding RNA that is involved in the post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of messenger RNA (mRNAs) [9, 10]. Multiple previous studies suggested that MIR31 plays a role in the pathology of skin wound healing, including the stimulation of wound contraction to enhance wound closure [11] and the promotion of skin wound healing by enhancing keratinocyte proliferation and migration [12]. Despite its potential to promote wound healing, MIR31 can also hinder the process by inhibiting wound healing promoters, such as Peroxisome Proliferator-Activated Receptor Gamma (PPARG), Interleukin-6 (IL6), and C-C Motif Chemokine Ligand 5 (CCL5) [13–15], and promoting wound healing inhibitors like MKI67, ACAN, and AHR [16–18]. Hence, further research is needed to fully understand the effects of MIR31 on wound healing. Currently, there is no research on whether MIR31 plays a role in VFWH, making it an area that requires further investigation.

In this study, a microarray was used to detect the expression changes of MIR31 in different stages of VFWH. In

addition, a co-expression analysis was conducted between MIR31 and the genes that presented significant expression changes in the vocal fold after injury. Our results indicated that the expression of MIR31 was stimulated by the wound and was significantly related to the vocal fold's wound healing process. This study guarantees further study to explore the role of MIR31 and VFWH.

2. Methods

2.1. mRNA and miRNA Data Extraction. The animal experiments in this study were performed by the regulations of the Peking University Animal Care and Use Committee (LSC-ZhangY-1) and license permit: 1116012800123. Male Sprague–Dawley (SD) rats aged 14–16 weeks and weighing 400–450 g underwent unilateral or bilateral vocal fold injury using procedures described in an earlier study [19]. The vocal fold was injured by separating and removing the lamina propria from the thyroarytenoid muscle. First, 20 animals were randomly divided into four experimental groups (five animals in each group) based on time of sacrifice: uninjured control, and 1, 4, and 8 weeks after injury. Following sacrifice, the larynx was harvested, and the bilateral vocal folds were dissected under magnification. Each specimen was immediately snap-frozen in liquid nitrogen and stored at −80°C for subsequent study.

Total RNA, including both mRNA and micro RNA (miRNA), was extracted from vocal folds using TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) and purified with an RNeasy mini kit (Qiagen, Valencia, CA, USA). Biotinylated complementary DNA (cDNA) was prepared according to the standard Affymetrix protocol from 250 ng of total RNA using an Ambion® WT Expression Kit. Following labeling, fragmented cDNA was hybridized for 16 hours at 45°C with the Clariom™ S Assay (rat, Affymetrix). GeneChips were washed and stained in the Affymetrix Fluidics Station 450. All arrays were scanned using an Affymetrix® GeneChip Command Console, which was installed in a GeneChip® Scanner 3000 7G.

The row data (.cel) were normalized using the TAC software (Transcriptome Analysis Console, Version 4.0.1) with the Robust Multichip Analysis (RMA) algorithm using Affymetrix default analysis settings and global scaling as the normalization method. Values presented are log2 RMA signal intensity. The microarray data discussed in this study are submitted to NCBI Gene Expression Omnibus with accession number GSE139383.

2.2. Screening of Differentially Expressed Genes. To analyze the mRNA and miRNA expression data, we initially utilized the Limma R package (version 3.36.5). Our goal was to examine the expression of MIR31 across various groups and identify the differentially expressed genes (DEGs) in the injured groups compared with the uninjured control group. To control the false discovery rate (FDR) for multiple tests, we employed the Benjamini–Hochberg procedure with a significance threshold of $q = 0.05$. The criteria for up- and downregulated genes were set as fold change >1.5 or <−1.5, with an FDR-corrected p-value <0.01. Subsequently, we employed one-way Analysis of Variance (ANOVA) to detect the expression changes of both MIR31 and other DEGs that exhibited significant expression variations in the three injured groups ($p < 0.01$). We further investigated the co-expression between MIR31 and the DEGs to explore potential associations between them.

For further analysis, we selected genes that met the following criteria: (1) they showed significance in each injured group compared with the control group (fold change >1.5 or <−1.5, and $p < 0.01$); (2) they also demonstrated significance when compared among the three injured groups ($p < 0.01$).

2.3. Functional Network and Pathway Analysis. A literature-based functional network analysis was carried out to investigate the genetic connection between MIR31 and wound healing. This analysis revealed molecules regulated by MIR31 that play a role in the wound healing process. The network analysis relied on Pathway Studio (http://www.pathwaystudio.com) for assistance. By comparing the downstream targets of MIR31 with the upstream regulators of wound healing, the genetic pathways driven by MIR31 were constructed. These pathways have the potential to either promote or inhibit the wound healing process. Additionally, a gene set enrichment analysis (GSEA) was conducted using Gene Ontology (GO) terms and Human Protein Atlas Expression Ontology (HPAEO). This analysis provided insights into the functional profile of MIR31 and its related DEGs. Pathways/GO items show significance were reported (FDR corrected $p < 0.05$ and overlap ≥2).

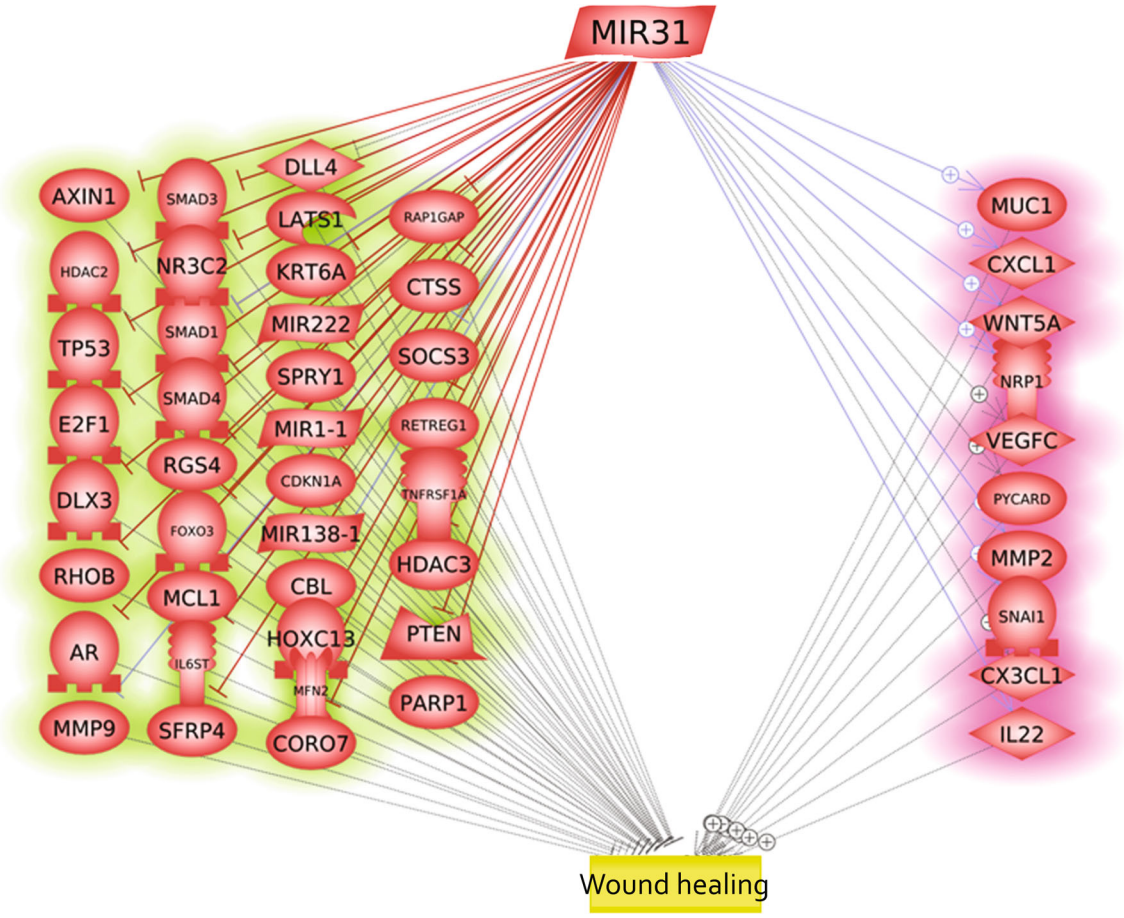
3. Results

3.1. Possible Negative Role of MIR31 on Wound Healing. Literature-based pathway analysis revealed that MIR31 can stimulate wound healing by activating 10 wound healing promoters and inhibiting 37 wound healing inhibitors, as illustrated in Figure 1(a).

However, as shown in Figure 1(b), MIR31 may also hinder the wound healing process by inhibiting seven wound healing promoters, including CXCL12, SPP1, STAT3, NOTCH1, PPARG, IL6, and CCL5. MIR31 can also promote seven wound healing inhibitors, including MKI67, ACAN, AHR, MIR34A, MIR24-1, MIR106B, and MIR26A1. Therefore, further research is needed to fully understand the effects of MIR31 on wound healing, especially for VFWH.

