

ORIGINAL RESEARCH ARTICLE

Prognostic evaluation and immune microenvironment profiling of Ewing sarcoma through characterization of lipid metabolism-related genes

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Abstract

Introduction: Lipid metabolism contributes to tumor progression and immune regulation in Ewing sarcoma (EwS), but its relationship with prognosis and tumor immune microenvironment (TIME) remains inadequately clarified.

Objectives: This study investigated lipid metabolism-related gene (LMRG) subtypes, their associations with immune microenvironmental characteristics, and the prognostic value of an LMRG-based risk model in EwS.

Methods: Transcriptomic datasets of EwS from GEO (GSE17679; training cohort) and ICGC (validation cohort) were analyzed using computational techniques. LMRG subtypes were identified by consensus clustering. The TIME was inferred using ESTIMATE, TIMER algorithm, and single-sample gene set enrichment analysis (ssGSEA). A multigene risk score model was developed through LASSO and multivariable Cox regression, evaluated with Kaplan–Meier survival curves and time-dependent receiver operating characteristic (ROC) curves. A nomogram was constructed based on the model integrated with clinicopathologic variables.

Results: Two molecular subtypes showed distinct survival; the immune-enriched, low-purity subtype had poorer outcomes. A five-gene signature (TXNRD1, FABP5, ORMDL1, RAB5A, DBI) stratified patients into high- and low-risk groups, with AUCs of 0.90–0.94 in the training cohort and 0.58–0.85 in the validation cohort. The risk score was associated with increased ESTIMATE and immune scores, along with reduced tumor purity. The integrated nomogram achieved a C-index of 0.759 with acceptable calibration.

Conclusion: Dysregulated lipid metabolism is intricately linked to the TIME and patient prognosis in EwS. The LMRG-based risk model provides a potentially useful tool for prognostic stratification and highlights potential therapeutic avenues targeting lipid metabolism and immune modulation.

Keywords: Ewing sarcoma; Lipid metabolism; Tumor immune microenvironment; Prognostic signature; Nomogram

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1. Introduction

Ewing sarcoma (EwS) is an aggressive mesenchymal-derived malignant tumor arising primarily in bone and deep soft tissues, most commonly diagnosed in children and

adolescents. Even with surgery, chemotherapy, and radiotherapy, the prognosis for patients with recurrent or metastatic EwS is poor, with five-year survival rates still under 25% in many series.¹⁻³ At the molecular level, EwS has a specific translocation between EWSR1 from chromosome 22 and FLI1 from chromosome 11, which drives oncogenic transcription and promotes cellular transformation and oncogenicity.⁴⁻⁶ However, the intricate and varied characteristics of the EwS tumor microenvironment (TME) pose substantial challenges to the success of targeted therapies.²

Accumulating evidence indicates that metabolic reprogramming is central to the biology of EwS, influencing tumor progression, survival, and therapeutic resistance.^{6,7} EwS cells have been shown to exhibit enhanced glycolysis and glutamine metabolism while simultaneously activating serine–glycine biosynthesis and one-carbon metabolism, reprogrammed in part by the oncogenic EWSR1-FLI1 fusion, which promotes serine–glycine biosynthesis, glutamine uptake, as well as glycolysis and lactate dehydrogenase activity.^{4,6} This metabolic shift not only supports rapid proliferation but also supplies intermediates for lipid, amino acid, and nucleotide synthesis. Compared with some other metabolic pathways that mainly support tumor-intrinsic biosynthetic demands, lipid metabolism is increasingly recognized as a critical interface between cancer cell metabolism and immune regulation within the TME. Dysregulated lipid metabolism affects fatty acid availability, redox homeostasis, inflammatory signaling, and immune-cell differentiation, thereby contributing more directly to immune microenvironmental remodeling.^{8,9} As a result, lipid metabolism has increasingly been recognized as an important regulator of tumor growth, survival, and immune evasion, while altered lipid utilization may reshape the TME, modulate immune cell infiltration, and promote immunosuppressive states.^{1,10}

The immune microenvironment of EwS is marked by substantial infiltration of myeloid cells, tumor-associated macrophages, and immunosuppressive mediators that are closely related to tumor progression, metastasis, and resistance to therapy.² Studies have demonstrated that neutrophil extracellular trap formation,¹¹ as well as stromal remodeling mediated by pro-tumorigenic MIF–CD74 signaling,¹² collectively foster an immunosuppressive milieu that suppresses antitumor immune activity in EwS. In parallel, metabolic genes—including those regulating glycolysis, lipid metabolism, and oxidative stress—have been identified as independent prognostic signatures in EwS, further underscoring their clinical significance.¹⁰

Despite increasing evidence linking metabolism to prognosis, the relationship between lipid metabolism

and the immune microenvironment in EwS has not been delineated. Lipid metabolism is being acknowledged as a key factor in immune regulation, affecting T cell activation, myeloid cell differentiation, and cytokine production.^{13,14} Thus, dysregulation of lipid metabolic pathways may not only drive tumorigenesis and progression in EwS but also contribute to mechanisms of immune evasion.

