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Aberrant Hematopoiesis and CD8$^+$ T-Cell Activation in Thymoma-Associated Pure Red Cell Aplasia

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ABSTRACT

Background: Thymoma-associated pure red cell aplasia (PRCA) is a rare autoimmune disorder characterized by selective erythroid lineage suppression. However, the underlying immune mechanisms remain unclear.

Methods: We performed single-cell RNA sequencing (scRNA-seq) on bone marrow cells from thymoma-PRCA patients and healthy controls to analyze hematopoietic cell populations. Additionally, we conducted bulk RNA sequencing of peripheral blood CD8 + T cells, flow cytometry analysis of CD8 + T-cell activation, and cytokine profiling of bone marrow supernatant.

Results: scRNA-seq revealed a significant reduction in erythroid progenitors (BFU-E, CFU-E, erythroblasts) and an increase in granulocyte-monocyte progenitors (GMP) in thymoma-PRCA patients. Differential gene expression analysis showed upregulation of *TMSB10*, *AREG*, and *SPN*, which are involved in immune modulation and T-cell activation. Bulk RNA sequencing of CD8 + T cells indicated enhanced expression of activation markers (*TNFRSF9, CTLA4, IRF4, CD38, MTHFD2*) and decreased expression of erythroid-related genes (*HBA1, HBA2, HBB*). Flow cytometry confirmed an increased CD8 + T-cell population in the bone marrow, with elevated levels of perforin, granzyme B, IFN-γ, and TNF-α. Cytokine analysis further demonstrated increased IFN-γ and TNF-α levels in the bone marrow microenvironment.

Conclusion: Thymoma-PRCA is associated with excessive CD8 + T-cell activation and an inflammatory bone marrow environment, leading to impaired erythropoiesis. These findings provide novel insights into the immune dysregulation underlying thymoma-associated PRCA and may help identify potential therapeutic targets.

1 | Introduction

Thymoma is a tumor originating from thymic epithelial cells, primarily occurring in the anterior mediastinum. While most thymomas grow slowly and are considered low-grade malignancies, some can be invasive. Approximately 30%–50% of patients are asymptomatic, while others may experience symptoms such as dyspnea or facial swelling due to tumor compression of the trachea or superior vena cava. In addition to these mechanical effects, thymoma is strongly associated

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with autoimmune diseases, particularly myasthenia gravis (MG) [1, 2].

Thymoma-related autoimmune diseases include MG, which affects 30%–50% of patients and results from autoantibodies targeting acetylcholine receptors at the neuromuscular junction [2]. Other associated conditions include pure red cell aplasia (PRCA) [3, 4], in which abnormal T cells suppress erythropoiesis, and Good syndrome [5], characterized by hypogammaglobulinemia due to impaired B-cell function. Additionally, thymoma has been linked to autoimmune hepatitis (AIH), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA), likely due to defective T-cell selection leading to autoimmune dysregulation [6].

PRCA is a rare bone marrow disorder characterized by the selective absence of red blood cell precursors, resulting in severe anemia while white blood cells and platelets remain unaffected. Among its various causes, autoimmune conditions, particularly thymoma, play a critical role [7, 8].

The mechanisms underlying autoimmune diseases in thymoma primarily involve abnormal T-cell development, aberrant antigen expression by thymic epithelial cells, and dysregulated B-cell function. Thymoma disrupts T-cell selection, allowing autoreactive T cells to escape into circulation and attack self-tissues. Additionally, thymic epithelial cells may abnormally express self-antigens, triggering autoantibody production. Furthermore, T-cell dysfunction can lead to impaired B-cell regulation, resulting in excessive autoantibody production and exacerbation of autoimmune responses [9–11].

In this study, we aim to investigate the mechanism of thymoma-associated PRCA through a multi-omics approach. Specifically, we will perform single-cell RNA sequencing on bone marrow cells from PRCA patients, sort CD8 + T cells from peripheral blood for bulk RNA sequencing, and analyze CD8 + T-cell activation using flow cytometry. These findings will provide valuable insights into the role of immune cells, particularly T cells, in the pathogenesis of PRCA in thymoma patients, potentially guiding future therapeutic strategies.

2 | Materials and Methods

2.1 | Single-Cell Data Processing and Quality Control

We collected bone marrow mononuclear cells (BMMNCs) from one patient with PRCA-thymoma and two healthy donors. Lin-cells were sorted from BMMNCs aspirates by immunomagnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) for single-cell RNA sequencing (scRNA-seq). Lin-cells were resuspended in phosphate-buffered saline (PBS) at the appropriate concentration and then loaded into the 10X Chromium Controller (10X Genomics) for barcoding. RNA from the barcoded cells was reverse-transcribed, and sequencing libraries were prepared using reagents from the Chromium Single Cell 3 v3 reagent kit (10X Genomics). Sequencing was carried out using the Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA). Cells with unique feature counts greater than 3500 or less than 200, or with mitochondrial counts exceeding 25%, were excluded due to low quality.

2.2 | Unsupervised Dimensionality Reduction

Dimensionality reduction, clustering, and visualization of scRNA-Seq data using Seurat v4 in R 4.4.0. Specifically, the raw data is first normalized using the NormalizeData function. Then, the data is normalized using the ScaleData function, and PCA is reduced. Next, the harmony function is used to remove the batch effect from the data. Recluster the cells using the FindNeighbors and FindClusters functions, and adjust the resolution range to 0.5 to identify finer subclusters. Potential marker genes are identified by the FindAllMarkers function, followed by UMAP (Uniform Manifold Approximation and Projection) analysis to visualize the clustering results. According to the typical marker genes, the cell clusters were annotated into 8 groups.