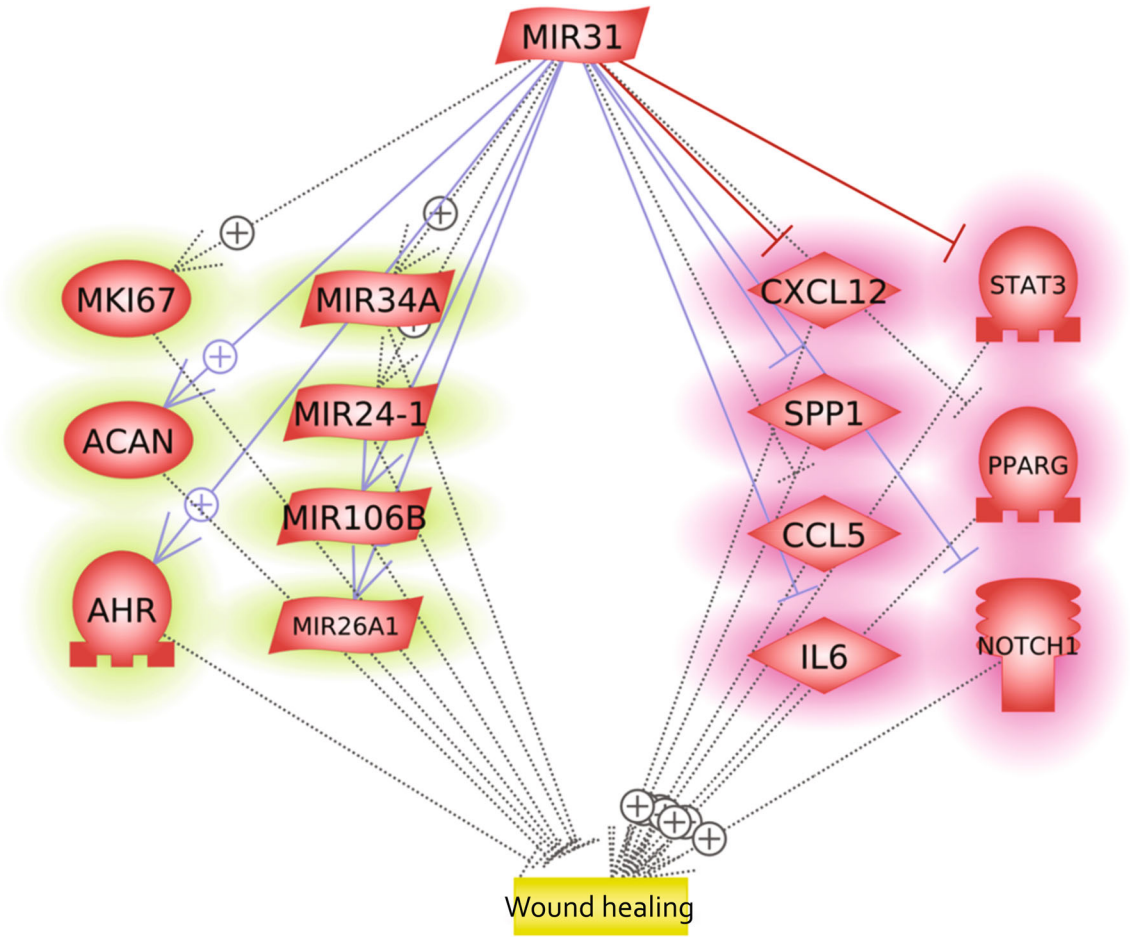
3.2. MIR31 Expression in VFWH. When compared with uninjured vocal fold tissues (control group, $n = 5$), MIR31 demonstrated significantly increased expression in injured vocal fold tissues at different time points (1, 4, and 8 weeks; $n = 5$ in each experimental group) with $p < 5.65 \times 10^{-5}$, as shown in Figure 2(a). It was found that the expression of MIR31 also varied significantly among injured vocal fold tissues at different time points ($p < 0.0054$; see Figure 2(b)).

3.3. Expression of 15 Significant Genes. Besides MIR31, our analysis also identified 17 other genes that present significance when compared among both four groups ($p < 0.0099$) and three injured groups ($p < 0.0075$), including *LOC100362109*, *Mex3b*, *Col5a2*, *C1qtnf2*, *Eva1b*, *H1fx*, *Col6a2*, *Oaf*, *Htra1*, *Col5a1*, *Rps13*, *LOC102549615*, *Prkaa1*, *LOC100909726*, *Serpinh1*, *Rps9*, and *LOC100360117*. The expression heat map of these genes and MIR31 was presented in Figure 3(a).



(a)

Figure 1: Continued.



(b)

Figure 1: MIR31-driven molecules related to wound healing. (a) MIR31-driven molecules promote wound healing, including 37 wound healing inhibitors (highlighted in green); and 10 wound healing promoters (highlighted in red). (b) MIR31-driven molecules hinder the wound healing process, including the 7 wound healing inhibitors (highlighted in green) and 6 wound healing promoters (highlighted in red).

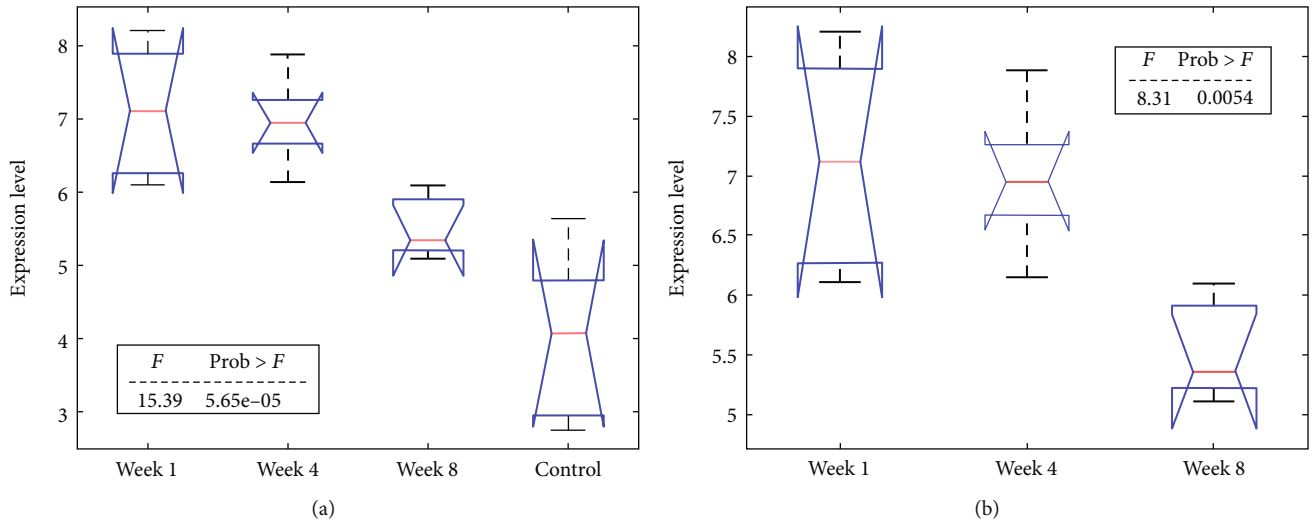


Figure 2: Comparison of expression levels of MIR31 in the four groups of rats. (a) The comparison results of four groups. (b) The comparison results of three injured groups.