In this study, we comprehensively profiled lipid metabolism-related genes (LMRGs) in EwS using public transcriptomic cohorts, explored their association with the tumor immune microenvironment (TIME), and assessed their prognostic potential. By integrating bioinformatics analysis with clinical outcome data, our work aimed to identify candidate biomarkers and potential therapeutic targets that link metabolic reprogramming to immune regulation in EwS.

2. Materials and methods

2.1. Data collection

RNA sequencing (RNA-seq) data and clinical characteristics of patients with EwS were retrieved from the Gene Expression Omnibus (GEO) database. The inclusion criteria included: (1) confirmed diagnosis of EwS; (2) availability of matched clinical data and gene expression matrices; and (3) sufficient clinical information, including survival time, survival status, age, and gender. The exclusion criteria included: (1) normal tissue samples; (2) samples lacking complete clinical information; (3) samples missing expression values for more than half of the genes; and (4) samples with biased expression profiles. A total of 88 samples from the GEO database (GSE17679) were designated as the training cohort, while 57 samples from the International Cancer Genome Consortium (ICGC) served as the validation cohort. To make the data comparable, the expression values were mapped to corresponding gene symbols based on the corresponding annotation files. The RNA-seq data from the ICGC cohort were normalized as $\log_2(\text{TPM} + 1)$ format, while the microarray probe values of GSE17679 were converted using $\log_2(X + 1)$ transformation. For genes matched by multiple probes, the average expression value was retained. Potential batch effects between cohorts were adjusted using the ComBat from the sva package.¹⁵ In addition, a total of 832 LMRGs were retrieved from the Molecular Signatures Database (MSigDB) website (<https://www.gsea-msigdb.org/gsea/msigdb/index.jsp>) using the term “Reactome Metabolism of Lipids”.¹⁶

2.2. Consensus clustering

Univariate Cox regression analysis was employed to initially screen genes, leading to the identification of 141 LMRGs

associated with EwS prognosis. Consensus clustering analysis was then performed to determine the LMRG subgroups using the R package “ConsensusClusterPlus,” with 1,000 replicates to ascertain stability. The optimal number of clusters was determined from the empirical cumulative distribution function plot.

2.3. LASSO Cox regression analysis and construction of LMRG prognostic signature

The prognostic significance of LMRGs in EwS was assessed by performing a least absolute shrinkage and selection operator (LASSO) Cox regression analysis with the R package glmnet on the training dataset, choosing the minimum lambda value as the optimal parameter. Subsequently, multivariate Cox regression was performed to construct the gene-based prognostic signature from the candidate LMRGs. The risk score for each patient was assigned using the following formula:

$$\text{Risk score} = \sum_{i=1}^n \text{Coef}_i * X_i \quad (1)$$

According to the median risk score, patients were categorized into high-risk and low-risk groups. The prognostic performance of the model was evaluated using Kaplan–Meier survival curves and time-dependent receiver operating characteristic (ROC) curves. The same formula was applied to calculate risk scores for patients in the validation cohort for validation.

2.4. Characterization of tumor immune microenvironment

To characterize the TIME, immune and stromal cells were inferred using the ESTIMATE algorithm, which generated immune score, stromal score, ESTIMATE score, and tumor-purity estimates for each sample. The TIMER database was utilized to thoroughly investigate the impact of these LMRGs genes on tumor-infiltrating immune-cell populations. Furthermore, the relative enrichment of 28 types of immune cells, sourced from the Molecular Signatures Database, was assessed through single-sample gene set enrichment analysis (ssGSEA).

2.5. Functional enrichment

The identification of differentially expressed genes (DEGs) between high-risk and low-risk groups was conducted using the R package limma, with the thresholds $|\log_2 \text{FC}| > 1.5$ and false discovery rate (FDR) < 0.05 . Functional enrichment was analyzed in R with the clusterProfiler package, including Gene Ontology (GO) analysis, Kyoto

Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and gene set enrichment analysis (GSEA).

2.6. Nomogram construction and evaluation

A model using a nomogram that incorporates risk scores and available clinical covariates was designed to estimate 1-, 3-, and 5-year overall survival in EwS patients. Model performance was assessed by the concordance index (C-index), calibration curves, and ROC analysis.

2.7. Statistical analyses

The statistical analysis and visualization tasks were carried out using R software, version 4.2.3. Wald tests were applied in both univariate and multivariate Cox regression models, while the log-rank test was used for comparisons in Kaplan–Meier survival analysis. Statistical significance was defined as a two-sided $p < 0.05$.

