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ORIGINAL

MONOCYTE-RELATED GENE SIGNATURES AND THEIR ROLE IN PRECISION THERAPY FOR ACUTE MYELOID LEUKEMIA: IMPLICATIONS FOR IMMUNE FUNCTION, PHYSICAL RESILIENCE, AND REHABILITATION

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ABSTRACT

Background: Acute Myeloid Leukemia (AML) is a hematologic malignancy characterized by immune dysfunction, metabolic alterations, and physical decline, significantly impacting patient resilience, rehabilitation, and overall functional capacity. Monocyte-associated gene signatures play a crucial role in shaping the tumor microenvironment, influencing immune response, and determining patient prognosis, all of which can affect treatment efficacy and physical rehabilitation outcomes. This study aims to investigate the clinical relevance of monocyte-related genes in AML, assess their impact on the tumor microenvironment, and explore potential strategies for precision therapy and integrative rehabilitation interventions. **Methods:** A weighted gene co-expression network analysis (WGCNA) was performed to identify and validate a prognostic model based on monocyte-associated genes. The biological enrichment pathways, somatic mutations, immune landscape, and metabolic characteristics were analyzed in high- and low-risk AML patient subgroups. Additionally, potential disease regulatory networks, small molecule drug

predictions, and drug sensitivity assessments were explored to provide insights into targeted therapy and immune-modulating interventions. **Results:** A four-gene monocyte-related prognostic signature (FBXL5, TIGAR, VDR, and ZCWPW1) was identified, effectively classifying AML patients into distinct risk subgroups with significant differences in clinical outcomes, immune activity, and metabolic profiles. The low-risk subgroup demonstrated a strong association with immune system activation and metabolic resilience, showing enhanced immune function, enriched biological pathways linked to immune-metabolic interactions, and a higher immune score. These findings suggest that monocyte-related gene expression is intricately linked to immune adaptation and metabolic fitness, both of which are essential for patient recovery and physical rehabilitation strategies. **Conclusion:** Monocyte-related gene signatures are closely associated with immune regulation, metabolic stability, and AML patient prognosis, with direct implications for precision therapy, rehabilitation, and physical resilience. These gene markers could serve as valuable prognostic biomarkers and potential therapeutic targets for immune-modulating and exercise-based interventions aimed at enhancing physical function, improving recovery outcomes, and optimizing integrative leukemia treatment protocols. Future research should explore how exercise, metabolic conditioning, and immune-targeted therapies can be incorporated into holistic rehabilitation programs to improve AML patient outcomes.

KEYWORDS: Acute Myeloid Leukemia, Monocyte, Tumor Microenvironment**,** Prognosis

1. INTRODUCTION

Acute Myeloid Leukemia (AML) is a highly aggressive hematologic malignancy characterized by the uncontrolled proliferation of myeloid precursor cells, leading to immune dysfunction, metabolic imbalances, and systemic inflammation. These pathological changes not only accelerate disease progression but also contribute to severe physical deterioration, reduced functional capacity, and impaired rehabilitation potential in AML patients. Given the increasing focus on integrative treatment approaches that combine precision therapy with metabolic and physical conditioning, understanding the role of monocyte-related gene signatures in AML is critical for developing targeted interventions that improve both clinical and functional outcomes. Monocytes play a crucial role in shaping the tumor microenvironment (TME) in AML, influencing immune surveillance, inflammatory responses, and metabolic regulation (Kukun et al., 2023; Shimony et al., 2023; Ugel et al., 2021). Recent studies indicate that dysregulated monocyte activity and gene expression profiles can determine disease progression, treatment resistance, and overall prognosis. Exploring these gene signatures can provide insights into how immune modulation, metabolic reprogramming, and structured physical activity interventions can enhance recovery, resilience, and rehabilitation outcomes in

AML patients (Sun et al., 2023). Monocytes and monocyte-derived macrophages are key regulators of immune activity, inflammation, and leukemic cell survival. Dysfunctional monocyte-related gene expression has been associated with increased oxidative stress, altered cytokine signalling, and metabolic dysfunction, all of which contribute to movement-related impairments, neuromuscular fatigue, and reduced physical activity levels in AML patients. Among the key genes identified, FBXL5, TIGAR, VDR, and ZCWPW1 have been shown to play a pivotal role in metabolic homeostasis, immune system regulation, and mitochondrial function. FBXL5 is involved in iron metabolism and oxidative stress response, impacting cellular energy production and neuromuscular adaptation. TIGAR regulates glycolysis and cellular stress resistance, which may influence endurance and fatigue in physically active AML patients. VDR plays a significant role in bone health, immune modulation, and muscle strength, making it particularly relevant for physical rehabilitation programs. ZCWPW1 is linked to inflammatory signalling and chromatin remodelling, which may affect immune function and physical recovery. Given the importance of these genes in immune-metabolic pathways, integrating precision therapy with structured exercise interventions can optimize physical resilience and enhance functional recovery in AML patients. AML treatment, including chemotherapy, targeted therapies, and hematopoietic stem cell transplantation (HSCT), often leads to severe fatigue, muscle atrophy, and impaired postural control, limiting a patient's ability to engage in regular physical activity. However, emerging evidence suggests that structured exercise interventions, when combined with precision medicine, can enhance immune function, improve metabolic stability, and promote long-term physical adaptation. Exercise plays a crucial role in modulating inflammatory cytokines, enhancing immune surveillance, and promoting hematopoietic function, all of which may improve treatment outcomes in AML. Additionally, given that monocyte-related gene signatures influence metabolic efficiency and oxidative stress regulation, tailored exercise and nutritional interventions may help AML patients improve endurance, reduce therapy-related fatigue, and support neuromuscular function (Newell & Cook, 2021; Schlenk, 2023).

By integrating exercise physiology with immunogenomics, rehabilitation programs can be customized to AML patients' metabolic and inflammatory profiles, optimizing functional recovery and improving their quality of life. This study aims to investigate the role of monocyte-related gene signatures (FBXL5, TIGAR, VDR, and ZCWPW1) in AML progression and their impact on immune function, metabolic adaptation, and physical resilience. Additionally, it seeks to assess the tumor microenvironment's role in immune activity and metabolic pathways, evaluate the implications of monocyte-related gene expression on targeted therapy response, and develop a framework for integrating exercise-based interventions with precision therapy. These insights will contribute to more effective rehabilitation strategies that align with personalized medicine approaches in leukemia treatment. Understanding how immune-metabolic

interactions influence physical performance and recovery in AML patients is crucial for developing interventions that enhance functional resilience, neuromuscular health, and post-treatment rehabilitation. This research is particularly relevant to sports science, rehabilitation, and exercise-based interventions, as it bridges the gap between molecular oncology and physical rehabilitation (Chen et al., 2023; Kayser & Levis, 2023). By identifying how monocyte-related gene signatures shape immune function and metabolic adaptation, this study provides valuable insights for healthcare professionals designing tailored exercise and rehabilitation programs for AML patients. Personalized rehabilitation strategies that align with precision therapy approaches can optimize immune recovery, improve neuromuscular function, and mitigate therapy-related fatigue. Future research should focus on how structured exercise programs, targeted nutritional strategies, and immunogenomics-based interventions can enhance functional independence and physical resilience in AML patients. Integrating metabolic and immune-targeted therapies into rehabilitation programs will ensure a more comprehensive approach to AML management, helping patients regain mobility, strength, and overall well-being.

2. Methods and Materials

2.1 Source and Accessibility of Data

In the training cohort, RNA sequencing transcriptomic data, along with relevant baseline clinical information, were obtained from 151 patients diagnosed with acute myeloid leukemia (AML) available in The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). Furthermore, patient data that included extensive transcriptional and clinical information were retrieved from the GSE37642 dataset obtained from the Gene Expression Omnibus (GEO) database (accessible at https://www.ncbi.nlm.nih.gov/geo/) for the evaluation cohort. The criteria for selecting eligible samples from the TCGA were as follows:

1) a confirmed AML diagnosis; 2) the availability of transcriptomic data; and 3) the provision of overall survival data along with pertinent clinical characteristics. In conclusion, this research included 130 bone marrow samples sourced from the TCGA database. The specific pre-processing procedures for the microarray data derived from the GSE37642 cohort are detailed below:

1) Samples lacking relevant follow-up data were excluded; 2) Probe identification numbers were transformed into Gene Symbol format; 3) Probes linked to multiple genes were discarded; 4) For genes that were represented by several probes, the mean expression value was determined. In the final analysis, a total of 140 bone marrow specimens from the GSE37642 cohort were included.

2.2 Analyzing Module Correlations and Constructing Co-Expression Networks

The establishment of a co-expression network was achieved by utilizing Weighted Gene Co-expression Network Analysis (WGCNA). To begin with, a weighted adjacency matrix was constructed using the power function (a_{mn} = |{mn}|^\beta), where (c_{mn}) signifies the Pearson correlation coefficient between genes m and n, while (a_{mn}) indicates the adjacency between these two genes. The parameter (\beta) acts as a soft threshold, enhancing the significant correlations among the genes. To ensure that the network conforms to a scale-free topology, an appropriate soft threshold parameter was chosen, ensuring that the goodness of fit remained above 0.8. Following this, the WGCNA approach was utilized to convert the immune cell characteristic data into a color-coded representation, which enabled the creation of a correlation heatmap that depicts the associations between gene modules and immune characteristics.