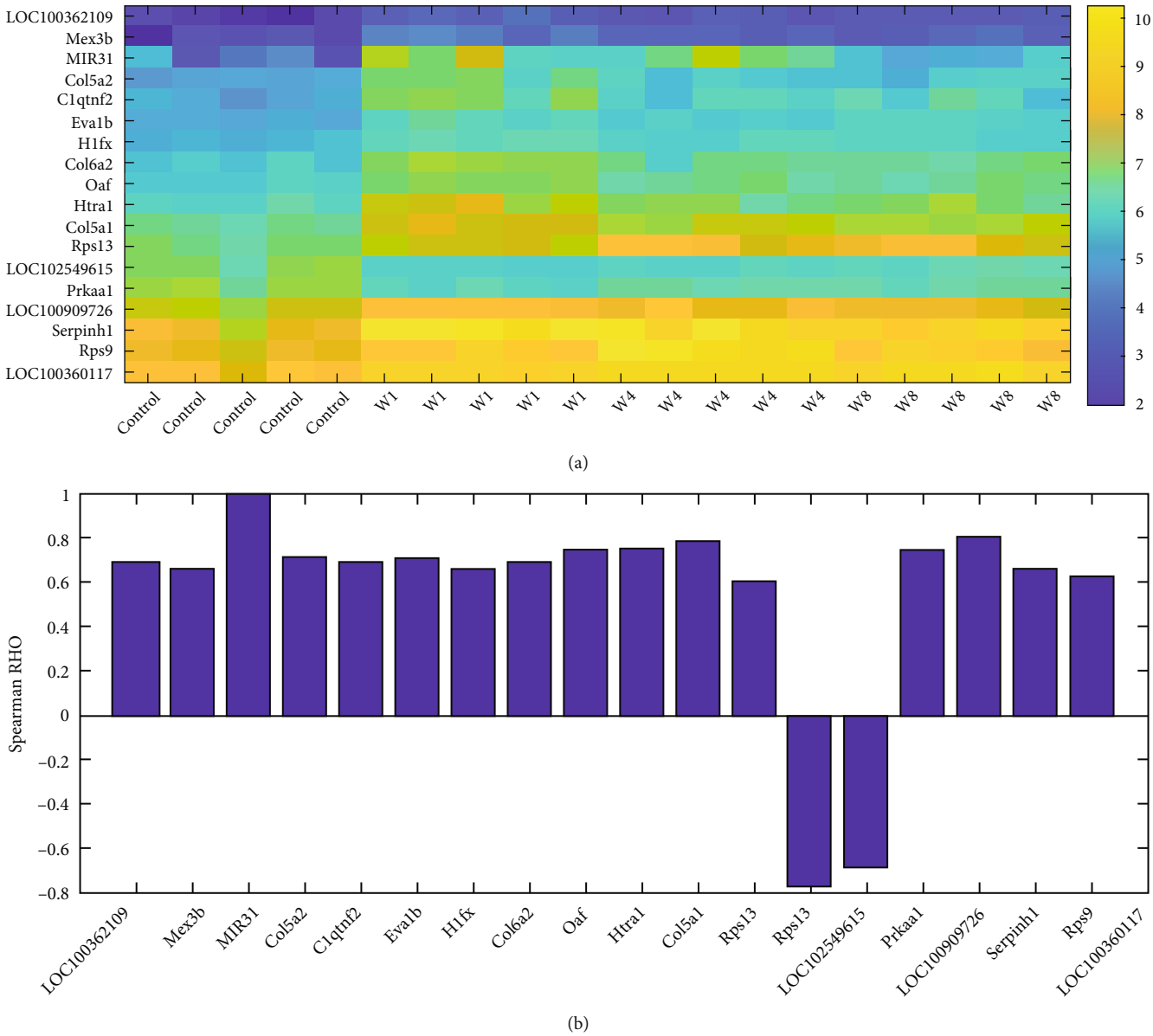


Figure 3: Expression and co-expression of 17 genes and MIR31 in the four groups of rats. (a) Heat map of expression levels. (b) Correlation between the 18 genes and MIR31.

Interestingly, 15 out of the 17 genes presented a strong positive correlation with MIR31 in terms of expression variation among the four groups (ROH $\in$ (0.63, 0.83)), and two showed a robust negative correlation with MIR31 (ROH = −0.78 and −0.70, respectively), as shown in Figure 3(b). These results suggested that MIR31 may regulate or co-function with these genes to influence the wound healing process of the vocal fold.

3.4. GSEA Results. Enrichment analysis was conducted using GSEA to compare the 17 genes (refer to Figure 3(a)) and MIR31 with the GO terms and HPAEO terms. The enrichment analysis utilized these 18 items as input. Results showed that these 17 genes were mainly involved in collagen-related GO terms, as shown in Figure 4. No HPAEO gene group was identified. Supplementary Material 1 presents comprehensive information on these pathways, encompassing the GO ID, the number of entities, the overlapping genes, as well as the p-values before and after FDR.

4. Discussion

Previous studies have suggested that MIR31 may facilitate skin wound healing by promoting the proliferation and migration of keratinocytes [11, 12]. However, MIR31 may also hinder the wound healing process by inhibiting seven wound healing promoters, namely CXCL12, SPP1, STAT3, NOTCH1, PPARG, IL6, and CCL5 [13–15]. Moreover, MIR31 can promote seven wound healing inhibitors, including MKI67, ACAN, AHR, MIR34A, MIR24-1, MIR106B, and MIR26A1 [16–18]. Therefore, further investigations are required to comprehensively comprehend the impact of MIR31 on wound

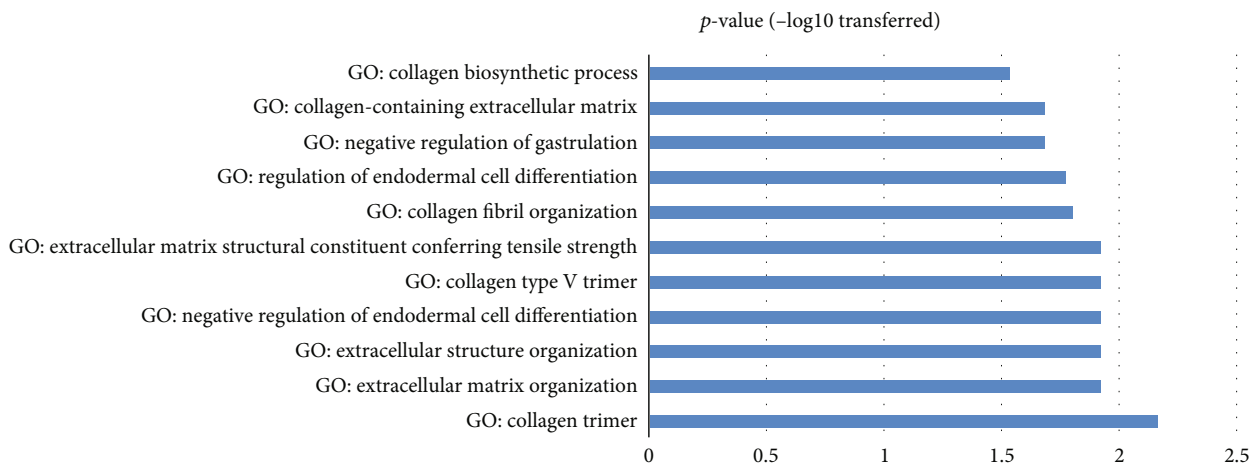


Figure 4: GSEA results of 17 genes and MIR31.

healing, particularly in the context of VFWH. Results from this study showed that MIR31 was stimulated by vocal fold wounds to demonstrate significantly high expression at the initial stage of vocal fold healing, which decreased in the late stage of the wound healing process. Moreover, MIR31 demonstrated a strong correlation with 17 genes showing significant expression variation during VFWH, which were mainly involved in collagen synthesis. Our results suggest that MIR31 is closely related to VFWH.

Expression analysis showed that MIR31 expression was significantly increased at the initial stage of VFWH (week 1), as shown in Figure 2(a). Upregulated MIR31 has been reported to alleviate inflammation in colon injury [20] and cardiac injury [21]. At the early stage of wound healing, inflammation is the initial response to cellular injuries and is the key process in wound healing [22]. MIR31 has also been shown to stimulate wound contraction and thus enhance wound closure [11]. Therefore, our results indicate that increased MIR31 expression could help at the early stage of the VFWH.

The up-regulated expression of MIR31 lasted to week 3 without an obvious decrease, as shown in Figure 2(b). MIR31 has been shown to promote skin wound healing by enhancing keratinocyte proliferation and migration, which may happen through suppressing its direct target gene, epithelial membrane protein 1, during wound healing [12]. MIR31 could also suppress the inhibitors of wound healing, including SMAD1 [23] and HDAC2 [24]. Moreover, up-regulation of MIR31 could lead to elevated cellular adenosine triphosphate (ATP) that is required for wound closure [25, 26]. Therefore, lasted overexpression of MIR31 could exert continued aid to the VFWH.

We also noticed that MIR31 expression significantly dropped 8 weeks after the vocal fold wound ($p < 0.0054$), which may be corresponding to the late stage of the wound healing process (Figure 2(b)). However, this may also reflect balanced factors that influenced VFWH. Figure 1(b) illustrates that MIR31 can impede the wound healing process by blocking various agents that promote wound healing, whereas encouraging the presence of certain factors that inhibit it. For instance, PPARG, a nuclear hormone receptor, plays a crucial role in wound healing by regulating inflammation, tissue remodeling, and cell signaling [13]. Activation of PPARG promotes wound healing by reducing oxidative stress, suppressing inflammation, and increasing the expression of wound-healing genes [27]. Additionally, PPARG deficiency or inhibition leads to impaired wound healing due to delays in apoptotic cell clearance, dysfunctional adipocytes, and fibrosis inhibition [28]. Consequently, the decline in MIR31 expression during later stages could facilitate the activation of these corresponding regulators that aid in wound healing. Nevertheless, additional research is required to fully understand the mechanism of MIR31 expression regulation during different stages of the VFWH process.

Co-expression analysis showed that MIR31 was strongly related to 17 genes that presented significant expression changes during VFWH (Figure 3). These genes were mainly involved in collagen biosynthetic and trimming, as shown in the GSEA results presented in Figure 4. Collagen has been shown to increase keratinocyte proliferation, positively acting on cell entry in the mitotic phase [29]. Thus, collagen synthesis and deposition into the wound are essential during wound healing [30]. Our results suggested that MIR31 may co-function with the 17 collagen production regulators to play a role in the VFWH process. In addition, our study also showed that MIR31 was related to multiple biological processes that were linked to wound healing, including fibrosis and keloid [31, 32].

Furthermore, investigation is required to address the limitations of this study in the future. First, it is crucial to validate the results obtained from animal studies by incorporating human data. Additionally, the pathway depicted in Figure 1 relies heavily on previous publications and should be subjected to human experimentation for further confirmation.

5. Conclusion

This study supports the promotion role of MIR31 in the process of VFWH, which may be through the regulation

of multiple biological processes, including keratinocyte proliferation, collagen production, ATP generation, and inflammation.