3. Results

3.1. Identification of LMRG subtypes

Consensus clustering based on prognostically relevant LMRGs separated the training cohort into two molecular subgroups, with most stable clustering at $k = 2$. Thirty-six samples were assigned to cluster 1 and 52 to cluster 2 (Figure 1A–1C). Heat map visualization demonstrated evident differences in LMRG-based transcriptomic partitions between the two subtypes (Figure 1D). Survival analysis based on the Kaplan–Meier method revealed that patients in cluster 2 had significantly improved overall survival compared to those in cluster 1 (Figure 1E). TIMER algorithm showed that cluster 1 displayed higher inferred infiltration of B cells, myeloid dendritic cells, and neutrophils, whereas CD4^+ T cells were relatively enriched in cluster 2 (Figure 2A–2F). Consistently, ssGSEA algorithm demonstrated that approximately half of the 28 tumor-infiltrating lymphocyte signatures (15/28) were clearly elevated in cluster 1 (Figure 2G and 2H). Furthermore, ESTIMATE analysis revealed that cluster 1 had significantly elevated immune and ESTIMATE scores, while its tumor purity was lower compared to cluster 2 (Figure 2I–2L). Notably, this immune-enriched phenotype was associated with worse survival, suggesting that the increased immune/stromal signal in cluster 1 may not represent effective antitumor immunity, but rather a dysfunctional immune context. Together, these findings indicate that LMRG-defined subtypes were closely linked to immune status, potentially affecting the overall survival of EwS patients.

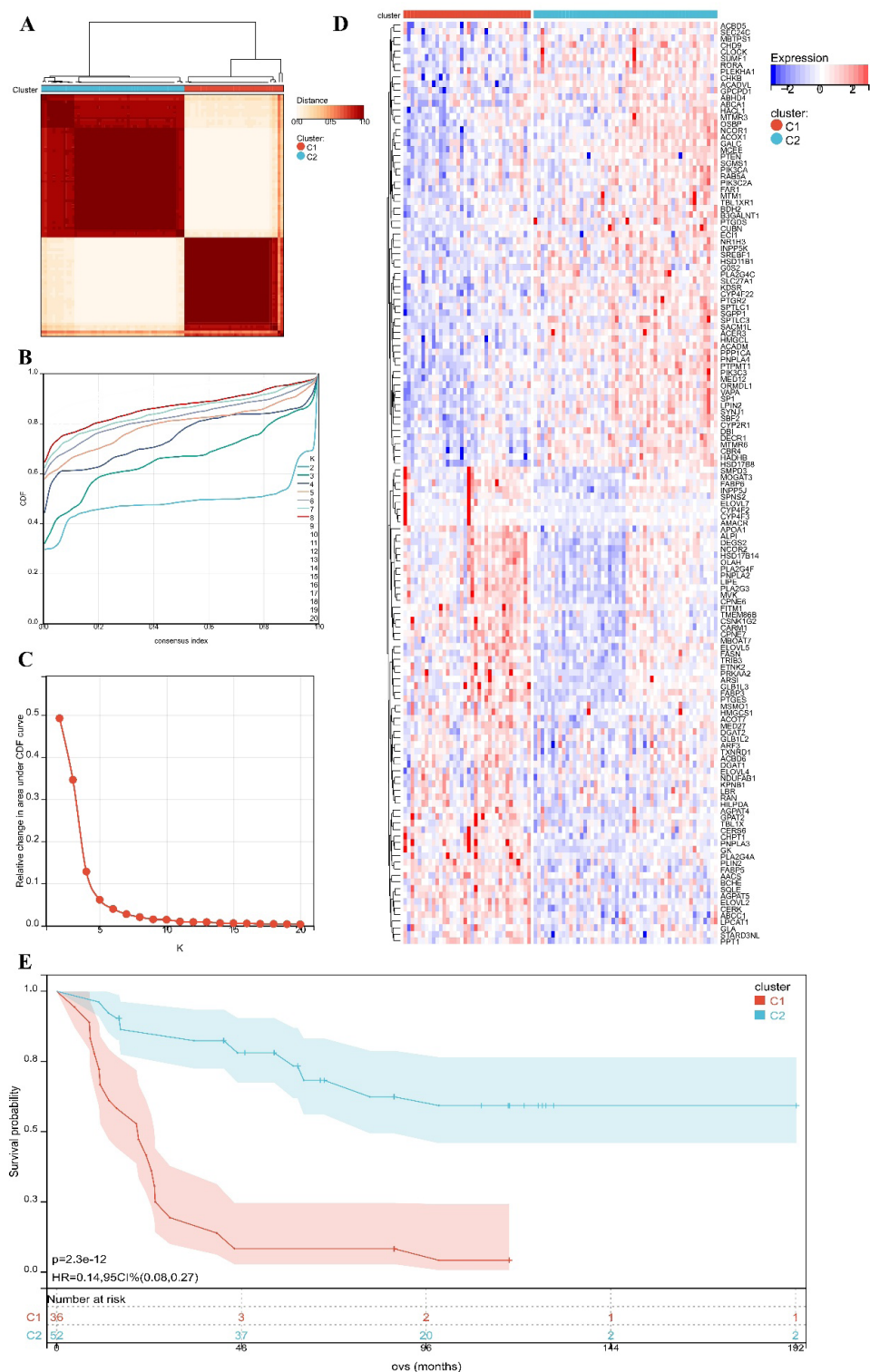


Figure 1. Characterization of lipid metabolism-related gene (LMRG)-based subtypes in Ewing sarcoma. (A) Consensus clustering matrix for the training cohort at $k = 2$. (B) Cumulative distribution function (CDF) curves across different clustering numbers (k). (C) Relative change in area under CDF curve for each k ; (D) Heatmap illustrating the expression profiles of prognostically relevant LMRGs in the two clusters. (E) Kaplan–Meier survival analysis of overall survival between the two clusters.