2.3 | Pseudotime Trajectory Analysis

Pseudotime analysis to determine the differentiation trajectory of cell development was performed using Monocle2. First, the UMI matrix was extracted from the Seurat object, and the newCellDataSet function was used to create the object. Genes with a mean expression greater than 0.1 were selected for trajectory analysis. This was followed by dimensionality reduction using the DDRTree method and cell ordering through the orderCells function.

2.4 | Identification and Visualization of Differentially Expressed Genes (DEGs)

The data of three thymoma-PRCA patients were analyzed for using the "limma" software package with five controls, respectively. DEGs were screened for p value <0.05 and $|\text{Log2FoldChange}|>1$. Next, "ggplot2" was utilized to plot the volcano map of DEGs to look for meaningful genes.

2.5 | GO and KEGG Analyses

GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses were performed using the "clusterProfiler" software package, with a threshold of $p<0.05$, to further investigate the most significant biological processes (BPs) and enrichment pathways of DEGs.

2.6 | Immunoinfiltration Analysis

We performed FPKM transformation of bulk data from three PRCA patients and five controls and used single-sample gene set enrichment analysis (ssGSEA) to estimate the relative abundance of different immune cell types in each sample. Specifically, this method estimates the relative enrichment of the gene set in the sample by comparing the gene expression data of each sample

with the immune cell gene set (http://cis.hku.hk/TISIDB/data/download/CellReports.txt).

2.7 | Sorting and RNA Sequencing of Bone Marrow CD8$^+$ T Lymphocytes

Bone marrow CD8 + T cells were isolated using immune magnetic bead sorting, followed by RNA sequencing to investigate gene expression profiles. For detailed methods, please refer to the references [12].

2.8 | CD3 + CD8 + Lymphocytes With Flow Cytometric Analysis

The thymoma-associated PRCA group enrolled five patients in our department, including two males and three females, with a median age of 59 (range: 52–75) years. Among them, there were three patients with newly diagnosed PRCA (ND-PRCA) and two patients with refractory PRCA (R-PRCA). The control group consisted of five healthy controls (two males and three females) with a median age of 45 (25–65) years.

Heparin anticoagulant sterile tube was used to collect bone marrow aspirates 2 mL from thymoma-associated PRCA patients and healthy controls. A volume of 100 μL of fresh sample was stained for 15 min in the dark at room temperature with the following antibodies, CD3-Percp (Biolegend, USA) and CD8-FITC (Biolegend, USA). Next, after red blood cell lysis, cells were washed with PBS. Data acquisition and analysis were performed using a FACSCalibur flow cytometer (BD Biosciences, USA) and Cell Quest software (Becton Dickinson, version 3.1). The SSC and FSC of cells were used to divide cells into three subgroups, namely, lymphocytes, monocytes, and granulocytes. The lymphocytes were gated. CD3 positive cells were defined as T lymphocytes. CD8 + CD3 + cells were defined as cytotoxic T lymphocytes. The number of CD8 + lymphocytes was measured by FCM assay.

2.9 | Perforin and Granzyme B of CD8 + Lymphocytes With Flow Cytometric Analysis

Perforin and granzyme B secreted by CD8 + T cells were measured using perforin-PE and granzyme-B APC monoclonal antibodies by the FACS Calibur flow cytometer (BD Biosciences, USA).

2.10 | Determination of IFN-γ and TNF-α Expression Levels in Bone Marrow Supernatant by ELISA

Bone marrow aspirates from both thymoma-associated PRCA patients and healthy controls were collected in EDTA anticoagulation tubes, and the supernatants were carefully extracted and subsequently frozen for analysis. The concentrations of IFN-γ and TNF-α were determined following the instructions provided by the ELISA commercial kit (Elabscience Biotechnology, China).

2.11 | Real-Time Quantitative Transcriptase-Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted using TRIzol reagent (Thermo Fisher, USA), and cDNA was generated using a reverse transcriptase kit (TIANGEN, China). The gene expressions were quantified by qPCR (2X SYBR Green qPCR Master Mix, Takara Bio, USA). The primer sequences were as follows: Perforin forward 5′-GACTGCCTGACTGTCGAGG-3′, reverse 5′-TCCCGGTAGGTTTGGTGGAA-3′; Granzyme forward 5′-TACCATTGAGTTGTGCGTGGG-3′, reverse 5′-GCCATTGTTTCGTCCATAGGAGA-3′; IFN-γ forward 5′-TCGGTAACTGACTTGAATGTCCA-3′, reverse 5′-TCGCTTCCCTGTTTTAGCTGC-3′; TNF-α forward 5′-GAGGCCAAGCCCTGGTATG-3′, reverse 5’-CGGGCCGATTGATCTCAGC; β-actin forward 5′-CCTGGCACCCAGCACAAT-3′, reverse 5′-GGGCCGGACTCGTCATAC-3′. To generate the relative quantification (RQ) of the gene expression, the $2^{-\Delta\Delta Ct}$ method was used: $\Delta\Delta Ct = (Ct_{target} - Ct_{\beta\text{-actin}})$ patients-$(Ct_{target} - Ct_{\beta\text{-actin}})$ controls.

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of Tianjin Medical University General Hospital (No. IRB2022-KY-128). Informed consent was obtained from all individual participants involved in the study.