Data Availability

Upon contacting the corresponding author, all data generated or analyzed during this study can be accessed.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Supplementary Materials

Supplementary Material 1 presents comprehensive information on these pathways, encompassing the GO ID, the number of entities, the overlapping genes, as well as the p-values before and after FDR. *(Supplementary Materials)*

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Research Article

Significance of PPARA as a Treatment Target for Chronic Lymphocytic Leukemia

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Peroxisome proliferator-activated receptor alpha (PPARA) has been suggested as a therapeutic target for chronic lymphocytic leukemia (CLL). However, the underlying molecular mechanism remains largely unclear. In this study, we analyzed DNA next-generation sequencing (NGS) data and clinical information from 86 CLL patients to identify gene markers related to treatment-free survival (TFS) length. We then constructed a genetic network that includes CLL promoters, treatment targets, and TFS-related marker genes. To assess the significance of PPARA within the network, we utilized degree centrality (DC) and pathway enrichment score (EScore). Clinical and NGS data revealed 10 TFS length-related gene markers, including RPS15, FOXO1, FBXW7, KMT2A, NOTCH1, GNA12, EGR2, GNA13, KDM6A, and ATM. Through literature data mining, 83 genes were identified as CLL upstream promoters and treatment targets. Among them, PPARA exhibited a stronger connection to CLL and TFS-related gene markers, as evidenced by its ranking at No. 13 based on DC, compared to most of the other promoters (>84%). Additionally, PPARA co-functions with 70 out of 92 in-network genes in various functional pathways/gene groups related to CLL pathology, such as regulation of cell adhesion, inflammation, reactive oxygen species, and cell differentiation. Based on our findings, PPARA is considered one of the critical genes within a large genetic network that influences the prognosis and TFS of CLL through multiple pathogenic pathways.

1. Introduction

Chronic lymphocytic leukemia (CLL) is a tumor originating from mature B lymphocytes [1]. It is the most common adult leukemia in Western countries [2]. In 2021, about 21,250 people in the United States were diagnosed with CLL, and about 4,320 died from this disease [3]. The clinical course of patients with CLL is highly heterogeneous, making it difficult to predict the likelihood that a patient will require treatment at the time of diagnosis. About 70%–80% of CLL patients are asymptomatic at the time of diagnosis, and around 30% will never require treatment for CLL [4]. Clonal diversity and complexity have consistently been associated with poor CLL prognosis [5, 6].

Nuclear receptor peroxisome proliferator-activated receptor alpha (PPARA) is a critical regulator of energy metabolism and mitochondrial and peroxisomal function [7]. Encoded by the PPARA gene, PPARA regulates the expression of genes involved in glucose and lipid metabolism and inflammatory processes [8] by binding to PPAR response elements in the promoter region of the genes [9]. PPARA has been shown to mediate glucocorticoid resistance and promote CLL [10] and has therefore been suggested as a therapeutic target for CLL [11]. A PPARA antagonist, NXT629, demonstrated the capability of reducing tumor burden in a mouse model of CLL [12], suggesting that PPARA may have a tumor-suppressive role in CLL. However, the precise mechanism by which PPARA regulates CLL is yet to be determined.

Besides PPARA, there are multiple genes that have been identified to play roles in the survival of CLL B cells and were suggested as therapeutic targets in CLL, including

NOTCH [13, 14], ZAP70 [15], CXCR4 [16], CD40 [17], CD44, and CD49d [18]. These genes could influence the prognosis of CLL patients and their treatment-free survival (TFS) length. It is worth noting that the proposed therapeutic targets for CLL, including PPARA, have been suggested by different studies conducted at various time intervals. Identifying and analyzing the relationship among these genes may help discover their significance and better understand their therapeutic roles in treating CLL [19].

In this study, we collected TFS-related gene markers by using DNA next-generation sequencing (NGS) data and clinical data collected from 86 CLL patients. We constructed the genetic network composed of the literature-identified CLL promoters and treatment targets, as well as CLL TFS-related marker genes. Degree centrality (DC) and pathway enrichment score (EScore) were used to evaluate the significance of each gene within the genetic network, including PPARA.

2. Materials and Methods

2.1. Patient Samples and Laboratory Testing. A total of 86 newly diagnosed CLL patients from Chongqing and other regions in western China from January 2019 to June 2021 in the Hematology Medical Center of Chongqing Xinqiao Hospital were collected. According to the iwCLL 2018 diagnostic criteria [20], these patients were diagnosed as CLL. The clinical and laboratory characteristics of all patients were collected, including gender, age, imaging examination, blood routine test, biochemical test, flow cytometry, and chromosome karyotype. Fluorescence in situ hybridization (FISH) image was used to detect del(17p), del(11q), and del(13q). Sanger sequencing of Polymerase Chain Reaction (PCR) amplification products was used to determine the immunoglobulin heavy chain variable region (IGHV) mutation status. Peripheral blood ($n = 39$) and bone marrow aspirate ($n = 47$) samples from 86 untreated CLL patients were collected for DNA–NGS data generation. According to the guidelines, patients without indications for treatment were followed up regularly, and appropriate treatment was given after reaching time-to-first treatment (TTFT). The TTFT for CLL patients is the time between diagnosis and the initiation of the first treatment and can vary depending on several factors. Close monitoring and appropriate treatment can help improve outcomes for patients with CLL. Patients are treated in a combination of hospital visits and follow-ups on the Internet management platform for CLL patients in the Hematology Medical Center of Chongqing Xinqiao Hospital. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Army Medical University (2020-Research No. 128-01).

2.2. NGS Data Collection. In this study, the CLL-related gene mutation detection kit (CLL 72) (Wuhan Steady Medical Laboratory Co., Ltd.; http://www.stdlbio.com/) was used to target 72 CLL-related genes based on NGS technology. The 72 genes cover chronic lymphoproliferative disease genes associated with Chinese onset recommended by authoritative guidelines/expert consensus at home and abroad, large-scale literature reports, and database accumulation, and are used to assist in routine screening of clinical CLL.

First, DNA extracted from blood and bone marrow samples was used as material to prepare a pre-library by fragmentation, adapter addition, PCR amplification, and other steps; then, biotin-labeled oligonucleotide probes were used to hybridize with the pre-library, and then Streptavidin magnetic beads are used to bind the probe to capture the target region; finally, the final capture library is obtained by PCR enrichment. The amplified library was sequenced using a gene sequencer (model: MiniSeq, MiSeq, NextSeq, NovaSeq, etc., produced by Illumina Corporation) for high-throughput sequencing.

Trimmomatic (v0.39) software was used to filter the raw data for quality control, and the filtered data were compared to the human reference genome (GRCh37) through the BWA-mem (v0.7.17) algorithm. Then, GATK (4.0.12.0) software was used to detect mutation sites, and Annovar (v20210122) software was used to annotate the mutations in combination with databases such as NCBI, COSMIC, Clinvar, ExAC, dbSNP (v138), and 1000 Genomes.

2.3. Statistical Method. Data analysis was performed using IBM SPSS Statistics 26. The measurement data conforming to the parametric distribution were expressed as the mean ± standard deviation; otherwise, the median (interquartile range) was expressed, and the enumeration data were expressed as the number of cases and percentages. TTFT was defined in months, from the date of initial diagnosis to the date of initial treatment. For all surviving patients, the time of statistical analysis by the researchers (March 2022) was taken as the last follow-up time. The COX risk regression model was used to evaluate the correlation analysis between patient characteristics and TTFT. First, the data were analyzed by univariate analysis, and the factors with univariate analysis $P < 0.1$ were included in the multivariate model for stepwise regression analysis. We reported and conducted further analysis on the factors that demonstrated significance ($P < 0.05$) regarding their association with gene mutations. R-4.1.2 was used for survival analysis and Kaplan–Meier curve drawing. Use the chi-square test function in R-4.1.2 to detect differential genes, and $P < 0.05$ criteria was used to determine the statistically significant genes related to TFS.

2.4. Construct the Genetic Therapeutic Network for CLL. Assisted by Pathway Studio (http://www.pathwaystudio.com), we conducted a literature data mining to uncover reported CLL therapeutic target genes and positive upstream regulators. The genes that were identified in previous studies as potential therapeutic or treatment targets for CLL are referred to as the "therapeutic targets." On the other hand, genes that have been shown to positively regulate CLL based on previous research are known as the "positive upstream regulators." To gather information on these genes and their relationship with CLL, a literature data mining approach was employed using a comprehensive biology database (http://www.pathwaystudio.com) that covers the entire PubMed abstracts and full-text journals from Elsevier and third-party publishers. This database is one of the largest in the world. For each gene–CLL relationship, there are

one or more supporting references that were manually reviewed for quality control.

Then we constructed the network connection to these genes, CLL, PPARA, and the TFS-significant genes identified from the NGS data and clinical data analysis.

2.5. Weighting PPARA in the Therapeutic Network of CLL. In graph theory, a vertex's centrality reflects the vertex's significance in the network. The simplest vertex centrality is DC, defined as the number of edges incident upon a vertex. For a graph $G := (V, E)$ with n vertices, the DC $C_D(v)$ for vertex v is defined in Equation (1).

$$C_D(v) = \frac{\text{Degree}(v)}{n-1}. \quad (1)$$

Besides DC, we also employed the EScore [21], which reflects a gene's importance in the significant pathways involved. The Escore for the "vth" gene is calculated as Equation (2).

$$\text{EScore}_v = \frac{\operatorname*{sum}_{i=1:m}(-\log_{10}(p_i))}{\max_{i=1:n}(-\log_{10}(p_i))}, \quad (2)$$

where p_i is the enrichment p-value of the "ith" pathway enriched, "n" represents the total number of pathways enriched, and "m" represents the number of pathways including the "vth" gene.