2.3 A Risk Signature Derived from a Central Module is Developed and Assessed

The Least Absolute Shrinkage and Selection Operator (LASSO) paired with Cox regression analyses represents a widely utilized statistical approach that integrates variable selection with regularization techniques. This integration improves both the accuracy of predictions and the clarity of the statistical models that have been developed. In the present investigation, these approaches were applied to identify independent risk factors linked to Acute Myeloid Leukemia (AML). The RMS programming package was utilized to create prognostic nomograms, which enabled an assessment of their effectiveness in predicting clinical outcomes. Subsequently, the viability of constructing a risk model based on diverse clinical attributes was investigated through Kaplan-Meier survival analysis. The precision of the model was further assessed by generating a receiver operating characteristic curve. To substantiate the risk signature, the GSE37642 dataset was used as an external validation cohort. A nomogram was then developed incorporating both clinical features and the risk signature to estimate the prognosis of AML.

2.4 Analysis of Functional Enrichment

In order to explore the signaling pathways and biological impact linked to the cluster of monocyte-associated genes, an analysis was performed using the Gene Ontology database. A significance threshold of 0.05 was set for this evaluation. Furthermore, Gene Set Enrichment Analysis (GSEA) was executed to analyze the data from the MSigDB collection (c2.cp.kegg.v7.4.symbols.Enrichment of gmt). The objective of this analysis

was to ascertain whether notable differences were present in the gene sets expressed between the high-risk and low-risk groups, with P-values less than 0.05 and a false discovery rate (FDR) below 0.25 considered as indicators of statistically significant differences.

2.5 Characterization of Somatic Mutations Across Subgroups and the Immune Landscape

The somatic mutation information related to acute myeloid leukemia (AML) specimens was acquired from the TCGA database. The distinct variations in somatic mutation characteristics among different sample subgroups were illustrated using a waterfall plot. In order to examine immune cell infiltration and evaluate the variations in the tumor microenvironment among the acute myeloid leukemia (AML) subgroups, we utilized multiple computational algorithms, such as XCell, quanTIseq, MCPCOUNTER, and CIBERSORT, to conduct an extensive analysis of immune infiltration.

2.6 The Establishment of a Regulatory Network Involving Microrna (Mirna) Genes and Transcription Factor (TF) Genes, along with the Identification of Potential Therapeutic Agents and an Analysis of Drug Sensitivity

In order to identify microRNAs (miRNAs) and transcription factors (TFs) that are associated with prognostic genes, the Network Analyst platform (https://www.networkanalyst.ca) was used. By doing this, we can develop regulatory networks that clarify miRNA-gene and TF-gene interactions. In order to discover prospective pharmacological compounds and examine the connections present in drug-gene interactions, the Drug-Gene Interaction Database (https://dgidb.org) was utilized. Given that a significant proportion of individuals diagnosed with acute myeloid leukemia demonstrate insufficient responses to pharmacological treatment, this investigation also included a drug sensitivity analysis aimed at proposing alternative therapeutic strategies for these patients. The drug sensitivity analysis was conducted using a comprehensive drug screening dataset accessible at (https://zenodo.org/records/7274740).

2.7 Analysis of Biomarker Expression at the Single-Cell Level and Their Subcellular Localization

In order to evaluate the expression patterns of particular biomarkers within bone marrow cells, we conducted an analysis utilizing single-cell and transcriptional datasets sourced from the Human Protein Atlas (HPA) database (accessible at http://www.proteinatlas.org/). Furthermore, we utilized the COMPARTMENTS database (https://compartments.jensenlab.org/) to forecast the subcellular distribution of the proteins associated with these biomarkers.

2.8 Analyses of Statistics

The independent samples t-test was employed to compare continuous variables between two separate groups, whereas categorical data were examined through the chi-square test. For the evaluation of patient survival and the construction of survival curves, the Kaplan – Meier method was applied alongside the log-rank test. A p-value of less than 0.05 was considered to reflect statistical significance.

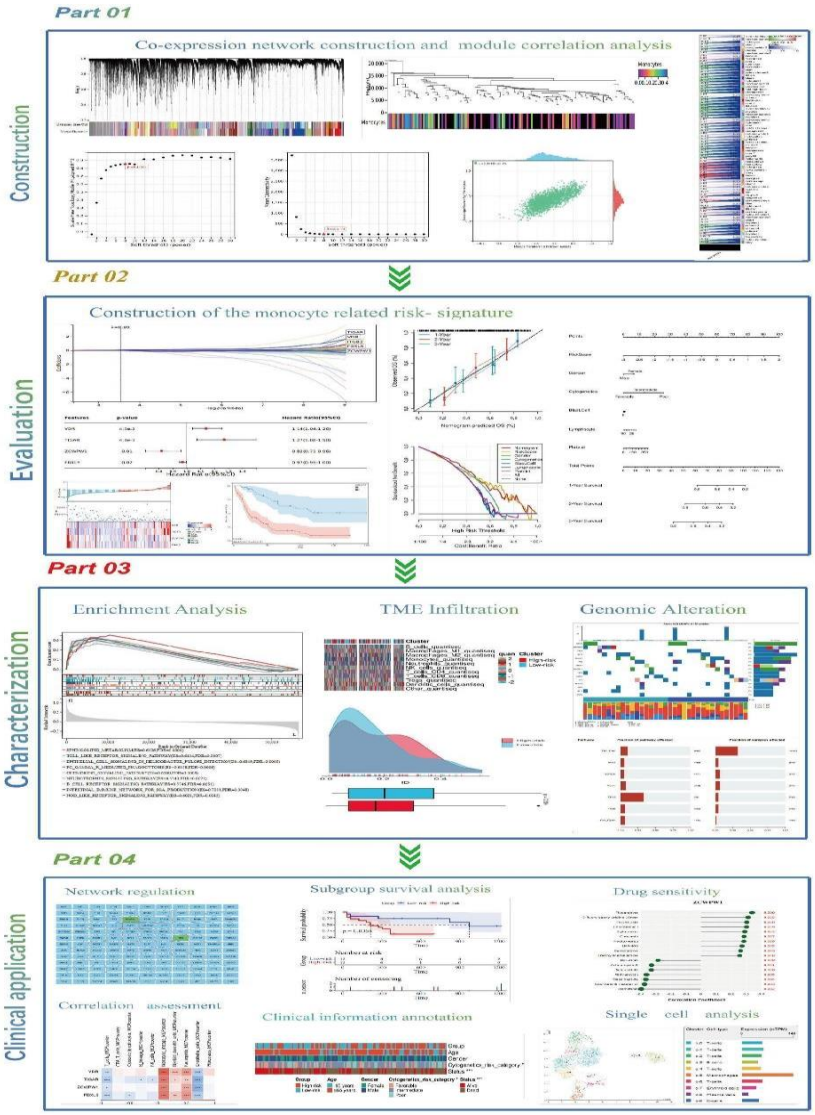

Figure 1: This Chart Illustrates the Process of the Study.

3. Result

3.1 Identification of the Hub Module Associated with Monocyte Infiltration via Weighted Gene Co-expression Network Analysis (WGCNA)

Clustering analysis was conducted utilizing the dynamic shear tree methodology, establishing a shear height threshold of 20,000 as the criterion

for tree pruning. The monocyte-related data were transformed into a color-coded format and subsequently represented in both a dendrogram and a corresponding heat map (refer to Figure 2A). Genes that satisfied the filtering criteria were employed to construct a scale-free network by assessing the strength of connectivity among them. The scale-free topology model was determined by calculating the scale-free R^2 values, which varied between 0 and 1. Figure 2B illustrates the correlation between the goodness of fit (R^2) and different soft threshold values; the red line signifies a goodness of fit R^2 of 0.85, indicating that an adequate fit is suggested when R^2 approaches or exceeds 0.9. Moreover, Figure 2C depicts the relationship between the average number of connections and varying soft thresholds, demonstrating that at a soft threshold β of 8, the R^2 value aligns with a point where the average connectivity of the network is nearly zero, thereby indicating that the gene distribution adheres to the characteristics of a scale-free network. The modules were examined concerning the characteristics of monocytes, leading to the creation of a module-feature association plot. This investigation uncovered a notable positive correlation between monocytes and the brown module, which consists of 132 genes (as shown in Figure 2D). Furthermore, a scatter plot was generated to illustrate the connection between the gene significance (GS) of the brown module and its module membership (MM), revealing a robust correlation with monocyte infiltration (Figure 2E, correlation coefficient = 0.82). As a result, we identified the brown module as the central module and proceeded to extract the genes within this module for subsequent analysis.