2.12 | Statistical Analysis

All data were analyzed using GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA) and are presented as mean ± standard deviation. Comparisons between two groups were performed using the Mann–Whitney *U* test, while comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA). Spearman rank correlation was used for correlation analyses. A p value of < 0.05 was considered statistically significant.

3 | Results

3.1 | Single-Cell Sequencing Reveals Altered Hematopoietic Cell Populations in Thymoma-PRCA

We selected Lin$^-$ sorted BMMNCs from one thymoma-PRCA patient and two healthy donors for single-cell RNA sequencing (Figure 1A). After quality control and batch correction, we performed dimensionality reduction and clustering, classifying the cells into eight distinct populations: hematopoietic stem and progenitor cells (HSPC), common myeloid progenitors (CMP), common lymphoid progenitors (CLP), granulocyte-monocyte progenitors (GMP), megakaryocyte-erythroid progenitors (MEP), burst-forming unit-erythroid (BFU-E), colony-forming unit-erythroid (CFU-E), and erythroblasts (Figure 1B). A bubble plot was used to illustrate the key markers associated with each cell population (Figure 1C).

Bubble stacking plot analysis, supported by Propeller software calculations, showed a significant reduction in BFU-E, CFU-E, and erythroblast populations, whereas GMP cells were notably increased in thymoma-PRCA bone marrow. Interestingly,

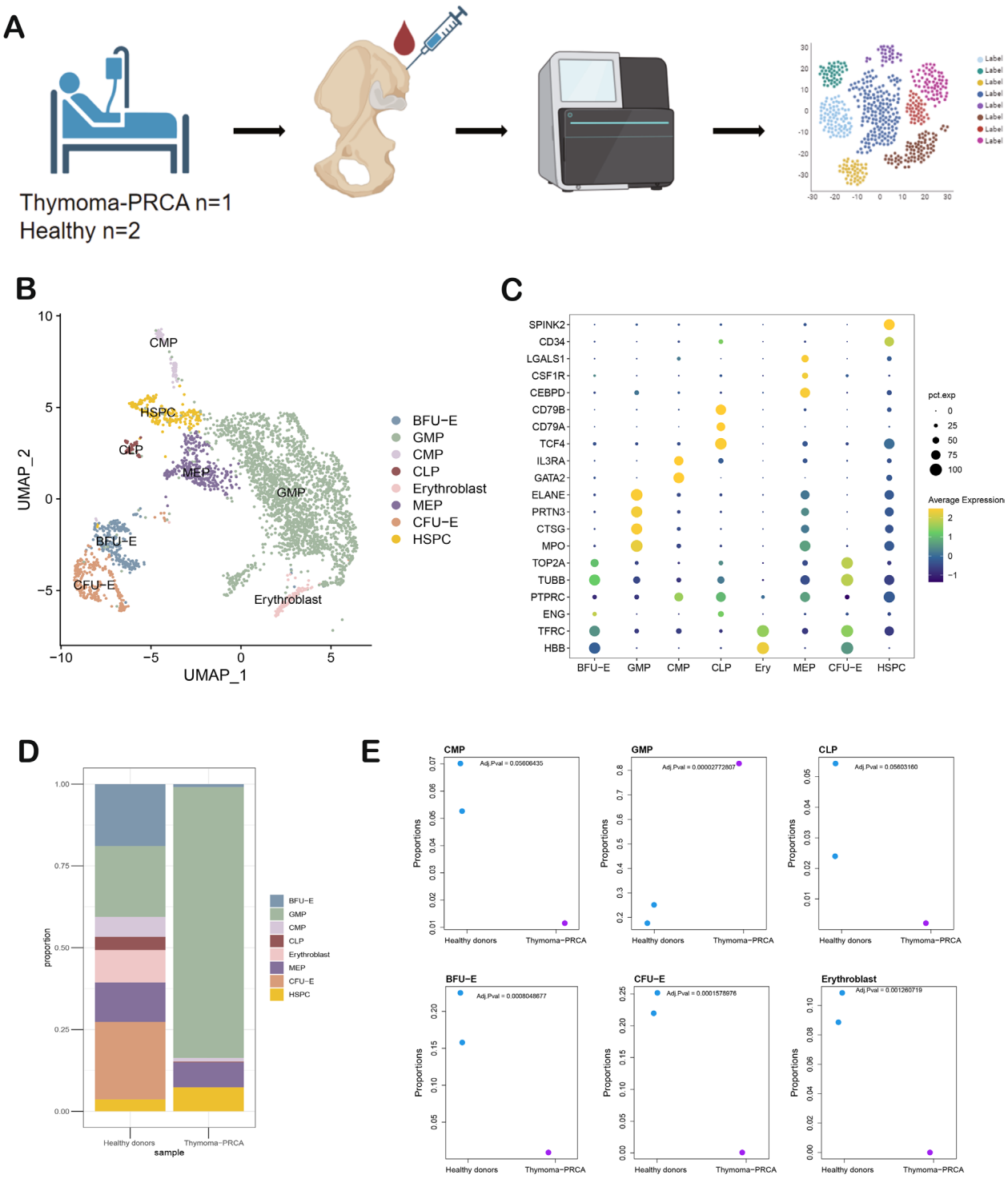

FIGURE 1 | Single-cell RNA sequencing reveals hematopoietic alterations in thymoma-associated PRCA. (A) Single-cell RNA sequencing (scRNA-seq) analysis of bone marrow mononuclear cells from a thymoma-associated PRCA patient and healthy controls. (B) UMAP visualization showing eight distinct cell populations, including hematopoietic stem and progenitor cells (HSPC), common myeloid progenitors (CMP), common lymphoid progenitors (CLP), GMP, megakaryocyte-erythroid progenitors (MEP), burst-forming unit-erythroid (BFU-E), colony-forming unit-erythroid (CFU-E), and erythroblasts. (C) Bubble plot illustrating key marker genes for each cell population. (D) Stacked bar plot showing the relative proportion of different cell types in PRCA patients and healthy controls. (E) Statistical analysis of significant differences in cell population proportions, highlighting reduced erythroid progenitors and increased GMPs in PRCA patients.