3. Results

3.1. Clinical Data of the 86 CLL Patients. The median age of the 86 patients was 57.34 ± 10.81 years old (range 34–81 years old), 61 males (70.9%), and 25 females. CLL-international prognostic index (CLL-IPI) score was high risk (including very high risk) in 12 cases (13.9%). There were 61 cases (70.9%) of IGHV mutations. NGS detected TP53 mutations in five cases (5.8%). FISH detected del(13q) in 12 cases (13.9%), del(11q) in 13 cases (15.1%), del(17p) in 5 cases (5.8%), and 12 amplifications in 2 cases (2.3%). The characteristics of the patients are shown in Table 1. Del(13q) refers to a chromosomal abnormality in which a portion of the long arm of chromosome 13 is missing. This deletion is a common finding in CLL and is the most frequent chromosomal abnormality in this type of leukemia. Del(11q) refers to a chromosomal abnormality in which a portion of the long arm of chromosome 11 is missing. This deletion is commonly found in patients with CLL and is one of the most significant genetic abnormalities associated with poor prognosis in this disease. Del(17p) refers to a chromosomal abnormality in which a portion of the short arm of chromosome 17 is missing. This deletion is also known as 17p deletion or TP53 deletion because it affects the TP53 gene located on this chromosome.

The median follow-up time of all patients was 22.00 months (95% CI 20.48–23.51), 31 cases (36.05%) achieved TTFT (TTFT-24) within 24 months, and the median TTFT (mTTFT) was 5.00 months (95% CI 2.31–7.69). A total of seven patients (8.14%) died, of which two patients who did not reach TTFT died of other causes (cardiovascular events and pulmonary infection) other than disease progression.

Table 1: Characteristics of 86 newly diagnosed chronic lymphoblastic leukemia patients.

Characteristics	*N* (%)
Sex	
Male	61 (70.93%)
Female	25 (29.07%)
Age (year)	
<45	9 (10.46%)
≥45, <65	51 (59.30%)
≥65	26 (30.23%)
CLL-IPI	
Low/intermediate risk	74 (86.05%)
High risk	12 (13.95%)
FISH	
del(13q)	12 (13.95%)
del(11q)	13 (15.11%)
del(17p)	5 (5.81%)
Trisomy 12	2 (2.32%)
TP53(NGS)	
TP53 mutation	5 (5.81%)
TP53 wt	78 (90.70%)
Unknown	3 (3.49%)
IGHV	
Mutated	61 (70.93%)
Unmutated	25 (29.07%)
Bulky disease	9 (10.465%)
Extranodal involvement	5 (5.814%)
Hepatomegaly	5 (5.814%)
Splenomegaly	42 (48.837%)
Initial WBC ($\times 10^9$/L)	
>100	3 (3.488%)
50–100	15 (17.442%)
<50	68 (79.07%)
Initial HGB (g/L)	
>110	69 (80.233%)
90–110	11 (12.791%)
<90	6 (6.977%)
Initial PLT ($\times 10^9$/L)	
≥100	77 (89.535%)
<100	9 (10.465%)
Initial LYM (%)	
>80	44 (51.163%)
50–80	31 (36.047%)
<50	11 (12.791%)
Initial LYMC ($\times 10^9$/L)	
>100	3 (3.488%)
50–100	8 (9.302%)
<50	75 (87.209%)

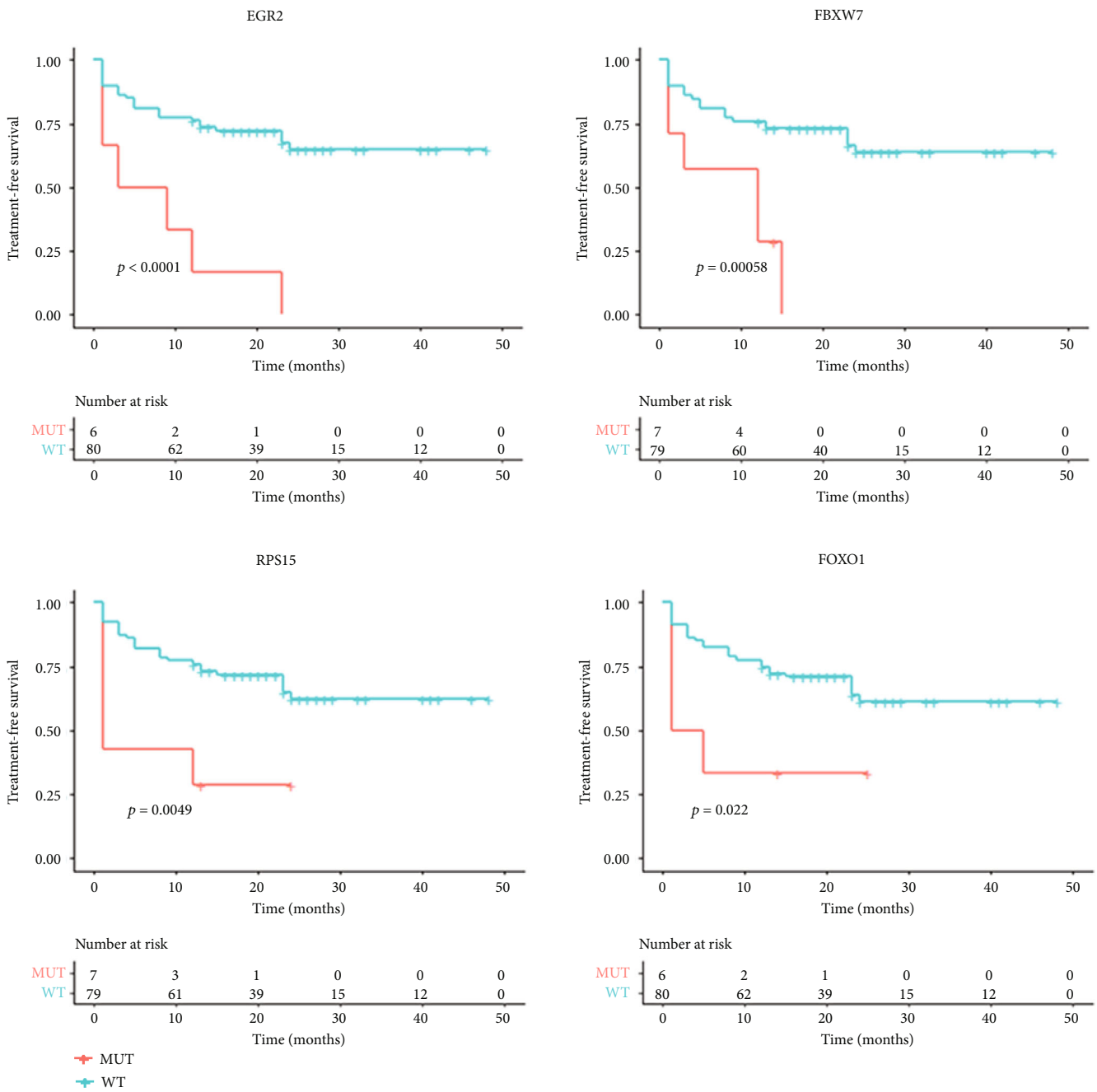


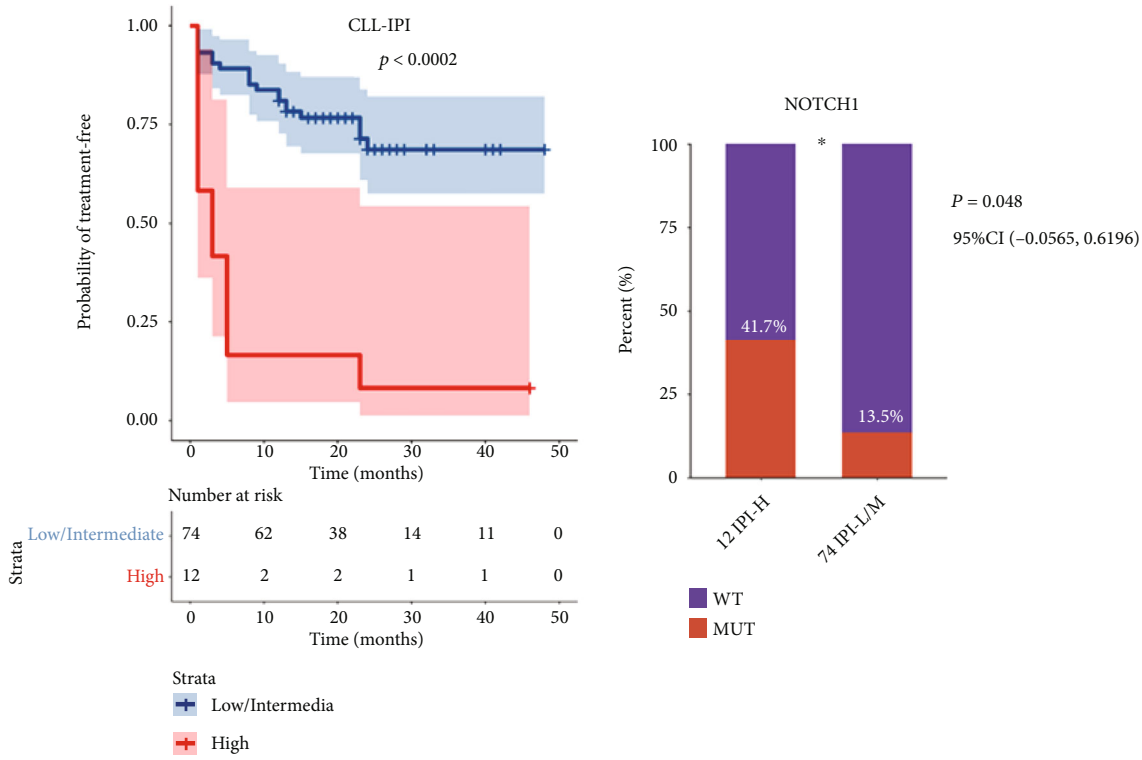
Figure 1: EGR2, FBXW7, RPS15, and FOXO1 mutations were shown to be associated with shorter treatment-free survival (TFS) in 86 patients.