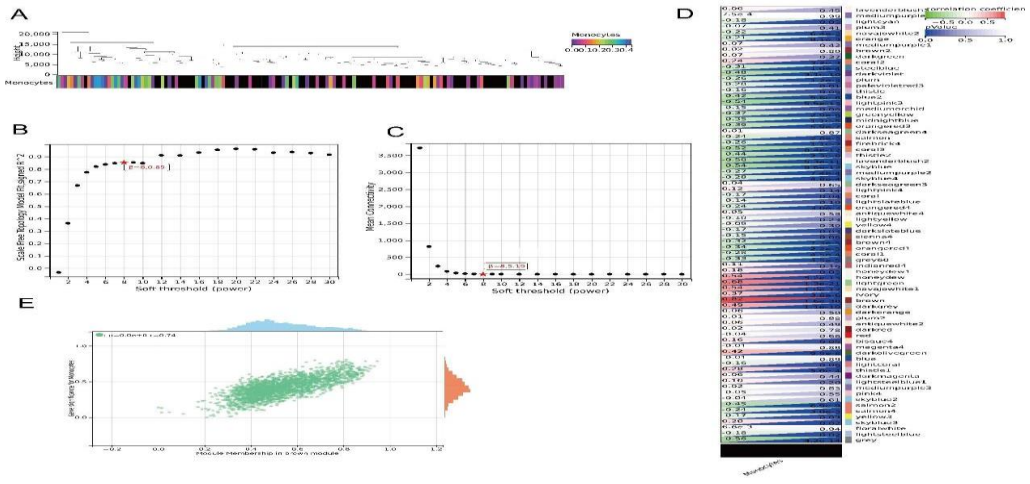

Figure 2: Identification of the Hub Module Associated with Monocyte Infiltration via Weighted Gene Co-expression Network Analysis (WGCNA). (A) A schematic representation demonstrating the clustering of modules. (B) A graphical depiction of the relationship between the goodness of fit and a range of soft threshold values. (C) An illustration of the correlation between average connectivity and varying levels of soft threshold. (D) A visual representation showcasing the clustering of modules. (E) A scatter plot illustrating the association between gene significance (GS) and module membership (MM) specifically within the green module.

3.2 Development of a Prognostic Signature Utilizing Genes from the Brown Module

To identify genes within the brown module associated with overall survival (OS) in acute myeloid leukemia (AML) patients from The Cancer Genome Atlas (TCGA), we employed LASSO regression analysis. This analysis revealed a total of five genes, which were subsequently subjected to univariate Cox regression analysis (refer to Figure 3A). To enhance the stability of the variables and mitigate the risk of overfitting, we further evaluated four genes identified through the univariate Cox regression (illustrated in Figure 3B). Ultimately, we identified four prognostic genes that were utilized to construct a risk signature. By employing the median risk score cutoff, individuals diagnosed with acute myeloid leukemia (AML) were divided into two separate classifications: high-risk and low-risk cohorts. It is important to highlight that the prognosis for individuals identified as high-risk was markedly worse in comparison to those classified as low-risk, as illustrated in Figure 3C. By integrating the risk signature with clinical characteristics derived from the TCGA cohort, we constructed a nomogram aimed at forecasting patient survival outcomes. The calibration curves demonstrated a strong concordance between the predicted survival rates at 1, 3, and 5 years and the actual observed survival rates, as depicted in Figure 3D.

Furthermore, the Kaplan-Meier survival analysis revealed a significant association between overall survival (OS) in acute myeloid leukemia (AML) patients and the risk score, indicating that the overall survival (OS) rate for individuals identified as high-risk was significantly lower than that of those categorized as low-risk. This observation underscores the role of a high-risk score as a detrimental prognostic factor for AML patients. The area under the receiver operating characteristic (ROC) curve was computed to be 0.76, 0.76, and 0.82 for the 1, 3, and 5-year intervals, respectively, suggesting that the risk signature is a reliable predictor of OS in AML patients, as illustrated in Figure 3E. Additionally, validation with the GEO dataset yielded similar findings (see Figure 3F), thereby reinforcing the robustness of our predictive model.

3.3 Enrichment and Analysis of Somatic Mutations

In our preliminary investigation, we identified signaling pathways associated with monocyte-related genes to elucidate the molecular mechanisms linked to prognosis. The results from Gene Set Enrichment Analysis (GSEA) indicated a significant enrichment of functional pathways related to cellular metabolism in patients classified as high-risk for acute myeloid leukemia (AML) (Figure 4A). Subsequent Gene Set Enrichment Analysis (GSEA) of the four prognostic genes revealed that within the high-risk cohort of acute myeloid leukemia (AML), these genes were involved in

biological processes related to necrosis, cellular apoptosis, and metabolic activities. This finding contributes to a deeper comprehension of the underlying mechanisms of disease pathogenesis (Figure 9A).

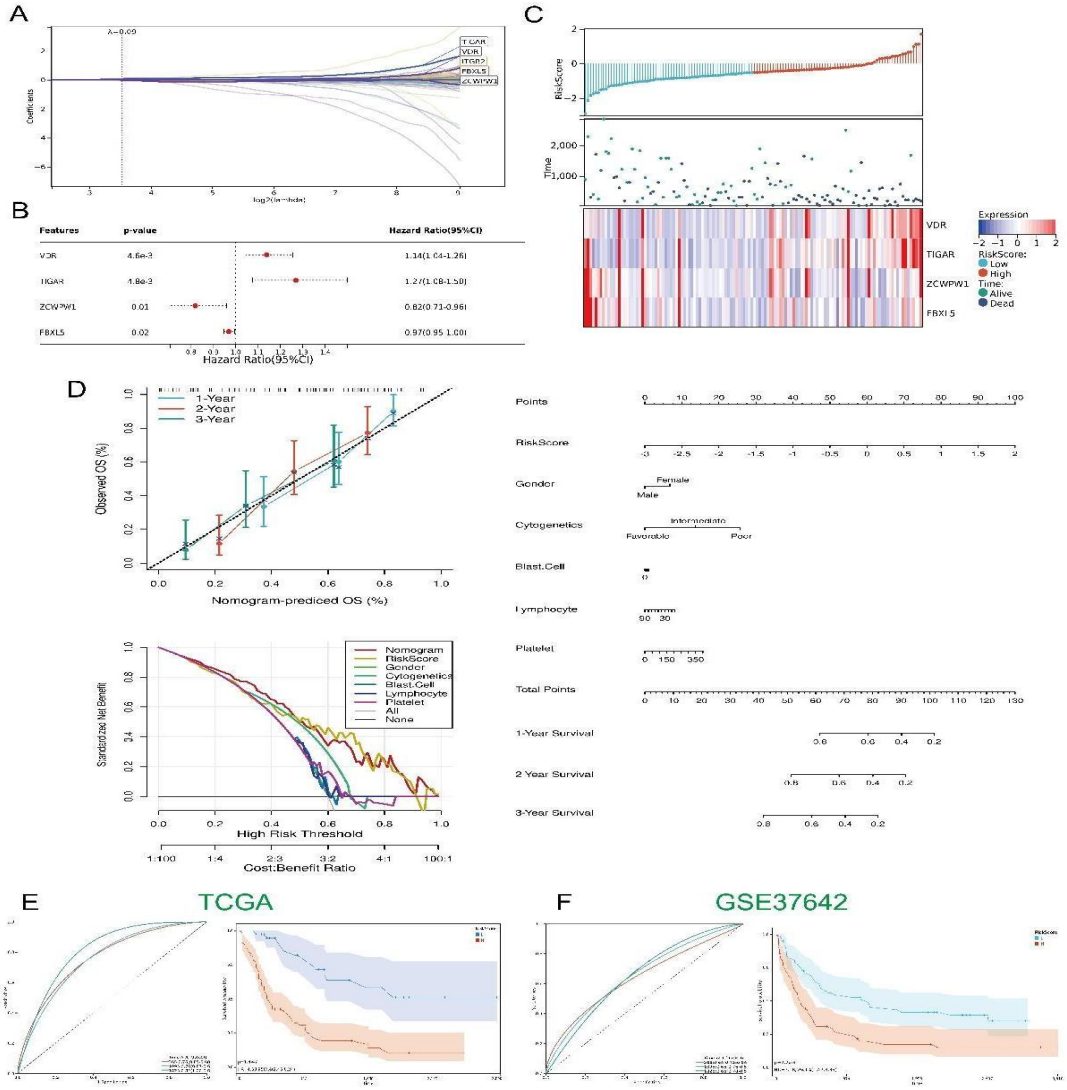

Figure 3: The development and validation of the monocyte-risk signature. (A) To identify the monocyte-risk signature, Lasso Cox regression analysis was utilized; (B) A univariate Cox regression analysis was conducted to evaluate the prognostic relevance of genes associated with B cells on an individual basis; (C) The risk score distribution, patient survival outcomes, and heatmaps illustrating the prognostic four-gene signature were generated utilizing data sourced from the TCGA database; (D) A monogram was developed, supplemented by Calibration and Decision Curve Analysis diagrams. In addition, time-dependent Receiver Operating Characteristic (ROC) curves along with Kaplan-Meier survival analyses were conducted for the TCGA cohort (E) as well as the GSE37642 cohort (F).