HSPC cells did not decrease significantly; instead, they exhibited an increasing trend, along with CMP and CLP populations (Figure 1D,E).

3.2 | Key Molecular Alterations and Enriched Pathways in Thymoma-PRCA

Differential gene analysis revealed that TMSB10 (thymosin beta 10) was significantly overexpressed across most cell populations (Figure 2A). This gene has been implicated in tumor cell proliferation, apoptosis, metastasis, and angiogenesis, and its overexpression is associated with a poor prognosis in certain cancers.

In the CLP and GMP populations, diagonal scatterplots of DEGs based on percentage differences showed that AREG (Amphiregulin) and SPN (sialophorin) were highly expressed (Figure 2B). AREG plays an immunomodulatory role at the interface between antigen-presenting cells and T cells, while SPN is crucial for antigen-specific T cell activation.

Pseudotime analysis using Monocle2 demonstrated a dynamic expression pattern of AREG and SPN, where their expression levels first increased, then decreased, and subsequently rose again during cell development. Notably, their expression in thymoma-PRCA patients remained consistently higher than in healthy controls throughout the developmental trajectory (Figure 2C).

Finally, GSEA enrichment analysis of each cell population indicated that most cell populations were enriched in the oxidative phosphorylation (OXIDATIVE_PHOSPHORYLATION) and TNF-α signaling via NF-κB (TNFA_SIGNALING_VIA_NFKB) pathways. Additionally, the interferon (IFN)-α and IFN-γ signaling pathways were upregulated in the BFU-E and CFU-E populations (Figure 2D).

3.3 | Transcriptomic Profiling of CD8 + T Cells in Thymoma-PRCA

We selected three thymoma-PRCA patients and five healthy controls, sorted CD8 + T cells from peripheral blood, and performed bulk RNA sequencing. Compared to the control group, Thymoma-PRCA patients exhibited elevated expression of genes related to T-cell regulation and activation, such as TNFRSF9, CTLA4, IRF4, CD38, and MTHFD2, while genes related to erythrocytes, such as HBA1, HBA2, and HBB, showed decreased expression (Figure 3A).

KEGG enrichment analysis revealed that the highly expressed genes in the thymoma–PRCA group were primarily enriched in pathways including PI3K-Akt, mTOR, TNF, TGF-beta, and T-cell receptor signaling (Figure 3B).

GO enrichment analysis indicated that the highly expressed genes in the thymoma-PRCA group were predominantly enriched in BPs related to the regulation of T-cell activation and T-cell proliferation (Figure 3C).

Using ssGSEA for immune infiltration analysis, we observed an increasing trend in activated CD8 + T cells in the PRCA patient group, while central memory CD8 + T cells and effector memory CD8 + T cells showed a decreasing trend (Figure 3D).

3.4 | Elevated CD8 + T-Cell Activity and Inflammatory Cytokines in the Bone Marrow of Thymoma-PRCA Patients

To validate the results of single-cell RNA sequencing and CD8 + T-cell sorting, we further examined the bone marrow CD8 + T-cell count in PRCA patients using flow cytometry. Our findings revealed a significant increase in the number of CD8 + T cells in the bone marrow of PRCA patients compared to healthy controls (Figure 4A).

Next, we assessed the levels of perforin and granzyme B in CD8 + T cells and found that both were markedly elevated in PRCA patients (Figure 4B). Further gene expression analysis of sorted CD8 + T cells confirmed a significant upregulation of perforin, granzyme B, IFN-γ, and tumor necrosis factor-α (TNF-α) in the bone marrow of PRCA patients (Figure 4C).

Cytokine analysis of bone marrow supernatant further confirmed an elevated inflammatory milieu in PRCA patients, as evidenced by significantly increased levels of IFN-γ and TNF-α (Figure 4D).

4 | Discussion

Single-cell RNA sequencing has provided valuable insights into the disrupted hematopoiesis in thymoma-PRCA. Our findings revealed a significant reduction in BFU-E, CFU-E, and erythroblast populations, suggesting a failure in erythroid lineage commitment and differentiation. This is consistent with previous studies describing PRCA as a condition where erythroid cells are selectively suppressed due to aberrant immune responses [13, 14]. The notable expansion of GMPs points toward a shift in hematopoiesis toward myeloid differentiation, potentially driven by the inflammatory environment in the bone marrow. Interestingly, we observed an increasing trend in HSPCs, CMPs, and CLPs, which could reflect compensatory hematopoietic responses or impaired lineage commitment.

In terms of molecular mechanisms, differential gene expression analysis highlighted TMSB10 (thymosin beta 10) as significantly upregulated across various cell populations. TMSB10 has been implicated in tumor progression, immune modulation, and angiogenesis [15, 16], and its elevated expression in thymoma-PRCA may contribute to the aberrant immune activity and hematopoietic suppression observed in these patients. Additionally, high expression of AREG (amphiregulin) and SPN (sialophorin) was found in the CLP and GMP populations. AREG plays an important role in the immunomodulation between antigen-presenting cells and T cells [17], while SPN is involved in antigen-specific T-cell activation [18]. The persistent upregulation of these genes in PRCA patients suggests that abnormal immune signaling may be a key factor in erythroid suppression.