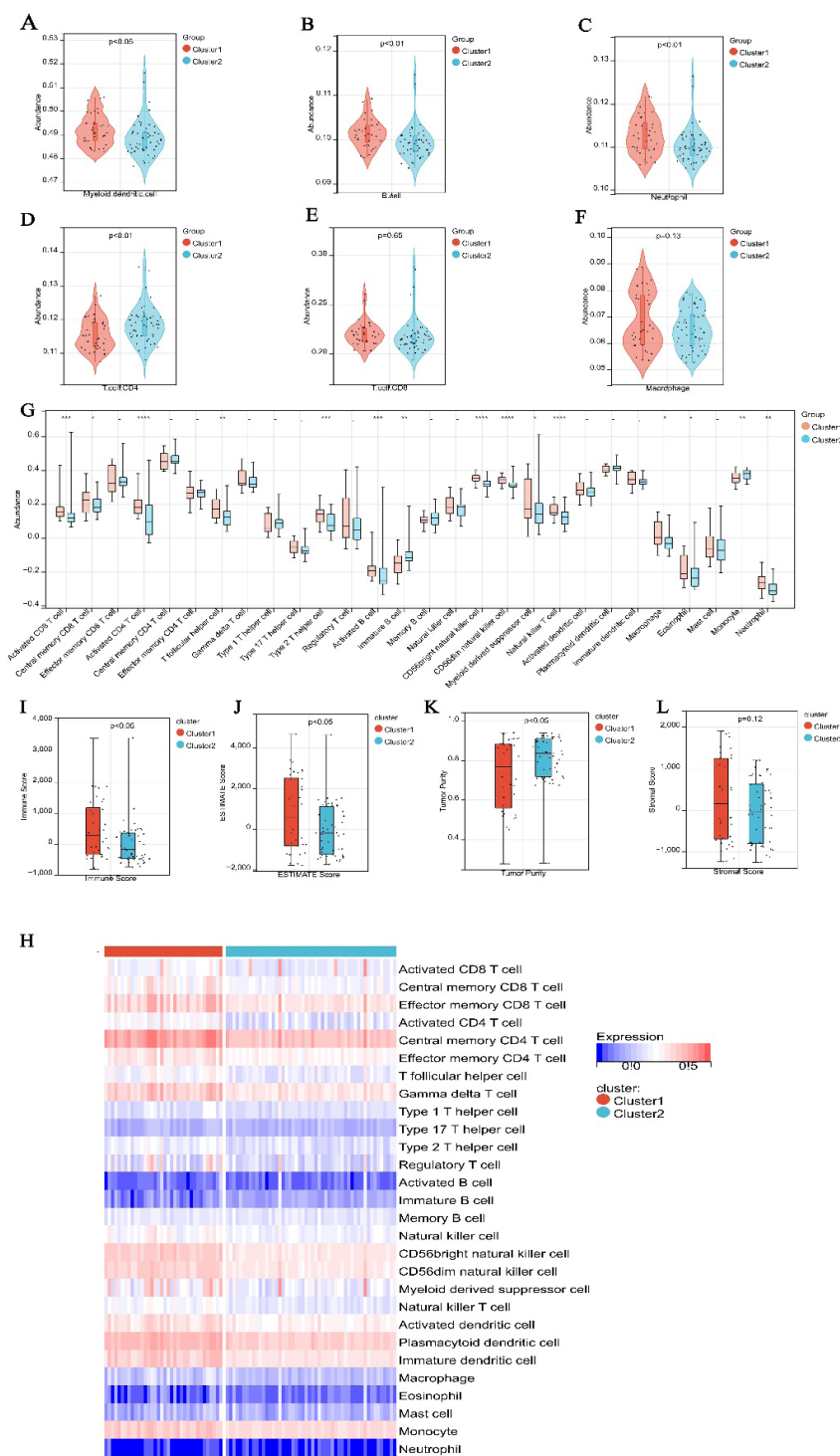


Figure 2. Immune-related analyses of the two lipid metabolism-related gene (LMRG)-based clusters in Ewing sarcoma. (A–F) Comparison of the abundance of myeloid dendritic cells, B cells, neutrophils, CD4⁺ T cells, CD8⁺ T cells, and macrophages between the two clusters as estimated by the TIMER algorithm. (G) Comparison of ssGSEA scores for 28 immune-cell signatures between the two clusters. (H) Heatmap display of the ssGSEA scores between the two clusters. (I–L) Comparison of immune score, ESTIMATE score, tumor purity and stromal score between the two groups, as calculated using ESTIMATE algorithm. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3.2. Construction of LMRGs risk signature in the training cohort