Subsequently, we mapped the chromosomal locations of these prognostic genes (Figure 4B). We conducted a comparative analysis of the pathways predominantly enriched in high-risk versus low-risk AML patients. Our

findings revealed distinct somatic mutation profiles across AML patient subgroups, with notable frequencies of genetic mutations in NPM1, RUNX1, TTN, DNMT3A, IDH2, and ELF4 among somatic mutations in AML patients. However, the relative frequencies of these mutations exhibited variability between subgroups. Furthermore, signaling pathways such as RTK-RAS, WNT, NOTCH, and Hippo were significantly enriched in the high-risk groups, suggesting potential critical pathogenic pathways that could inform precision therapeutic strategies, including targeted therapies (Figure 4B-G). Additionally, we observed that the genetic mutations in AML patients with somatic alterations predominantly consisted of nonsense mutations, primarily manifesting as single nucleotide polymorphisms (SNPs) (Figure 4H-I).

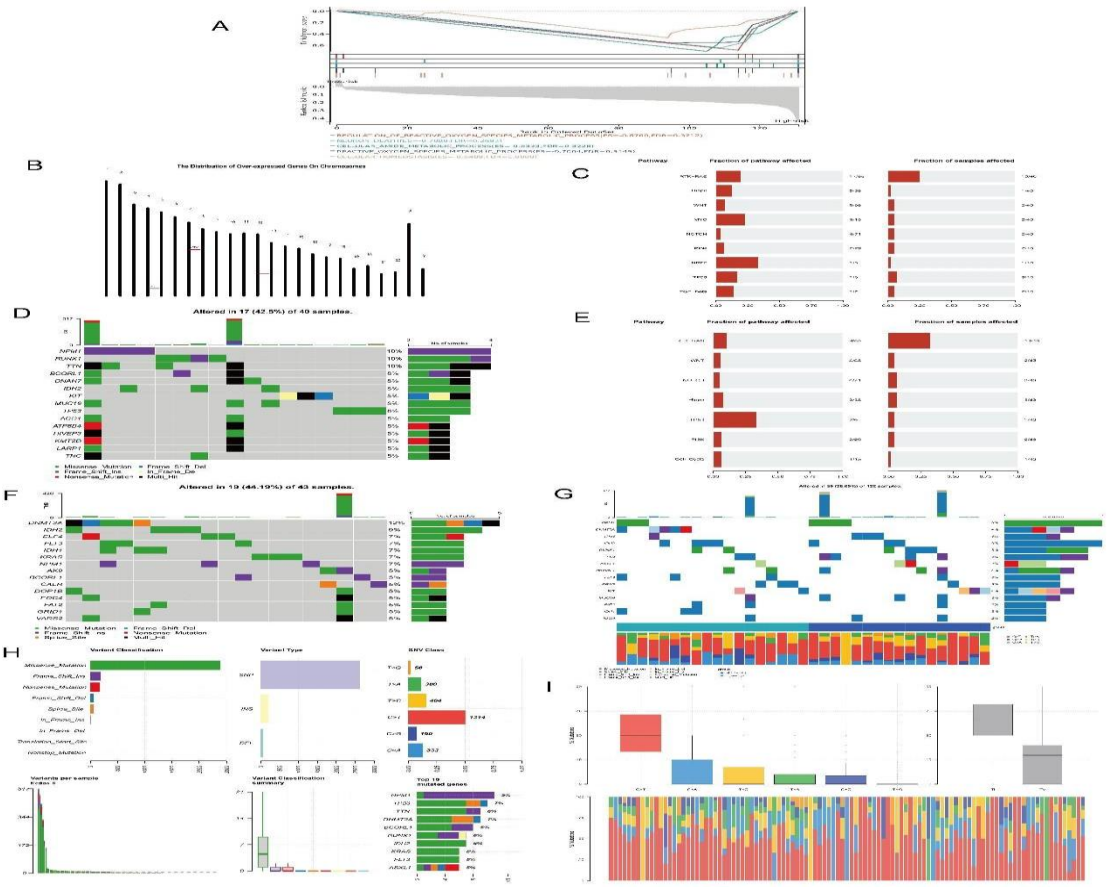

Figure 4: Enrichment and Analysis of Somatic Mutations. (A) GSEA analysis identifies potential signaling pathways in AML high risk-group subtype; (B) The chromosomal locations of four prognostic genes; (C-D) Shared pathogenic pathways and mutated genes identified within the high-risk cohort; (E-F) Shared pathogenic pathways and mutated genes observed in the low-risk cohort. The prevalent types and categories of genetic mutations in AML patients exhibiting somatic mutations are depicted in (G-I).

3.4 Analysis of the Tumor Microenvironment

Considering the pivotal function of monocytes in orchestrating the anti-tumor immune response, we undertook an extensive analysis of the tumor

microenvironment across various subgroups by employing several analytical methodologies, such as CIBERSORT, MCPCOUNTER, quanTIseq, and XCell. Our initial focus was on evaluating the expression levels of different immune cell types within these subgroups. In the high-risk cohort of acute myeloid leukemia (AML) patients, we identified a comparatively higher proportion of immune cells, particularly B cells and monocytes. Conversely, the cohort identified as low-risk demonstrated a greater frequency of T cells, as depicted in Figure 5A-D. In addition, when assessing the immune and microenvironmental scores in connection with the groups classified as high-risk and low-risk, it became evident that low-risk AML patients exhibited significantly elevated immune scores (Figure 5G) and microenvironment scores (Figure 5F), while high-risk AML patients showed increased Stroma Scores (Figure 5E). These results highlight the complex interplay between monocyte-associated risk factors and the tumor microenvironment.

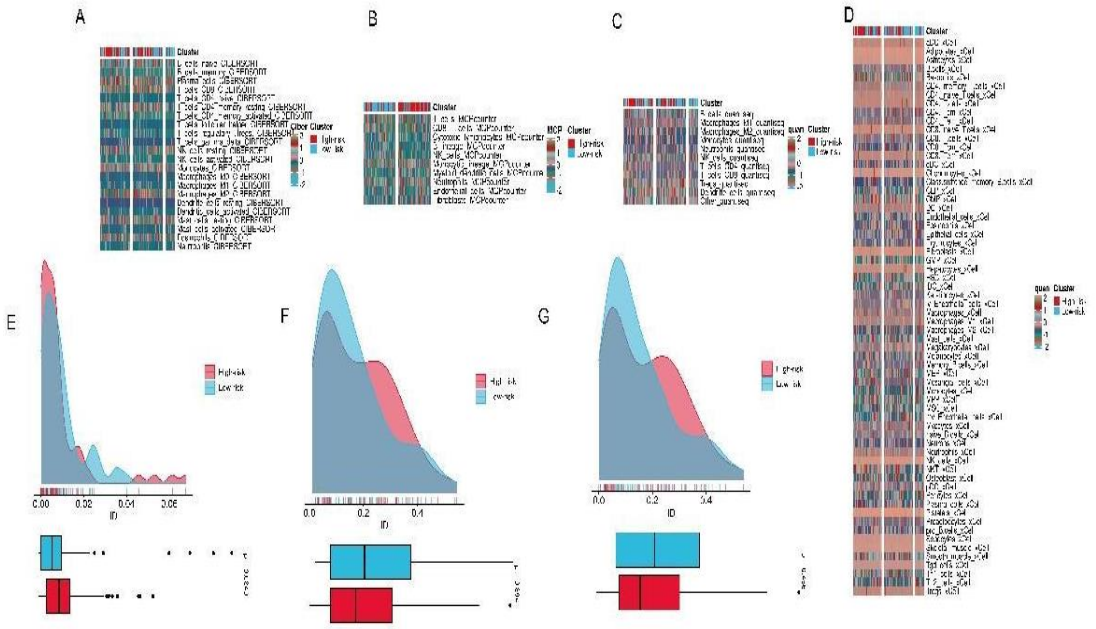

Figure 5: Immune landscapes between subgroups. (A-D) Relative proportion of immune cell infiltration in subgroups by XCell、quan Tlseq、MCPCOUNTER and CIBERSORT algorithm; (E-G) Comparison of Stroma Score、Micro environment Score and Immune Score in subgroups.