GSEA further revealed the activation of inflammatory and metabolic pathways, including oxidative phosphorylation and

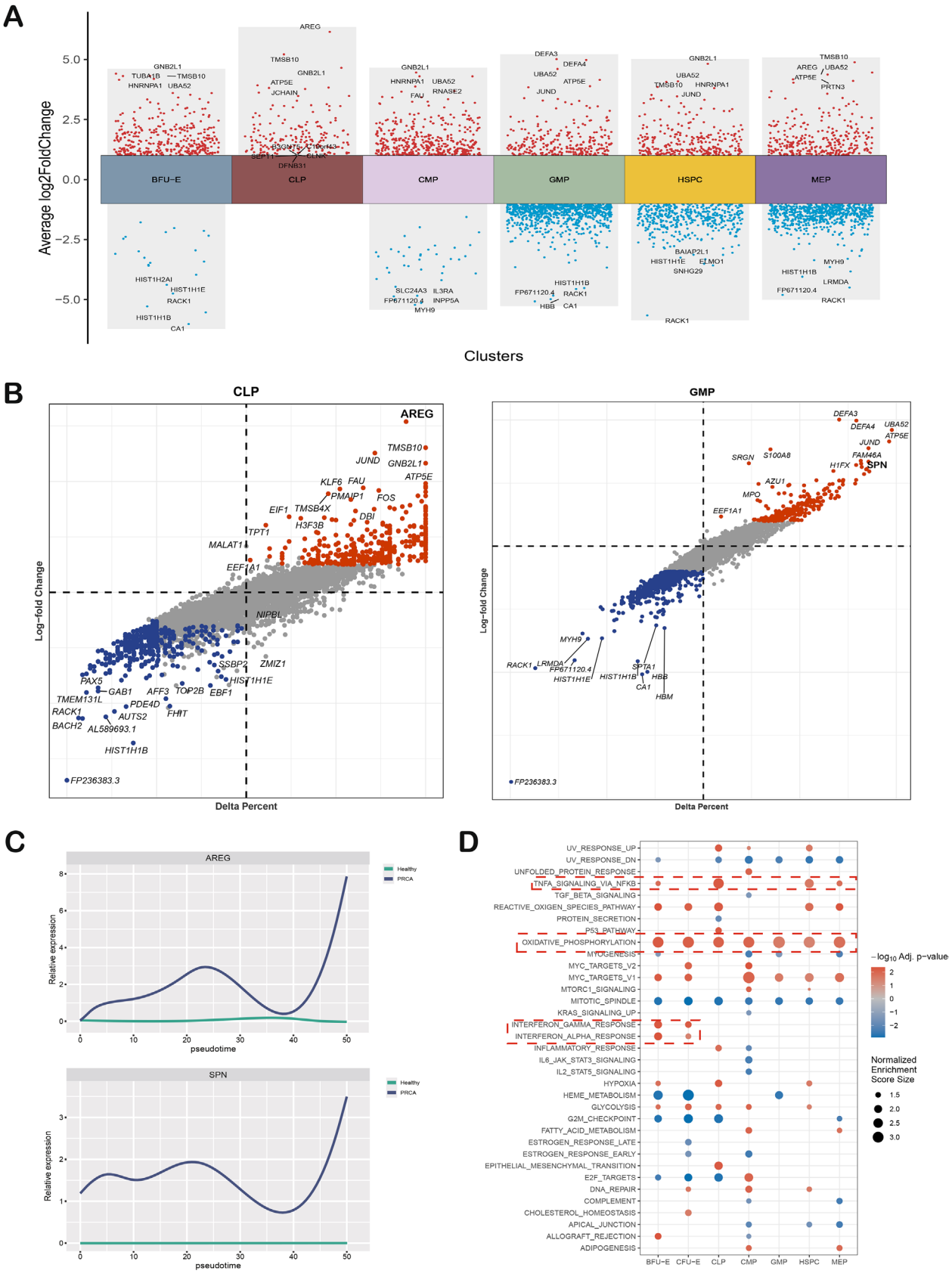

FIGURE 2 | Legend on next page.

FIGURE 2 | Differentially expressed genes (DEGs) and pathway enrichment analysis in thymoma-associated PRCA. (A) Manhattan plot showing DEGs across different cell populations, with significantly upregulated and downregulated genes in PRCA patients. Only $\log_2$FoldChange($\log_2$FC) > 1 and $p < 0.01$ points are displayed. Visualize the top 5 genes in the up-down and downregulated genes. (B) Diagonal scatter plots displaying DEGs in common lymphoid progenitors (CLP) and granulocyte-monocyte progenitors (GMP). The abscissa is used for the pct difference, the ordinate is used for $\log_2$FC, red for upregulated genes, and blue for downregulated genes. (C) Pseudotime trajectory analysis using monocle2 demonstrates altered gene expression patterns of AREG and SPN genes during the differentiation process in PRCA patients. (D) Gene set enrichment analysis (GSEA) indicating upregulation of oxidative phosphorylation, TNF-α/NF-κB signaling, and interferon pathways in PRCA bone marrow cells.