To quantify the prognostic relevance of LMRGs in EwS, a multigene risk signature model was constructed. Thirteen candidate genes were retained using LASSO analysis with the minimal lambda values (Figure 3A). Subsequently, five candidate genes were identified through multivariate Cox analysis: *TXNRD1*, *FABP5*, *ORMDL1*, *RAB5A* and *DBI*. Among them, *TXNRD1* and *FABP5* were identified as risk factors, as both had positive coefficients in the risk formula, whereas *ORMDL1*, *RAB5A*, and *DBI* were identified as protective factors, as they had negative coefficients. The final LMRG-based risk score for each sample was calculated as follows:

$$\text{Risk score} = 0.238 * \text{FABP5} + 0.085 * \text{TXNRD1} - 0.489 * \text{ORMDL1} - 0.14 * \text{RAB5A} - 0.013 * \text{DBI} \quad (2)$$

By employing the median risk score as the cutoff, EwS patients were successfully stratified into high-risk and low-risk groups. The ranking plots and the heatmap showed that the risk factors *TXNRD1* and *FABP5* were more highly expressed in the high-risk group, whereas the protective factors *ORMDL1*, *RAB5A* and *DBI* were more highly expressed in the low-risk group (Figure 3B). The Kaplan–Meier analysis revealed that patients in the high-risk group had notably worse overall survival (Figure 3C). Additionally, the area under the ROC curve (AUC) values for the risk signature were 0.94, 0.90, and 0.90 at 1, 3, and 5 years, respectively (Figure 3D). The ssGSEA algorithm indicated that patients in the high-risk group exhibited significantly increased infiltration of most immune cell signatures (Figure 3E). Consistently, ESTIMATE analysis showed higher immune and ESTIMATE scores along with lower tumor purity in the high-risk group (Figure 3F–3I). Collectively, these outcomes implied that the LMRG-based signature has prognostic relevance and associates with TIME in EwS.

3.3. Validation of the LMRG risk signature

The performance of prognostic risk score model was further validated in the validation cohort. Using the aforementioned formula, EwS cases in the validation cohort were categorized into high- and low-risk groups. The heatmap confirmed the expression of the five candidate genes (Figure 4A). The Kaplan–Meier analysis showed that the high-risk group experienced worse overall survival (Figure 4B; $p = 0.02$). The ROC analysis indicates that the risk model achieved AUC values of 0.85, 0.65, and 0.58 at 1, 3, and 5 years, respectively (Figure 4C). These results indicate that the model had relatively

favorable short-term predictive performance, whereas its long-term predictive accuracy in the external cohort was more limited, especially at 5 years. Consistent with the training cohort, ssGSEA revealed that numerous immune-cell signatures were significantly lower in the low-risk group compared with the high-risk group (Figure 4D). These findings suggest that the developed LMRG-based risk model has prognostic relevance in EwS, although its predictive performance appears to be stronger in the short term than in the long term.

3.4. Development and evaluation of an integrated nomogram

Subsequently, a nomogram integrating the LMRG-based risk score with clinicopathologic variables, including metastasis and recurrence, was designed to provide a more comprehensive prognostic model for patients with EwS. As shown in Figure 5A, the risk score and each clinicopathologic characteristic were assigned a specific value proportional to its prognostic contribution, and the summed points correspond to estimated 1-, 3-, and 5-year survival probabilities. The C-index for the nomogram estimating EwS survival was 0.759, while the ROC curve revealed AUC values of 0.93, 0.95, and 0.92 at 1, 3 and 5 years, respectively (Figure 5B), suggesting better overall prognostic performance than the gene signature alone. Furthermore, calibration curves showed good agreement between the predicted and observed survival probabilities at 1, 3 and 5 years (Figure 5C). These findings suggest that the nomogram has potential utility for individualized prognostic assessment in EwS.

3.5. DEG and functional enrichment analyses

To understand the mechanisms behind the prognostic impact of the LMRG-based model in EwS, differential expression analysis was conducted between the high- and low-risk groups. As depicted in Figure 6A, 23 genes were significantly upregulated, whereas 89 genes were downregulated. According to the GO analysis, these DEGs were mainly connected to functions related to the immune function or nervous system, such as inflammatory response, myeloid leukocyte migration, mononuclear cell migration, leukocyte chemotaxis, nervous system development, and glial cell development (Figure 6B). The analysis of KEGG pathway demonstrated that the DEGs were predominantly enriched in pathways related to immune and inflammatory processes, in addition to tumor-associated signaling, such as IL-17 signaling pathway, rheumatoid arthritis, legionellosis, and PPAR signaling pathway (Figure 6C). In addition, GSEA analysis found that pathways such as microglia differentiation; positive regulation of CD4⁺ αβ T-cell proliferation; positive regulation of antigen