3.5 Risk Models in Clinical Practice

To assess the efficacy of formulating monocyte-associated risk signatures, we investigated the association between the risk score and the expression levels of different types of immune cells. Our findings indicated a notable positive association between the risk score and the expression of several immune cell types, specifically Monocytic cells, Fibroblasts, and Myeloid dendritic cells. In contrast, immune cell types such as T cells and NK cells displayed a negative correlation with the risk score. Overall, the four

prognostic genes exhibited a robust relationship with a majority of immune cell types, particularly Monocytic cells, Endothelial cells, and Neutrophils (refer to Figure 6 A-B). Following this, we created a comprehensive heatmap and a donut chart to depict the differences in clinical characteristics among acute myeloid leukemia (AML) patients classified into high and low-risk categories, thereby emphasizing the successful establishment of risk signatures associated with monocyte genes and their clinical implications (see Figure 6 C-D). Finally, we performed a subgroup survival analysis of AML patients, categorized by sex, age, prognostic classification, and clinical events, to confirm the applicability of this predictive model (illustrated in Figure 6 E1-E7). Furthermore, we evaluated the expression levels of the four prognostic genes within both high-risk and low-risk groups of acute myeloid leukemia (AML), as demonstrated in Figure 9B.

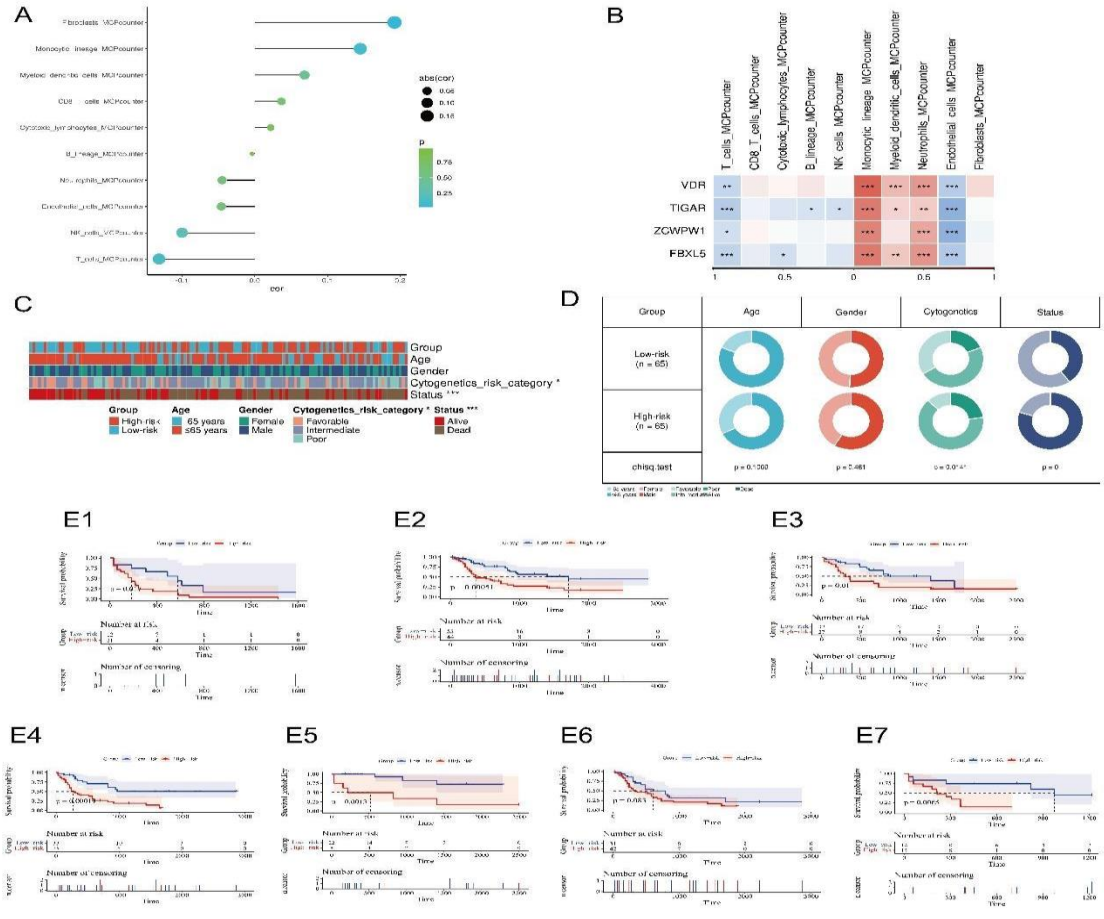

Figure 6: Clinical Utilization of Risk Scores. (A) An in-depth evaluation of the relationship between risk scores and the expression levels of diverse immune cell types. (B) An investigation into the connection between a four-gene signature and the expression profiles of different immune cell types; (C) Extensive heatmaps depicting the variations in clinical data distribution between high-risk and low-risk patient cohorts; (D) A comparative evaluation of patient demographics, encompassing age, sex, cytogenetic characteristics, and survival outcomes among distinct subgroups; (E 1-7) An analysis of survival outcomes across the various subgroups.

3.6 Regulatory Network Construction, Small-Molecule Drug Prediction, Drug Sensitivity Analysis of the Prognostic Genes

miRNAs are a type of naturally occurring short non-coding RNA that play a crucial role in the degradation of messenger RNA (mRNA). In parallel, transcription factors (TFs) are proteins that specifically bind to designated DNA sequences, thereby exerting control over gene expression. In order to enhance our understanding of the pathogenesis associated with acute myeloid leukemia (AML), we employed Network Analyst software to forecast the microRNAs and transcription factors that impact prognostic genes. This methodology allowed for the development of regulatory networks that depict the relationships between microRNAs and genes, in addition to the interactions between transcription factors and genes.Our findings uncovered complex regulatory dynamics involving approximately 143 transcription factors and 114 miRNAs that are linked to prognostic genes (see Figure 7A-B). Following this, we directed our attention towards identifying potential therapeutic agents for AML. We conducted a drug sensitivity analysis focused on these four prognostic genes. The findings indicated that chemotherapeutic agents such as Selumetinib and PD-98059 exhibited significant therapeutic efficacy against the VDR gene. In contrast, the ZCWPW1 gene demonstrated heightened sensitivity to agents like Fludarabine and Fenretinide, suggesting their therapeutic potential. Additionally, for the FBXL5 gene, agents such as Fludarabine and Nelarabine were identified as having sensitive therapeutic effects. Conversely, the TIGAR gene showed increased sensitivity to chemotherapeutic drugs including Cobimetinib (isomer 1) and Dabrafenib. Collectively, these results provide valuable insights that may inform potential treatment strategies for patients with AML (see Figure 7C-F).

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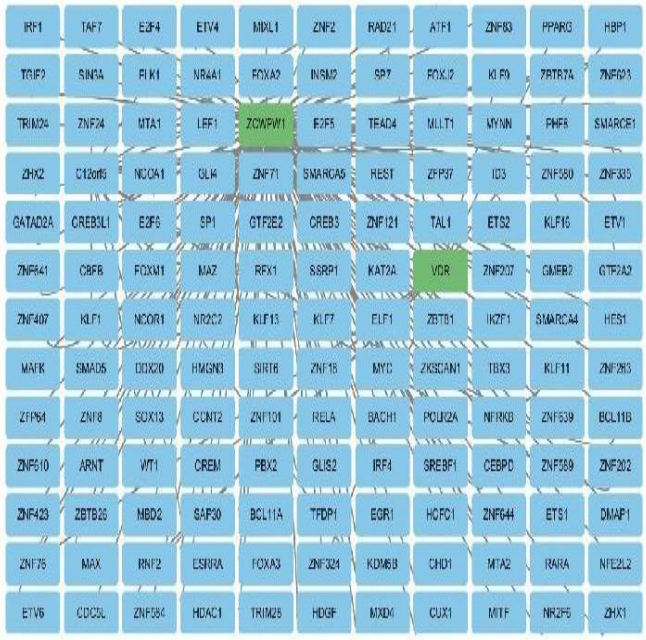