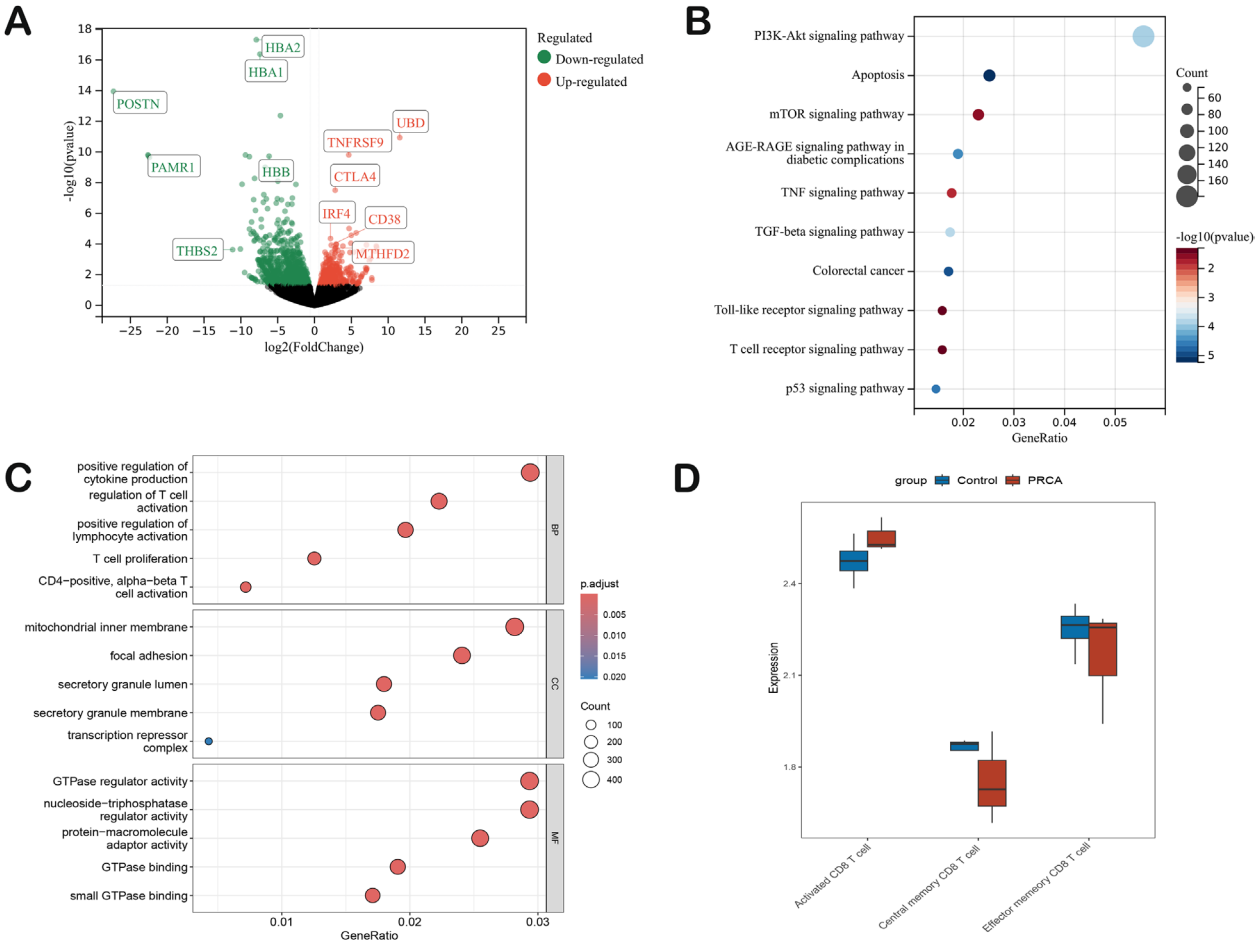

FIGURE 3 | Bulk RNA sequencing and immune infiltration analysis of CD8 + T cells in PRCA patients and controls. (A) Volcano plot of Differentially expressed genes (DEGs) of CD8 + T cells in three patients with thymoma-PRCA and five controls. p value < 0.05 and |$\log_2$FoldChange| > 1 were identified as significant DEGs. The red dots represent the upregulated genes, and the green dots represent the downregulated genes. (B) KEGG pathway analysis showing enrichment in PI3K-Akt, mTOR, TNF, and T-cell receptor signaling pathways. (C) GO enrichment analysis revealing biological processes associated with T-cell activation and proliferation. (D) Boxplot to visualize the expression of CD8 + T-related immune cells in thymoma-PRCA and controls.

TNF-α signaling via NF-κB. Notably, the upregulation of IFN-α and IFN-γ signaling pathways in the BFU-E and CFU-E populations points to the potential role of excessive inflammatory signaling in directly impairing the survival and differentiation of erythroid progenitors.

Bulk RNA sequencing of peripheral blood CD8 + T cells confirmed significant upregulation of genes related to T-cell activation, such as TNFRSF9, CTLA4, IRF4, and CD38. These genes are associated with T-cell proliferation, immune regulation, and exhaustion, suggesting that CD8 + T cells in PRCA patients exhibit an activated phenotype, potentially contributing to the autoimmune process [19]. Additionally, downregulation of erythroid-related genes, such as HBA1, HBA2, and HBB, reinforces the concept that immune dysregulation plays a crucial role in erythroid suppression in thymoma-PRCA.