Richter's transformation (diffuse large B-cell lymphoma) was present in 4 of 31 TTFT-24 patients.

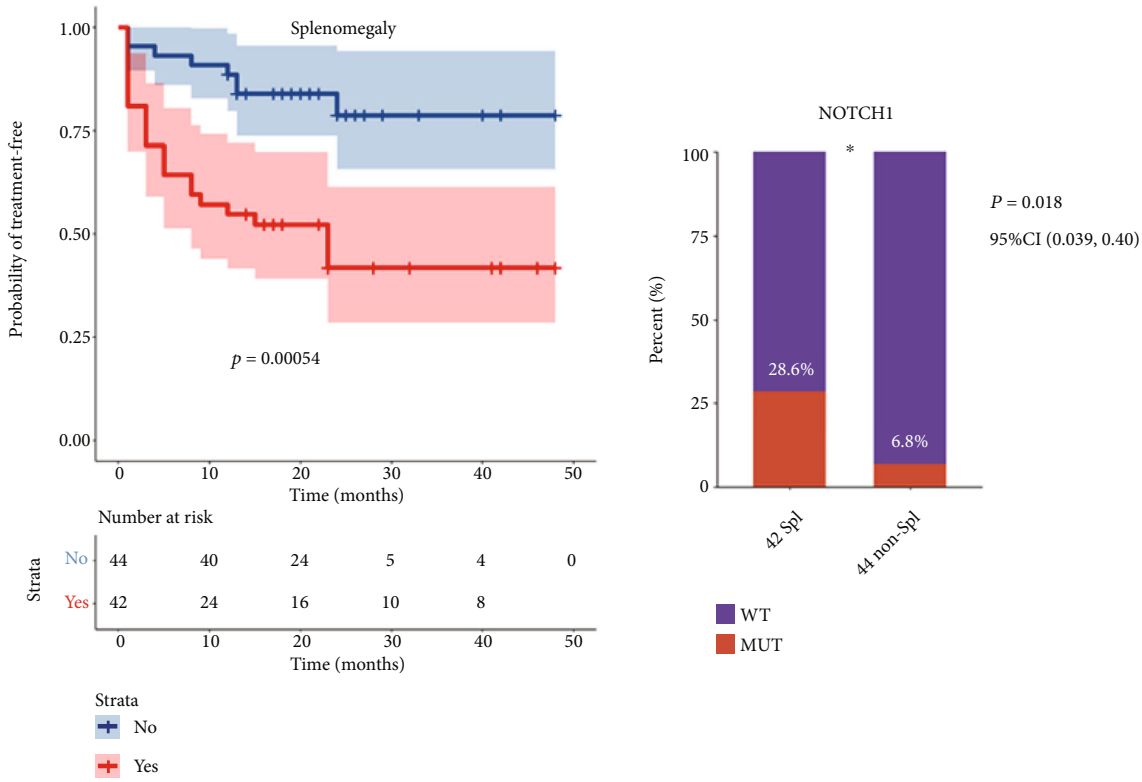
3.2. TFS-Related Clinical Parameters and Genes. We presented the spectrum of gene mutations of 86 patients in Supplementary Figure 1. DNA-seq data analysis showed that the mutations of four genes presented significant associations with shorter TFS (Figure 1), including EGR2, FBXW7, RPS15, and FOXO1.

The results of multivariate analysis showed that four factors are associated with shorter TFS and can be used as independent prognosis decision factors, including CLL-IPI score ($P = 0.025$, 95% CI 1.14, 7.02), del(11q) ($P = 0.002$, 95% CI 1.62, 8.81), splenomegaly ($P = 0.009$, 95% CI 3.24, 7.08), and newly diagnosed platelets ($<100 \times 10^9$/L) ($P = 0.003$, 95% CI 1.63, 11.17). In Supplementary Table 1, we presented the analysis results for all factors. However, due to limited space, we only emphasized the discussion on the four significant factors.

The chi-square test showed that NOTCH1 mutation ($P = 0.048$, 95% CI −0.06, 0.62) was linked to the CLL-IPI score. Associated with del(11q) were ATM mutation ($P = 0.010$, 95%

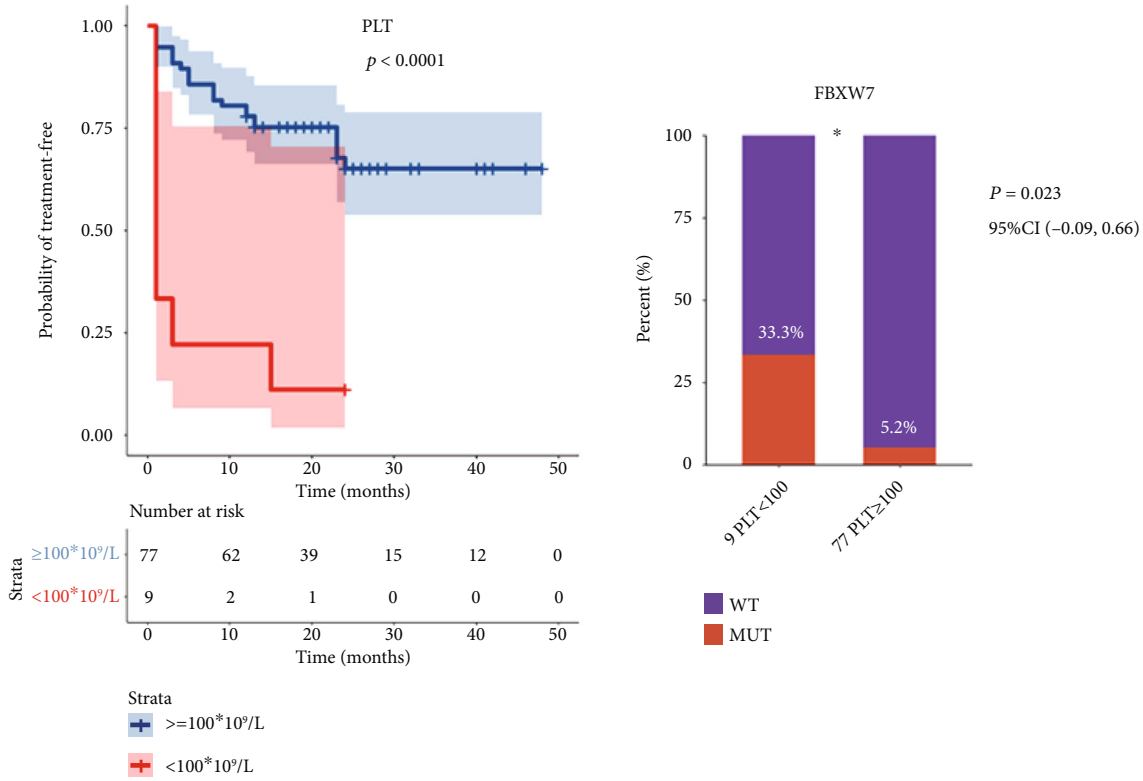


(a)



(b)

Figure 2: Continued.



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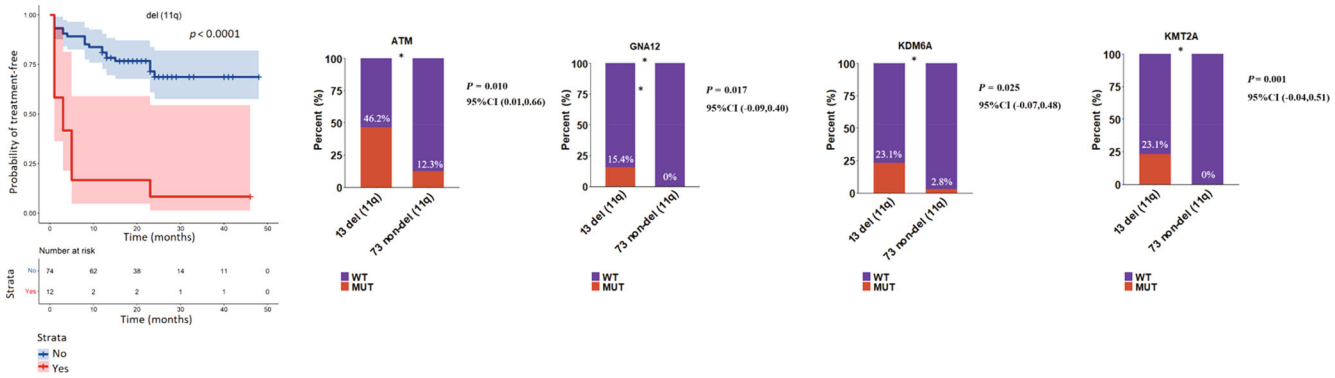

(d)

Figure 2: The four clinical factors associated with shorter TFS and their associated genes. (a) CLL-IPI related gene; (b) Splenomegaly related gene; (c) platelets related gene; (d) del(11q) related genes.