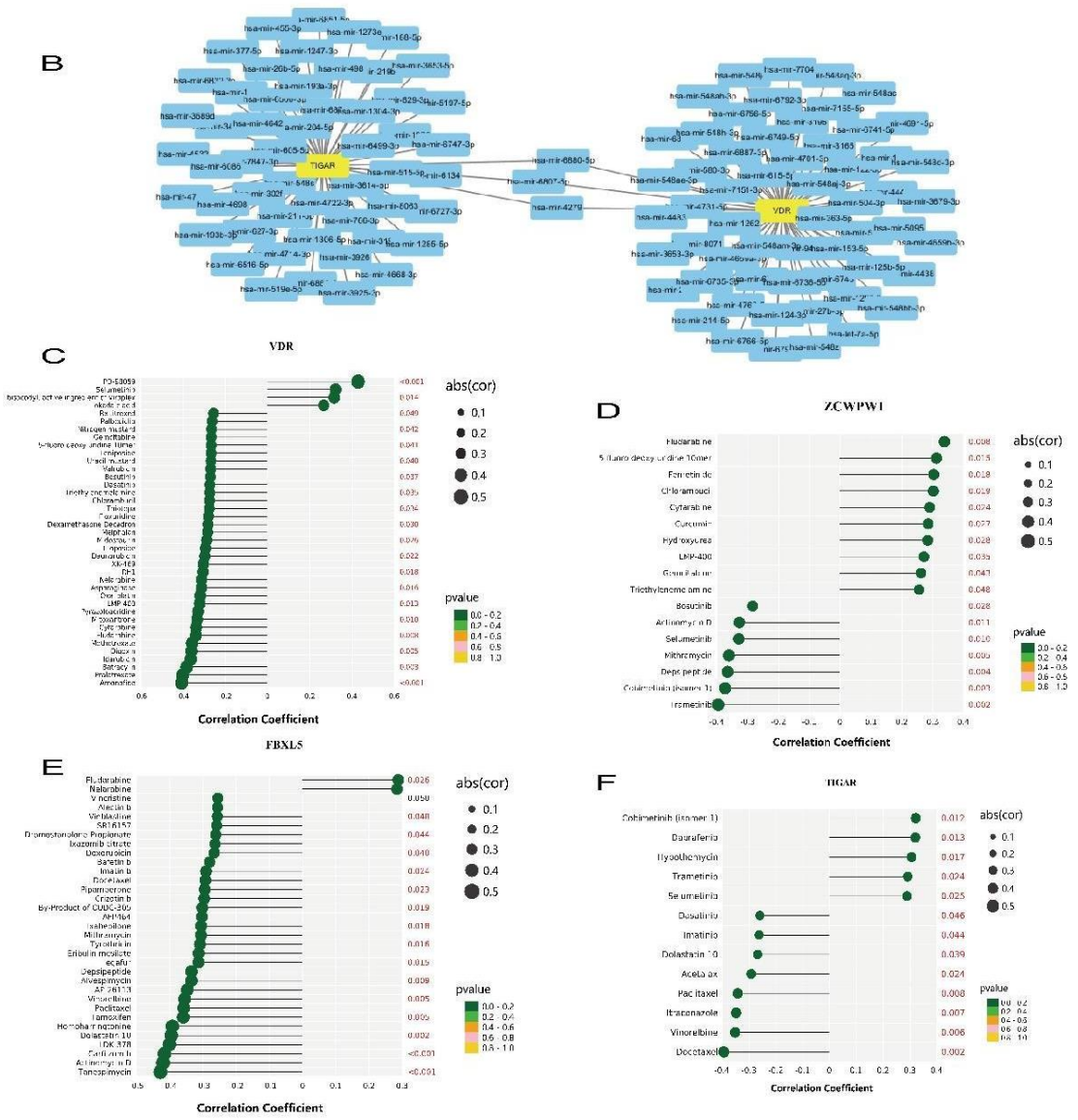

Figure 7: Regulatory network construction, small-molecule drug prediction, drug sensitivity analysis of the prognostic genes. (A-B) The miRNA-gene and transcription factor-gene regulatory networks; **(**C-F) Drug sensitivity analysis of the prognostic genes.

3.7 The Analysis of Single Cells and Subcellular Localization of Prognostic Genes

To achieve a more accurate depiction of the expression profiles of prognostic genes within human bone marrow tissue, we utilized single-cell RNA sequencing (scRNA-seq) alongside the Human Protein Atlas (HPA) database to characterize the various cellular populations present within the bone marrow. The clustering analysis conducted in our study identified nine unique subsets of myeloid cells, which are illustrated in the UMAP visualization. The findings additionally indicated that FBXL5 is predominantly found in macrophages, T lymphocytes, and B lymphocytes, whereas TIGAR is predominantly detected in macrophages, plasma cells, and T cells. Moreover, VDR exhibits substantial

expression in both macrophages and plasma cells, while ZCWPW1 shows elevated expression levels in T cells and B cells (Figure 8A). Proteins carry out a variety of biological functions that are closely associated with their specific cellular localization. The results further demonstrated that FBXL5 is primarily located in macrophages, T cells, and B cells. The findings suggest that FBXL5 is mainly localized in the cytosol, TIGAR is primarily linked to the glucose transporter complex, VDR is predominantly found in the nucleus, and ZCWPW1 is chiefly located in the mitochondria (Figure 8B).

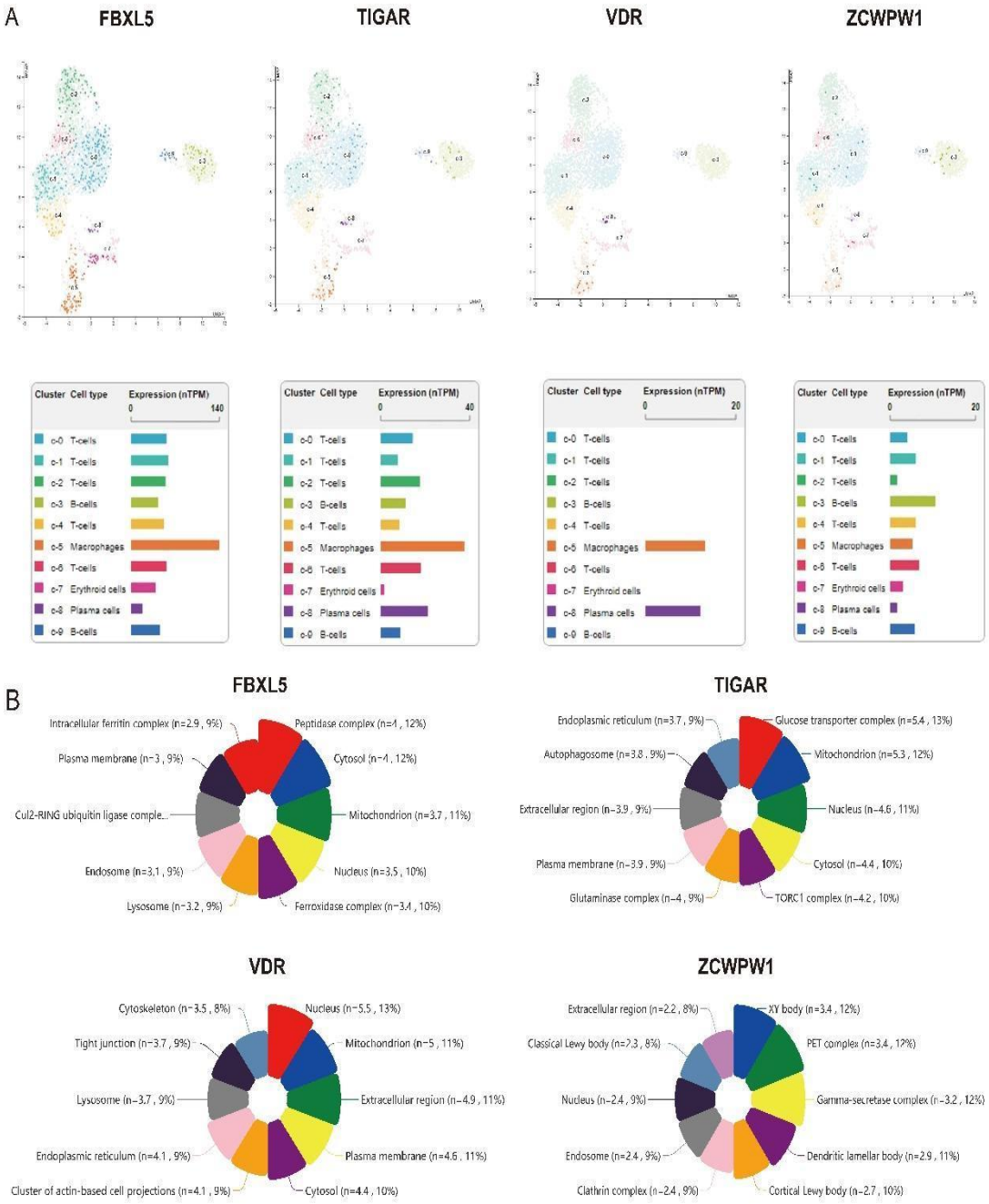

Figure 8: The analysis of single cells and subcellular localization of prognostic genes. (A) Analysis of gene expression at the single-cell level (B) Examination of the subcellular localization of key genes.