KEGG and GO enrichment analyses of the upregulated genes in PRCA patients revealed significant enrichment in pathways related to immune activation and inflammation, including PI3K-Akt, mTOR, TNF, TGF-β, and T-cell receptor signaling. These pathways suggest a complex interaction between immune activation, inflammatory responses, and hematopoietic suppression. Furthermore, the increase in activated CD8 + T cells in

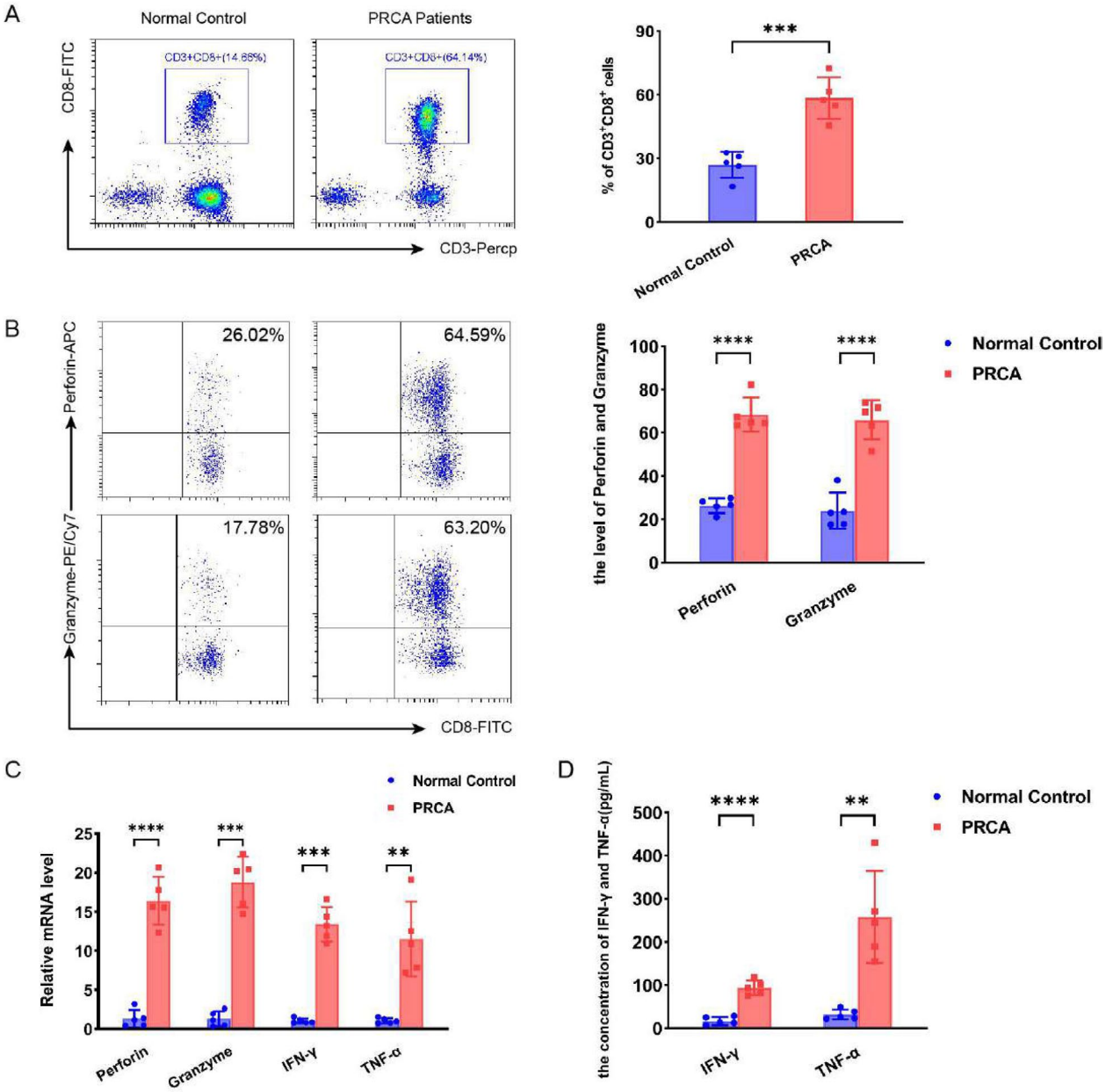

FIGURE 4 | The increased proportion and enhanced killing function of $CD8^+$ T cells was observed in thymoma-associated PRCA patients. The proportion of $CD8^+CD3^+$T lymphocytes (A), and the expression of perforin and granzyme (B) in $CD8^+$ T lymphocytes from bone marrow aspirates of thymoma-associated PRCA patients ($n = 5$) and controls ($n = 5$). (C) Comparison of mRNA levels of perforin, granzyme, IFN-γ, and TNF-α between thymoma-associated PRCA patients ($n = 5$) and controls ($n = 5$). (D) Comparison of IFN-γ and TNF-α levels in bone marrow supernatants using ELISA. $^{****}p < 0.0001$; $^{***}p < 0.001$; $^{**}p < 0.01$.

thymoma-PRCA patients highlights the potential role of dysregulated T-cell subsets in driving autoimmunity and bone marrow dysfunction [12].

Flow cytometry analysis of bone marrow samples confirmed a significant increase in $CD8^+$ T cells [9], with elevated levels of perforin and granzyme B, both key mediators of cytotoxicity. This suggests that CD8 + T cells may directly contribute to the immune-mediated destruction of erythroid progenitors. Additionally, cytokine analysis of the bone marrow supernatant revealed significantly elevated levels of IFN-γ and TNF-α, further supporting the presence of a proinflammatory microenvironment that could exacerbate erythropoietic suppression.