CI 0.01, 0.66), GNA12 mutation ($P = 0.016$, 95% CI −0.09,0.39), KDM6A mutation ($P = 0.025$, 95% CI −0.07, 0.48), and KMT2A mutation ($P = 0.001$, 95% CI −0.04, 0.51). The differential gene mutation associated with newly diagnosed patients with platelet $<100 \times 10^9$/L was FBXW7 mutation ($P = 0.023$, 95% CI −0.09, 0.66). NOTCH1 mutations were associated with splenomegaly ($P = 0.018$, 95% CI 0.039, 0.40). Two gene mutations, EGR2 ($P = 0.014$, 95% CI −0.17, 1.00) and GNA13 ($P = 0.030$, 95% CI −0.31, 0.81), were associated with Richter's transformation. We presented the clinical parameters associated with shorter TFS and their associated genes in Figure 2.

3.3. CLL-Regulation Network. Literature data mining identified 83 genes as CLL upstream promoters and treatment targets, including PPARA. Their connections with CLL and the 10 CLL TFS-related genes were employed to construct the CLL-regulation network, as shown in Figure 3. In Supplementary Table 2, we outline each relationship in detail, including the relation type, relation direction, source node, and target node. The 10 genes associated with CLL TFS were marked in yellow, while the 83 CLL upstream promoters, including PPARA, were marked in red. The network was established in such a way that links the 83 CLL upstream promoters to both the 10 CLL TFS-related genes and CLL itself. All the relationships in the network were supported by one or more references. Utilizing this constructed network, we evaluated and compared the centrality scores of each of the 83 CLL promoters to determine their association with CLL and its TFS length.

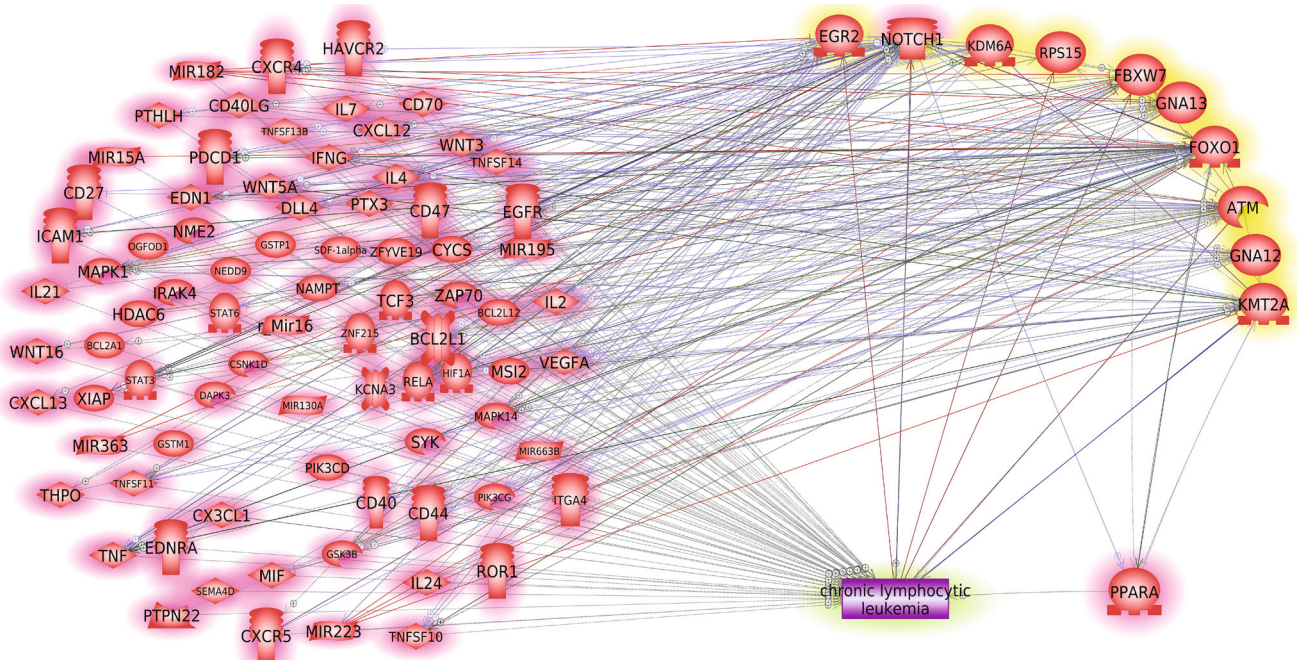

Figure 3: CLL-regulation network composed of 83 CLL upstream promoters (highlighted in red) and 10 CLL TFS-related genes (highlighted in yellow). NOTCH1 was in both groups and highlighted by mixed colors (red and yellow).

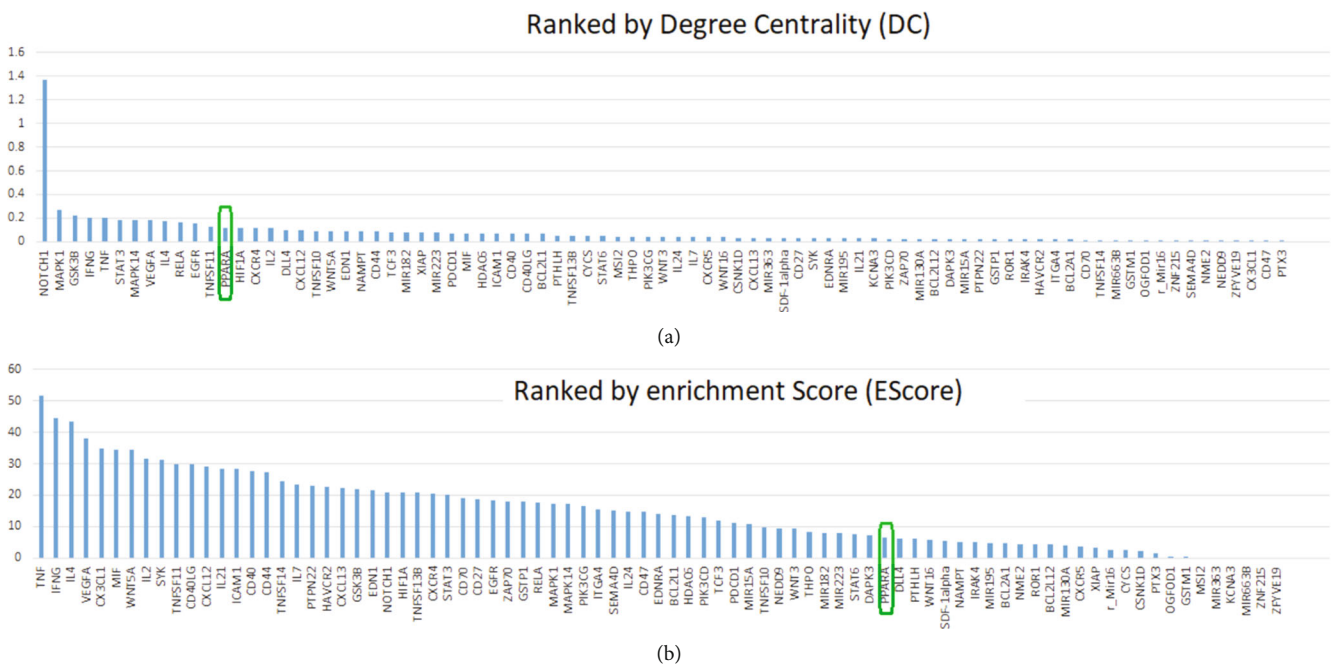

Figure 4: PPAR ranking. (a) Using degree centrality (DC); (b) using enrichment score (EScore).

3.4. DC and EScore of PPARA. Ranked by DC, PPARA is the No. 13 in 83 CLL promoters, as shown in Figure 4(a). The results suggest that PPARA presents a tighter connection to CLL and TFS-related gene markers than most (>84%) of the previously reported CLL promoters, indicating the significance of PPARA as a treatment target for CLL.

Ranked by EScore, PPARA is indexed as No. 57 out of 83 CLL promoters, as shown in Figure 4(b). The relatively low EScore indicated that the role of PPARA influencing CLL TFS might be mainly focusing on a limited number of functional areas, such as regulation of leukocyte cell–cell adhesion and inflammatory response, as shown in Figure 4. To note, a high EScore signifies that the gene participates in a broader range of pathways, whereas a lower EScore suggests that the gene is more concentrated on specific functional areas. Its importance is determined by not only how many