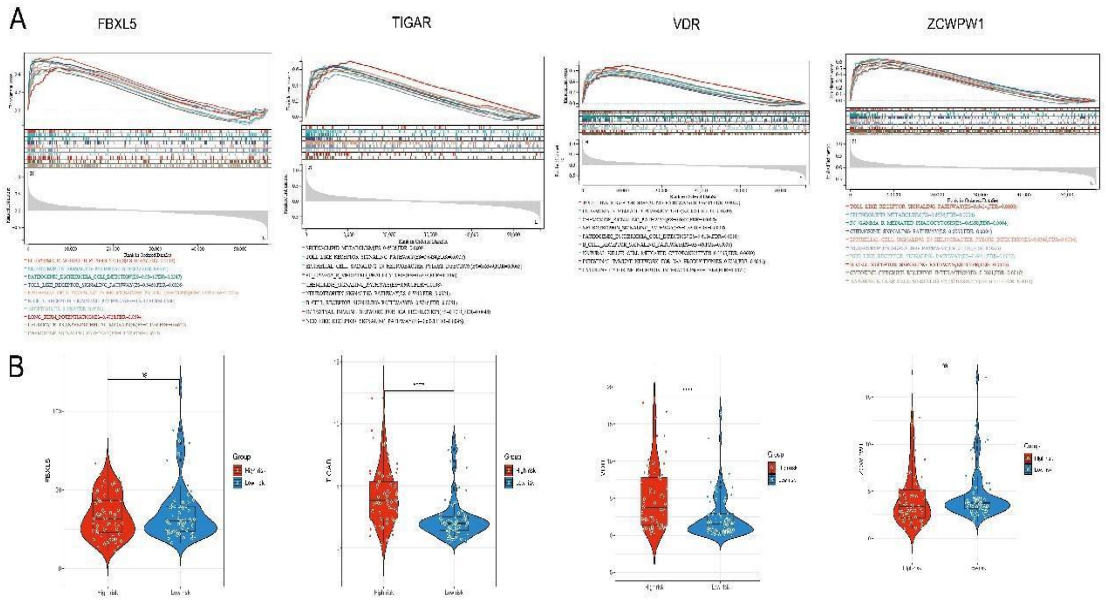

Figure 9: Prognostic gene enrichment analysis and comparison of expression levels between groups. (A) Conducting gene set enrichment analysis for the signature comprising four genes; (B) Evaluating the expression levels of the pivotal genes included in the prognostic signature among different risk groups.

4. Discussion

Monocytes play a crucial role within the tumor immune microenvironment, exhibiting a complex and significant function. Primarily, they intensify inflammatory responses by modulating the activities of immune cells. Furthermore, monocytes are capable of releasing numerous growth factors that facilitate the proliferation and growth of tumor cells, while also enhancing their capacity to evade detection by the immune system (Olingy et al., 2019). Despite their critical role, the precise contributions of monocytes in acute myeloid leukemia (AML) remain insufficiently characterized. This study aims to clarify the mechanisms through which monocyte-associated genes function in AML and evaluate their potential for therapeutic intervention, thereby optimizing the therapeutic opportunities associated with targeting monocytes in the treatment of AML. Initially, we conducted an extensive examination of the tumor microenvironment, which then prompted our exploration of acute myeloid leukemia (AML). Our prognostic model, constructed via weighted gene co-expression network analysis (WGCNA) with a specific emphasis on monocytes, revealed that the high-risk cohort exhibited significantly increased expression levels of B cells and a variety of immune cell types, including monocytes. Conversely, the low-risk cohort exhibited an increased level of T cell expression. Building on existing literature, we posited a potential immunological pathogenesis for AML, thereby laying the groundwork for future immunotherapeutic strategies. T cells serve as pivotal agents of immune surveillance, adept at recognizing and eliminating malignant cells. However,

within the tumor microenvironment of AML patients, these malignant cells may evade detection and attack by T cells through multiple mechanisms. AML cells can compromise T cell functionality by secreting immunosuppressive factors or by fostering the development of immunosuppressive cells, such as regulatory T cells (Tregs). This immunosuppressive environment significantly enhances the survival and proliferation of AML cells. B lymphocytes are essential to immune surveillance, evasion, and therapeutic strategies in AML, primarily through their involvement in antibody production, antigen presentation, and contributions to immunotherapy. B cells can function as antigen-presenting cells (APCs), presenting antigens from AML cells to T cells, thereby stimulating an anti-cancer immune response. Nevertheless, AML cells can also impede the functionality of B cells through various mechanisms, thereby creating an immunosuppressive microenvironment that enables them to evade immune detection (Engelhard et al., 2021; Laumont et al., 2022). Monocytes, a subtype of leukocyte produced in the bone marrow, traverse the circulatory system and have the potential to differentiate into macrophages or dendritic cells, both of which are essential components of the immune response. The relationship between monocytes and acute myeloid leukemia (AML) is marked by a variety of interactions and influences. Initially, monocytes and their differentiated derivatives significantly contribute to the tumor microenvironment, potentially facilitating or hindering the proliferation and spread of AML cells. They exert influence on AML cell behavior through the release of cytokines, chemokines, and growth factors. Furthermore, monocytes can transform into antigen-presenting cells, such as dendritic cells, which are vital for immune surveillance(Mantovani et al., 2014; Olingy et al., 2019). These cells have the capability to recognize and analyze antigens derived from acute myeloid leukemia (AML) cells, which in turn stimulates T cells and initiates an immune reaction targeting the leukemia. Concurrently, AML cells may employ various strategies to evade detection by monocytes and other immune components. For example, AML cells can suppress monocyte functionality, thereby reducing their effectiveness in antigen presentation and T cell activation, which aids in the evasion of immune attacks. In conclusion, immune cells demonstrate intricate and diverse roles in the advancement and treatment of acute myeloid leukemia (AML).A more thorough exploration of the relationships between immune cells and AML has the potential to yield significant understanding and therapeutic strategies that could enhance the treatment and outlook for this disease. This area will be a central emphasis of our future research endeavors. For numerous years, conventional cytotoxic chemotherapy has been the predominant therapeutic approach for acute myeloid leukemia (AML). However, the enduring heterogeneity inherent in AML has led to stagnant survival rates among affected individuals. Therefore, it is essential to undertake thorough investigations into the molecular mechanisms that regulate the growth and survival of acute myeloid leukemia (AML) cells, as this will contribute to the development of varied therapeutic approaches. This research highlights the

progress made in understanding the key signaling pathways implicated in AML, thereby providing a theoretical framework for the creation of innovative therapeutic options. Mutations in genes linked to the RTK-RAS signaling pathway, particularly those involving NRAS and KRAS, are acknowledged as significant factors in the development of AML (Mustafa Ali et al., 2023). These genetic alterations can disrupt signal transduction processes, promoting the growth and survival of leukemic cells. Importantly, mutations in NRAS and modifications in the NF1 gene may serve as prognostic markers that influence the survival outcomes of patients diagnosed with AML. The WNT signaling pathway is crucial in the context of acute myeloid leukemia (AML), as it significantly impacts disease progression by regulating the self-renewal and proliferation of leukemic stem cells (LSCs). Recent studies have uncovered an autocrine loop unique to AML, which involves T cell immunoglobulin mucin-3 (TIM-3) and its ligand, galectin-9 (Gal-9). This loop activates the canonical WNT pathway, thereby facilitating the self-renewal and proliferation of LSCs. Furthermore, the NOTCH signaling pathway plays a complex role in AML, markedly promoting the self-renewal and proliferation of LSCs through the activation of β-catenin (Abdel-Rahman et al., 2023). Inhibition of NOTCH signaling has been demonstrated to upregulate the negative regulator of ERK signaling, leading to a subsequent reduction in ERK activation, which may indicate potential therapeutic strategies for AML management (Gruszka et al., 2019). The Hippo signaling pathway represents a critical regulatory network that oversees cell growth, division, survival, differentiation, and tissue homeostasis in multicellular organisms. Research into this pathway has yielded new insights regarding the regulation of organ size, tissue regeneration, and cancer development (Harvey et al., 2013; Zheng & Pan, 2019). Although the specific role of the Hippo pathway in AML remains inadequately investigated, findings from studies on other cancers provide valuable perspectives for understanding its function in the context of AML. To obtain a more profound understanding of the mechanisms underlying acute myeloid leukemia (AML), we examined the relationship between prognostic genes and tumor biology. FBXL5 has been identified as a gene significantly linked to cancer, playing a pivotal role in its initiation and advancement. As an E3 ubiquitin ligase and a member of the FBOX protein family, FBXL5 has been demonstrated to physically interact with the PTEN protein, thereby negatively modulating its expression while simultaneously enhancing the expression and phosphorylation of PI3K, AKT, and mTOR. This regulatory mechanism fosters cellular proliferation, tumor development, and inhibits apoptotic processes. Furthermore, FBXL5 facilitates epithelial-mesenchymal transition (EMT) by influencing the degradation and accumulation of E-cadherin at the cell membrane, which subsequently promotes tumor invasion and metastasis (Park et al., 2021; Vinas-Castells et al., 2013; Wu et al., 2015). In contrast, the ZCWPW1 gene plays a role in carcinogenesis and tumor progression primarily through the ubiquitination of its substrates, leading to their degradation

(Andrews et al., 2018). TIGAR (Tp53-induced glycolysis and apoptosis regulator) is a protein that is overexpressed in numerous human cancers, influencing the viability and growth of cancerous cells through modifications in metabolic pathways and their reactions to oxidative stress. Specifically, TIGAR redirects cellular metabolism from glycolysis to the pentose phosphate pathway by lowering fructose-2,6-bisphosphate concentrations, which results in increased NADPH production, thereby reducing reactive oxygen species (ROS) levels and enhancing the resistance of cancer cells to ferroptosis (Bensaad et al., 2006; Liu et al., 2022; Liu et al., 2019). The association between the vitamin D receptor (VDR) and cancer predominantly pertains to the potential preventive and therapeutic benefits of vitamin D. Acting as the receptor for vitamin D, VDR modulates gene expression by binding to its active forms (such as calcitriol), thus impacting the growth, maturation, and programmed cell death of neoplastic cells (Campbell & Trump, 2017; Carlberg & Velleuer, 2022). The results of this study establish a fundamental framework for the subsequent advancement of targeted and individualized treatment strategies for acute myeloid leukemia (AML).