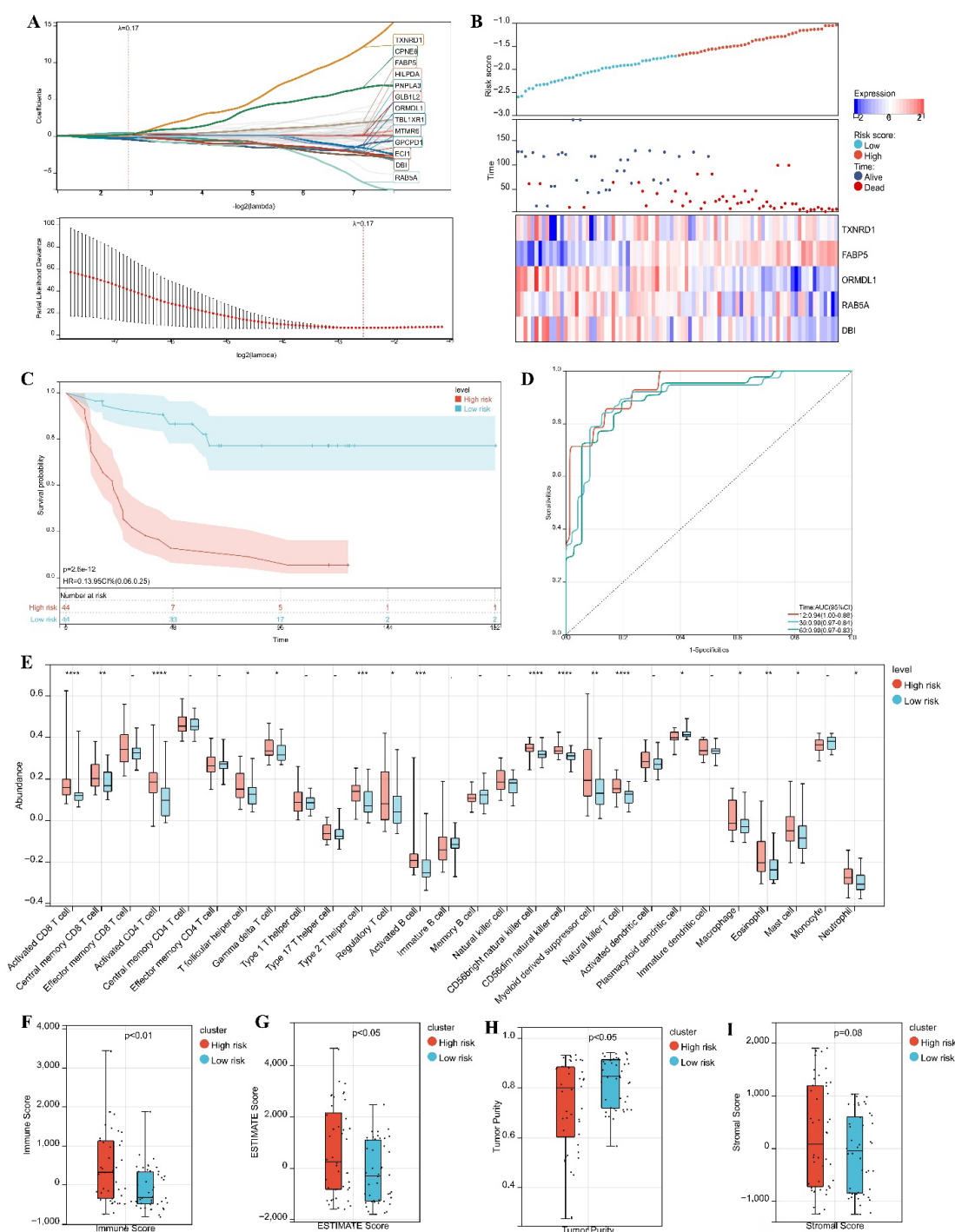


Figure 3. The lipid metabolism-related genes (LMRGs)-based risk signature in the training cohort. (A) LASSO coefficients of the thirteen prognostic LMRGs. (B) Distribution of risk score, survival status, and expression of the five prognostic LMRGs in the high and low risk groups, showing that the risk factors (*TXNRD1* and *FABP5*) were more highly expressed in the high-risk group, whereas the protective factors (*ORMDL1*, *RAB5A*, and *DBI*) were more highly expressed in the low-risk group. (C) Kaplan–Meier survival curve of the Ewing sarcoma in the high- and low-risk groups. (D) Time-dependent ROC curves evaluating the prognostic performance of the LMRGs-based signature in the training cohort. (E) Comparison of the ssGSEA scores for immune cells signatures between the high- and low-risk groups. (F–I) The relationship between risk score and immune score, stromal score, ESTIMATE score, and tumor purity. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