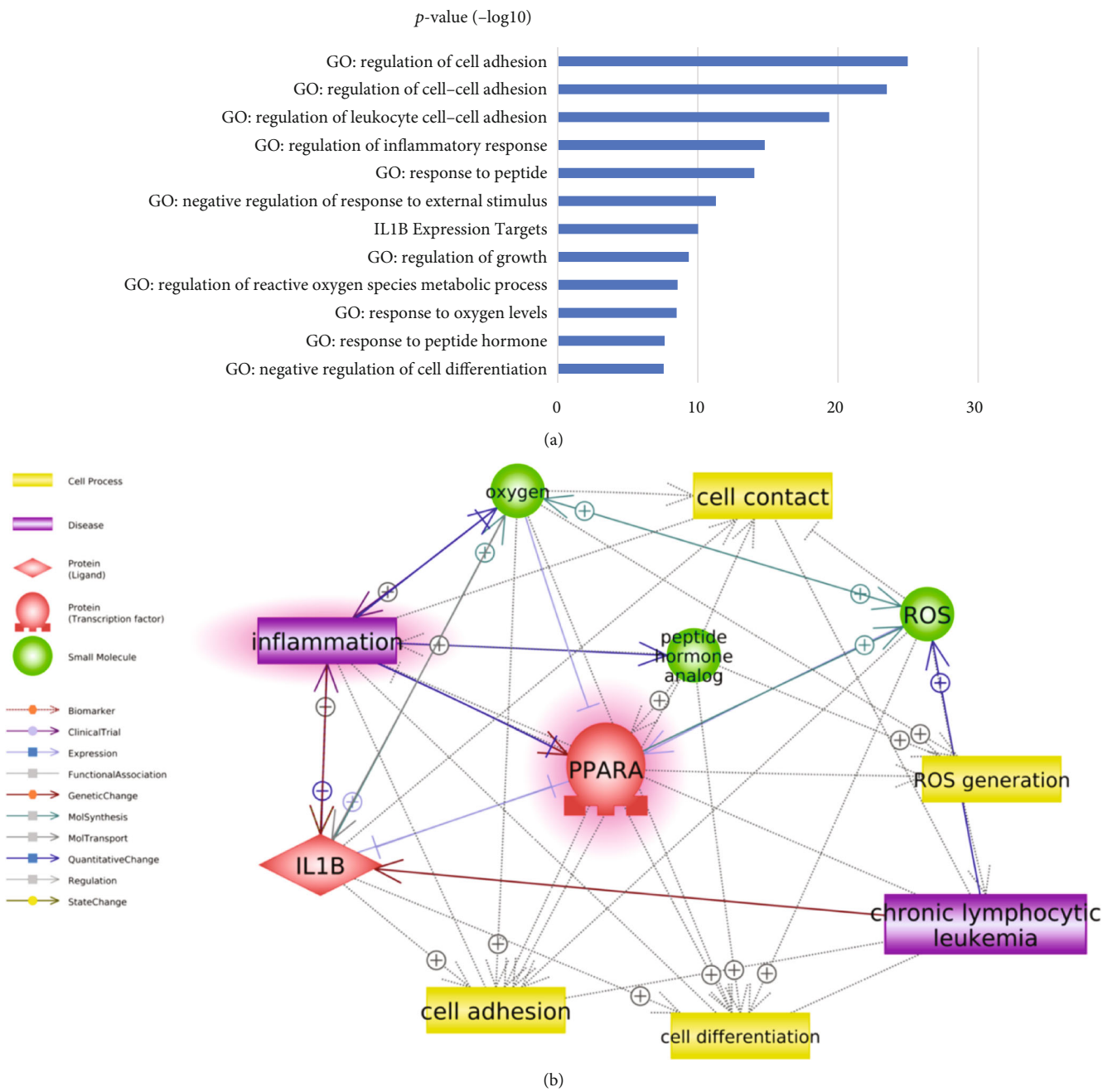

Figure 5: Functional connection between PPARA and chronic lymphocytic leukemia (CLL). (a) The significantly enriched pathways/GO terms involving PPARA; (b) corresponding literature-based pathways connecting PPARA and CLL.

pathways are involved but also how crucial these biological pathways are to CLL. Our investigation revealed that the majority of the pathways enriched by PPARA are crucial in the pathological development of CLL, such as cell adhesion, cell differentiation, and inflammation (as illustrated in Figure 5(b)). Figure 5 illustrates the role of PPARA in terms of the pathways it is involved in. Specifically, Figure 5(a) depicts the pathways that involve PPARA, while Figure 5(b) shows how these pathways (functional groups) are related to PPARA and CLL, providing a comprehensive overview of the role of PPARA in CLL. It is also worth mentioning that PPAR was functionally linked with most of these 83 genes (70/83 = 84.34%) to play roles in these pathways associated with the pathology of CLL.

4. Discussion

Numerous investigations utilizing animal models and human cell lines have explored the link and function of PPARA in CLL. The findings indicate that PPARA promotes the development of CLL, and thus, targeting PPARA gene regulation may serve as a potential therapeutic strategy for managing CLL [10–12, 22]. PPARA was found to be expressed by circulating CLL cells and highly associated with advanced-stage of

CLL [11]. In 2015, Messmer et al. developed a PPARA antagonist, NXT629, which inhibits agonist-induced transcription of PPARA-regulated genes, demonstrating target engagement in CLL cells [12]. Another inhibitor of PPARA, MK886, has also been shown to kill CLL cells [23]. However, the molecular mechanism of PPARA as a treatment target for CLL remains unclear.

Meanwhile, dozens of genes have also been suggested as CLL promoters or treatment targets in past years, and PPARA is one of them. According to its DC value, which indicates the significance of a vertex within a network, PPARA ranks 13th among 83 CLL promoters (as shown in Figure 4(a)). The result indicated that PPARA is more closely related to CLL and its TFS-related gene markers than most CLL promoter genes. Moreover, PPARA co-functions with most other CLL promoters in multiple pathways/gene groups related to the pathology of CLL. Our results support the importance of PPARA as a treatment target for CLL.

To explore the influence of PPARA in the prognosis and treatment of CLL at the molecular level, we first identified the CLL TFS-related gene markers by analyzing DNA–NGS data and clinical data of 86 CLL patients. Our results showed that mutations of four genes (EGR2, FBXW7, RPS15, and FOXO1) were directly related to shorter TFS, and six genes (NOTCH1, ATM, GNA12, KDM6A, KMT2A, and FBXW7) were linked to four clinical factors associated with shorter TFS, including CLL-IPI, del(11q), splenomegaly, and newly diagnosed platelets $<100 \times 10^9$/L. Moreover, mutations of two genes (EGR2 and GNA13) were associated with Richter's transformation of CLL. Altogether, our data suggested 10 CLL TFS-related gene markers. Interestingly, 6 out of these 10 CLL TFS-related gene markers have been implicated with CLL in previous studies (Figure 3), and 1 gene (NOTCH1) has also been reported as a CLL promoter in previous studies [23]. These results support the validity of the clinical/NGS data analysis.

Literature data mining also revealed 83 CLL promoters, which include PPARA. Out of these 83 genes, 69 genes present more or less linkage to the 10 CLL TFS-related gene markers, supporting their role in the prognosis and treatment of CLL. To note, ranked by DC, PPARA is superior to most of these genes by indexing No. 13. These results suggested the significance of PPARA as a treatment target for CLL.

Enrichment analysis showed that 91 out of 92 genes (CLL promoters and CLL TFS-related gene markers) were significantly enriched within 152 pathways/gene sets (False Discovery Rate (FDR) corrected p-value$<3.4 \times 10^{-8}$). Out of these pathways, PPARA was enriched within 12 pathways (Figure 5), and three of them were within the top 10 out of 152 pathways (FDR corrected p-value$<1.03 \times 10^{-19}$), which are linked to cell adhesion in general and leukocyte cell–cell adhesion. However, PPARA was not involved in the positive regulation of cell adhesion and cell migration, suggesting that PPARA might be involved in the negative cell contact and migration within the CLL pathology to influence the prognosis of CLL. It has been shown that CLL B cells induce alteration in cytoskeleton formation and vesicle transportation pathways in T cells by cell–cell contact [24], leading to T-cell dysfunction [25]. PPARA expression was found to be elevated in CLL patients and associated with an advanced disease stage [26]. There is evidence that activation of PPARA and PPARG has anti-inflammatory and immunomodulatory effects. Their agonists, such as troglitazone, inhibit the release of inflammatory cytokines from monocytes and induce apoptosis of T-lymphocytes [27].

The pathways where PPARA was enriched also include inflammation regulation, regulation of reactive oxygen species, regulation of cell differentiation, and their related pathways and gene groups (IL1B expression, peptide hormone, and oxygen response). All these pathways have been implicated in previous studies to play essential roles in the pathology of CLL [28–30]. Our findings indicate a potential association between PPARA and the prognosis of CLL, along with several possible underlying mechanisms.

It is worth pointing out that, together with PPARA, 70 out of the 92 CLL promoters and TFS-related genes (entities in the network presented in Figure 3) were enriched in these significant pathways mentioned above. Our results indicated that PPARA functionally collaborated with a large number of genetic markers to promote CLL. Other pathways not involving PPARA (140/153) may reflect the complex nature of CLL, and the relatively low EScore of PPARA suggests that the PPARA only plays roles in a piece of the whole pathology mechanism of CLL.

This study has several limitations. First, the genetic markers related to TFS length were identified through the analysis of NGS data from only 72 genes, which are used for routine screening of clinical CLL. Therefore, it is necessary to test more genes to explore CLL TFS length-related genetic markers thoroughly. Next, the regulation network of CLL TFS was constructed by integrating the findings of various independent studies. To confirm the accuracy of the network, it is recommended that all of the genetic markers be tested in the same batch of experiments.

5. Conclusion

Our findings reinforce the notion that PPARA is a crucial treatment target for CLL that is strongly linked to its TFS. PPARA collaborates with a considerable number of genetic markers and impacts the prognosis and TFS of CLL through several essential pathways, such as the regulation of cell adhesion, inflammation, reactive oxygen species, and cell differentiation.

Data Availability

The data of this study are available from the corresponding author upon reasonable request.

Ethical Approval

This study was reviewed by the ethics committee of Xinqiao Hospital of Army Medical University (2020-research No. 128-01).

Consent

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Materials. Supplementary Figure 1 presents the spectrum of gene mutations of 86 CLL patients.

Supplementary Materials. Supplementary Table 1 provides the results from univariate analysis and multivariate analysis of prognostic factor of chronic lymphocytic leukemia.

Supplementary Materials. Supplementary Table 2 outlines the underlying information of each relationship presented in Figure 3, including the relation type, relation direction, source node, and target node.

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