In conclusion, our study provides new insights into the immune-mediated mechanisms underlying thymoma-PRCA, highlighting the dysregulation of hematopoiesis, aberrant T-cell activation, and an inflammatory bone marrow microenvironment. These findings suggest potential therapeutic targets and underline the importance of targeting immune-mediated processes in the treatment of PRCA. Future studies with larger patient cohorts are necessary to validate these results and explore targeted therapeutic strategies for thymoma-PRCA.

Author Contributions

H.W. and H.N. conceived and designed the study, collected, assembled, analyzed, and interpreted data, and wrote the manuscript. M.L. and X.D. contributed patients, analyzed and interpreted data, and wrote the manuscript. H.Z. and X.H. contributed patients, analyzed and interpreted data. Y.L., L.Y., Y.W., Y.L., and P.T. contributed patients and interpreted data. All authors read and approved the manuscript.

Ethics Statement

This study received approval from the ethics committee of our hospital, and informed consent was obtained from each patient according to the Helsinki Declaration.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

1. F. C. Detterbeck and A. Zeeshan, "Thymoma: Current Diagnosis and Treatment," *Chinese Medical Journal* 126, no. 11 (2013): 2186–2191.

2. E. A. Engels, "Epidemiology of Thymoma and Associated Malignancies," *Journal of Thoracic Oncology* 5, no. 10 Suppl 4 (2010): S260–S265.

3. S. Moriyama, M. Yano, H. Haneda, et al., "Pure Red Cell Aplasia Associated With Thymoma: A Report of a Single-Center Experience," *Journal of Thoracic Disease* 10, no. 8 (2018): 5066–5072.

4. T. Murakawa, J. Nakajima, H. Sato, M. Tanaka, S. Takamoto, and M. Fukayama, "Thymoma Associated With Pure Red-Cell Aplasia: Clinical Features and Prognosis," *Asian Cardiovascular & Thoracic Annals* 10, no. 2 (2002): 150–154.

5. M. Zaman, A. Huissoon, M. Buckland, et al., "Clinical and Laboratory Features of Seventy-Eight UK Patients With Good's Syndrome (Thymoma and Hypogammaglobulinaemia)," *Clinical and Experimental Immunology* 195, no. 1 (2019): 132–138.

6. N. Noël, A. Le Roy, A. Hot, et al., "Systemic Lupus Erythematosus Associated With Thymoma: A Fifteen-Year Observational Study in France," *Autoimmunity Reviews* 19, no. 3 (2020): 102464.

7. P. Fisch, R. Handgretinger, and H. E. Schaefer, "Pure Red Cell Aplasia," *British Journal of Haematology* 111, no. 4 (2000): 1010–1022.

8. M. Liu, T. Zhang, X. Dong, and H. Wang, "Acquired Pure Red Cell Aplasia: Unraveling the Immune Pathogenesis," *Journal of Bio-X Research* 6, no. 4 (2023): 138–148.

9. V. Hoffacker, A. Schultz, J. J. Tiesinga, et al., "Thymomas Alter the T-Cell Subset Composition in the Blood: A Potential Mechanism for Thymoma-Associated Autoimmune Disease," *Blood* 96, no. 12 (2000): 3872–3879.

10. C. Bernard, H. Frih, F. Pasquet, et al., "Thymoma Associated With Autoimmune Diseases: 85 Cases and Literature Review," *Autoimmunity Reviews* 15, no. 1 (2016): 82–92.

11. S. Shelly, N. Agmon-Levin, A. Altman, and Y. Shoenfeld, "Thymoma and Autoimmunity," *Cellular & Molecular Immunology* 8, no. 3 (2011): 199–202.

12. Y. Liu, M. Liu, X. He, et al., "Molecular Landscape of $CD8^+$ T Cells in Pure Red Cell Aplasia," *Annals of Hematology* (2025), https://doi.org/10.1007/s00277-025-06220-5.

13. R. T. Means, "Pure Red Cell Aplasia," *Blood* 128, no. 21 (2016): 2504–2509.

14. Y. Liu, H. Niu, J. Ren, et al., "Single-Cell RNA Sequencing Reveals Abnormal Transcriptome Signature of Erythroid Progenitors in Pure Red Cell Aplasia," *Genes & Diseases* 11, no. 1 (2024): 49–52.

15. C. Song, Z. Su, and J. Guo, "Thymosin β 10 Is Overexpressed and Associated With Unfavorable Prognosis in Hepatocellular Carcinoma," *Bioscience Reports* 39, no. 3 (2019): BSR20182355.

16. X. Zhang, D. Ren, L. Guo, et al., "Thymosin Beta 10 Is a Key Regulator of Tumorigenesis and Metastasis and a Novel Serum Marker in Breast Cancer," *Breast Cancer Research* 19, no. 1 (2017): 15.

17. C. Berasain and M. A. Avila, "Amphiregulin," *Seminars in Cell & Developmental Biology* 28 (2014): 31–41.

18. S. J. Mentzer, E. Remold-O'Donnell, M. A. Crimmins, B. E. Bierer, F. S. Rosen, and S. J. Burakoff, "Sialophorin, a Surface Sialoglycoprotein Defective in the Wiskott-Aldrich Syndrome, Is Involved in Human T Lymphocyte Proliferation," *Journal of Experimental Medicine* 165, no. 5 (1987): 1383–1392.

19. H. Niu, L. Yan, L. Yang, et al., "High TOX Expression on $CD8^+$ T Cells in Pure Red Cell Aplasia," *Annals of Hematology* 102, no. 5 (2023): 1247–1255.