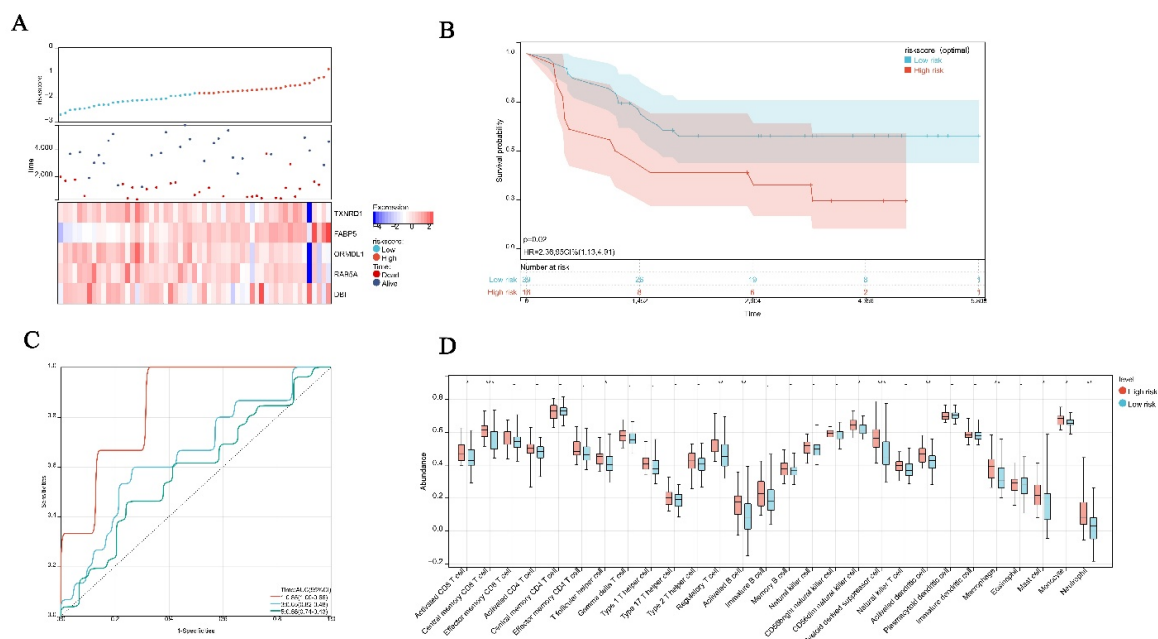


Figure 4. Validation of the risk model in the validation cohort. (A) Distribution of risk score, survival status, and expression of the prognostic lipid metabolism-related genes (LMRGs) in the validation cohort. (B) Kaplan–Meier survival curve of the Ewing sarcoma in the high- and low-risk groups in the validation cohort. (C) Time-dependent ROC curves evaluating the prognostic performance of the LMRG-based signature in the validation cohort. (D) Comparison of the ssGSEA scores for immune cells between the high- and low-risk groups in the validation cohort.

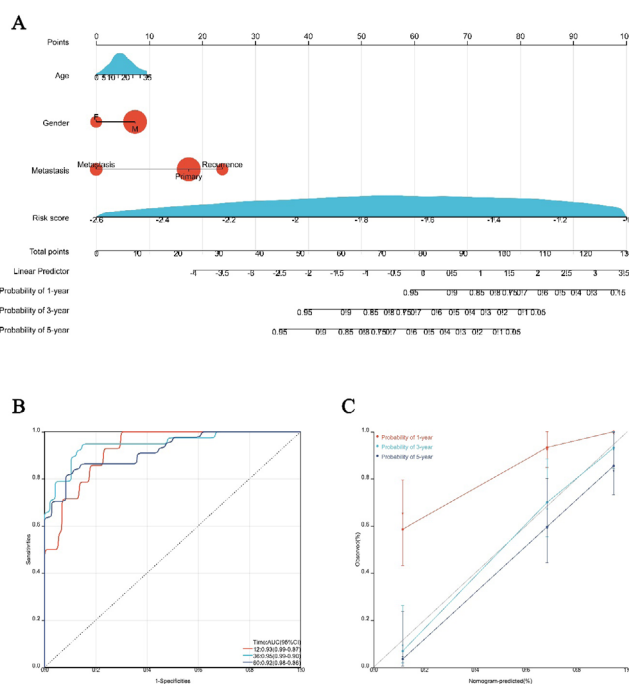


Figure 5. Construction and evaluation of the prognostic nomogram in Ewing sarcoma. (A) Nomogram construction based on risk score and clinicopathologic variables for predicting 1-, 3-, and 5-year overall survival. (B) Time-dependent ROC curves evaluating the predictive performance of the nomogram. (C) Calibration curves of the nomogram for 1-, 3-, and 5-year overall survival prediction.