5. Conclusion

This study underscores the critical role of monocyte-related gene signatures in shaping immune function, metabolic regulation, and rehabilitation potential in Acute Myeloid Leukemia (AML) patients. By identifying key prognostic genes—FBXL5, TIGAR, VDR, and ZCWPW1—this research highlights how immune-metabolic interactions influence disease progression, treatment response, and physical resilience. Given that AML treatment often leads to muscle atrophy, fatigue, and impaired neuromuscular function, understanding the molecular mechanisms underlying monocyte-driven inflammation and metabolic dysfunction can provide valuable insights for optimizing precision therapy and rehabilitation strategies. The tumor microenvironment (TME) and monocyte-related immune activity play a crucial role in shaping the physical capacity, recovery trajectory, and long-term well-being of AML patients. This study highlights that low-risk patient subgroups, identified through monocyte-related gene expression patterns, exhibit stronger immune resilience, metabolic stability, and greater potential for physical recovery. These findings suggest that integrating metabolic reprogramming, immune-targeted therapy, and structured physical activity interventions could significantly enhance neuromuscular function, exercise tolerance, and rehabilitation outcomes in AML patients. From a sports science and rehabilitation perspective, these findings have important implications for developing exercise-based interventions tailored to immune and metabolic profiles in AML patients. Regular physical activity has been shown to enhance immune surveillance, reduce inflammatory cytokine levels, and improve neuromuscular coordination, all of which are essential for supporting functional independence and quality of life post-treatment. Additionally, targeted exercise

regimens focusing on resistance training, aerobic conditioning, and functional mobility exercises could help mitigate therapy-related fatigue, enhance metabolic efficiency, and promote recovery. Future research should focus on evaluating the impact of structured rehabilitation programs on immune modulation and metabolic adaptation in AML patients. Additionally, longitudinal studies assessing the interaction between monocyte-related gene expression, exercise physiology, and treatment outcomes could provide deeper insights into how precision therapy and rehabilitation can be integrated for improved long-term survivorship. By bridging oncology, immunology, and sports science, this study provides a foundation for developing multidisciplinary strategies that optimize both clinical outcomes and physical resilience in AML patients. Implementing personalized rehabilitation programs based on immune-metabolic profiling will be key to enhancing physical function, reducing treatment-related complications, and improving overall recovery in leukemia patients.

Declaration

Approval from an ethics review board and the acquisition of participant consent: This manuscript does not fall under the category of a clinical trial; therefore, the requirements for obtaining ethical approval and obtaining consent from participants are not relevant.

Availability of Data and Materials

The foundational data utilized in this study is accessible through the GEO database (https://www.ncbi.nlm.nih.gov/geo/), with the corresponding accession number being GSE37642, as well as the TCGA database (https://portal.gdc.cancer.gov/).

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References

Abdel-Rahman, S. A., Talagayev, V., Pach, S., Wolber, G., & Gabr, M. T. (2023). Discovery of Small-Molecule TIM-3 Inhibitors for Acute Myeloid Leukemia Using Pharmacophore-Based Virtual Screening. *J Med Chem*, *66*(16), 11464-11475.

Andrews, S. J., Ismail, Z., Anstey, K. J., & Mortby, M. (2018). Association of

Alzheimer's genetic loci with mild behavioral impairment. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *177*(8), 727-735.

Bensaad, K., Tsuruta, A., Selak, M. A., Vidal, M. N. C., Nakano, K., Bartrons, R., Gottlieb, E., & Vousden, K. H. (2006). TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *cell*, *126*(1), 107-120.

Campbell, M. J., & Trump, D. L. (2017). Vitamin D receptor signaling and cancer. *Endocrinology and metabolism clinics of North America*, *46*(4), 1009-1038.

Carlberg, C., & Velleuer, E. (2022). Vitamin D and the risk for cancer: A molecular analysis. *Biochemical Pharmacology*, *196*, 114735.

Chen, X., Li, Y., Xia, H., & Chen, Y. H. (2023). Monocytes in Tumorigenesis and Tumor Immunotherapy. *Cells*, *12*(13).

Engelhard, V., Conejo-Garcia, J. R., Ahmed, R., Nelson, B. H., Willard-Gallo, K., Bruno, T. C., & Fridman, W. H. (2021). B cells and cancer. *Cancer cell*, *39*(10), 1293-1296.

Gruszka, A. M., Valli, D., & Alcalay, M. (2019). Wnt Signalling in Acute Myeloid Leukaemia. *Cells*, *8*(11).

Harvey, K. F., Zhang, X., & Thomas, D. M. (2013). The Hippo pathway and human cancer. *Nat Rev Cancer*, *13*(4), 246-257.

Kayser, S., & Levis, M. J. (2023). The clinical impact of the molecular landscape of acute myeloid leukemia. *Haematologica*, *108*(2), 308-320.

Kukun, H., Jia, W., Yang, F., & Wang, Y. (2023). Clinical Utility of Automated Structural Brain Volume Analysis in MRI for Evaluating Temporal Lobe Epilepsy in Fitness Athletes. *Revista multidisciplinar de las Ciencias del Deporte*, *23*(91).

Laumont, C. M., Banville, A. C., Gilardi, M., Hollern, D. P., & Nelson, B. H. (2022). Tumour-infiltrating B cells: immunological mechanisms, clinical impact and therapeutic opportunities. *Nat Rev Cancer*, *22*(7), 414-430.

Liu, M.-Y., Li, H.-M., Wang, X.-Y., Xia, R., Li, X., Ma, Y.-J., Wang, M., & Zhang, H.-S. (2022). TIGAR drives colorectal cancer ferroptosis resistance through ROS/AMPK/SCD1 pathway. *Free Radical Biology and Medicine*, *182*, 219-231.

Liu, Z., Wu, Y., Zhang, Y., Yuan, M., Li, X., Gao, J., Zhang, S., Xing, C., Qin, H., & Zhao, H. (2019). TIGAR promotes tumorigenesis and protects tumor cells from oxidative and metabolic stresses in gastric cancer. *Frontiers in oncology*, *9*, 1258.

Mantovani, A., Vecchi, A., & Allavena, P. (2014). Pharmacological modulation of monocytes and macrophages. *Curr Opin Pharmacol*, *17*, 38-44.

Mustafa Ali, M. K., Williams, M. T., Corley, E. M., AlKaabba, F., & Niyongere, S. (2023). Impact of KRAS and NRAS mutations on outcomes in acute myeloid leukemia. *Leuk Lymphoma*, *64*(5), 962-971.

Newell, L. F., & Cook, R. J. (2021). Advances in acute myeloid leukemia. *Bmj*, *375*, n2026.

Olingy, C. E., Dinh, H. Q., & Hedrick, C. C. (2019). Monocyte heterogeneity and functions in cancer. *J Leukoc Biol*, *106*(2), 309-322.

Park, C. K., Heo, J., Ham, W. S., Choi, Y.-D., Shin, S. J., & Cho, N. H. (2021). Ferroportin and FBXL5 as prognostic markers in advanced stage clear cell renal cell carcinoma. *Cancer Research and Treatment: Official Journal of Korean Cancer Association*, *53*(4), 1174-1183.

Schlenk, R. F. (2023). Acute myeloid leukemia: introduction to a series highlighting progress and ongoing challenges. *Haematologica*, *108*(2), 306-307.

Shimony, S., Stahl, M., & Stone, R. M. (2023). Acute myeloid leukemia: 2023 update on diagnosis, risk-stratification, and management. *Am J Hematol*, *98*(3), 502-526.

Sun, Y., Tao, H., He, Z., & Liu, T. (2023). Percutaneous Kyphoplasty Enhanced with Calcium and Zoledronic Acid for Managing Thoracolumbar Compression Fractures in Athletes with Osteoporosis and Fitness Concerns. *Revista multidisciplinar de las Ciencias del Deporte*, *23*(91).

Ugel, S., Canè, S., De Sanctis, F., & Bronte, V. (2021). Monocytes in the Tumor Microenvironment. *Annu Rev Pathol*, *16*, 93-122.

Vinas-Castells, R., Frias, A., Robles-Lanuza, E., Zhang, K., Longmore, G. D., Garcia de Herreros, A., & Diaz, V. M. (2013). Nuclear ubiquitination by FBXL5 modulates Snail1 DNA binding and stability. *Nucleic acids research*, *42*(2), 1079-1094.

Wu, W., Ding, H., Cao, J., & Zhang, W. (2015). FBXL5 inhibits metastasis of gastric cancer through suppressing Snail1. *Cellular Physiology and Biochemistry*, *35*(5), 1764-1772.

Zheng, Y., & Pan, D. (2019). The Hippo Signaling Pathway in Development and Disease. *Dev Cell*, *50*(3), 264-282.