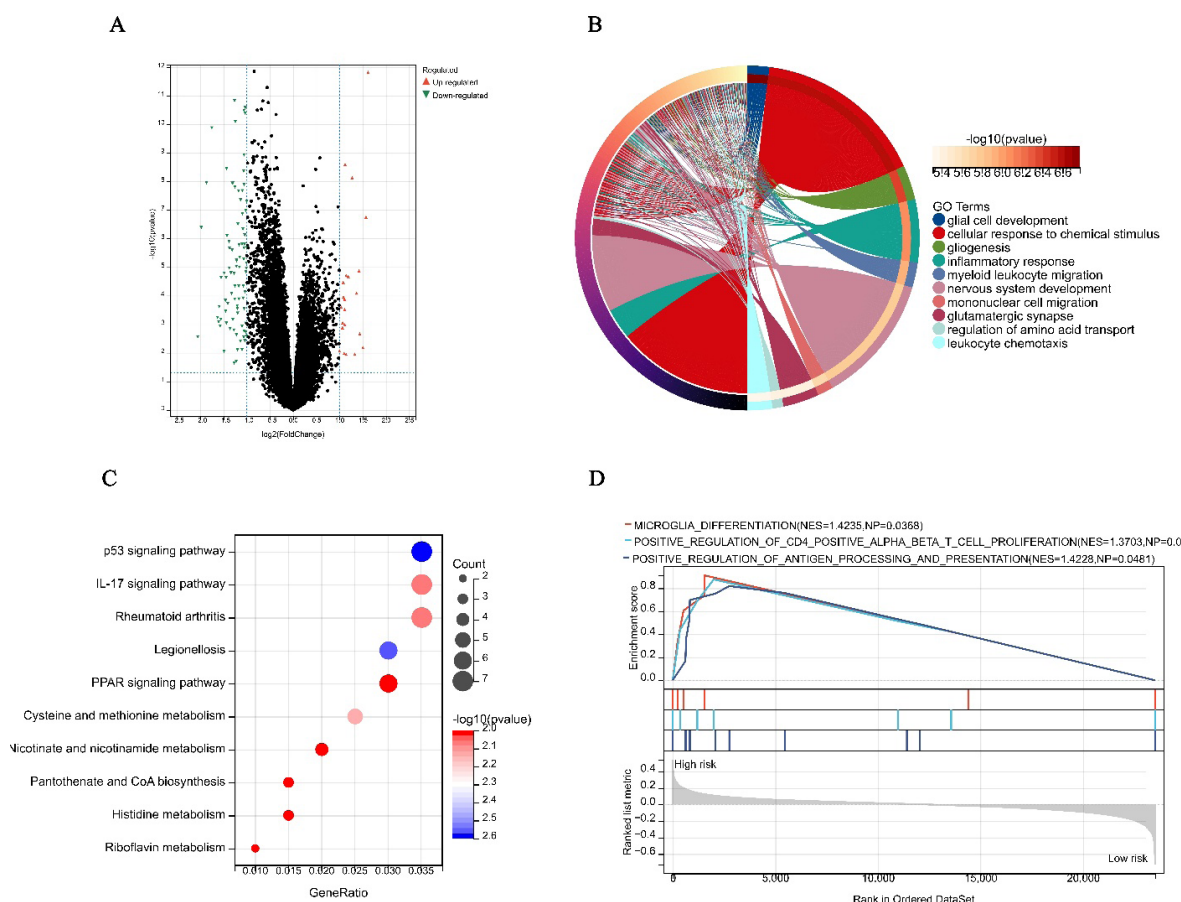


Figure 6. Differentially expressed genes (DEGs) analysis and functional analyses between the high- and low-risk groups. (A) Volcano plot showing DEGs expression between the high- and low-risk groups. (B) Circle plot of the top 10 GO pathway analysis enrichment results. (C) Bubble diagram of enriched KEGG pathways. (D) GSEA of representative biological functions between the high- and low-risk groups. Abbreviations: GO: Gene Ontology; GSEA: Gene set enrichment analysis; KEGG: Kyoto Encyclopedia of Genes and Genomes.

processing and presentation were significantly more enriched in the high-risk group than in the low-risk group (Figure 6D).

4. Discussion

Ewing sarcoma (EwS) is an extremely aggressive bone malignancy primarily targeting children, adolescents, and young adults. Despite advances in multimodal therapies, the survival outcomes for EwS patients remain unsatisfactory, and it is imperative to identify key genes and establish a risk classification strategy for providing personalized, targeted treatment. In the present study, we identified lipid metabolism-related molecular subtypes of

EwS, developed and validated a prognostic risk model, and revealed a strong connection between dysregulated lipid metabolism and TIME.

Using consensus clustering, we identified two LMRG-based molecular subtypes with distinct survival outcomes and immune characteristics. Patients in subtype with higher immune scores were more likely to exhibit poorer prognosis and lower tumor purity than those with better outcomes, suggesting that immune enrichment in EwS may reflect a dysfunctional or immunosuppressive microenvironment rather than effective antitumor immunity. We further developed and externally validated an LMRG-based prognostic model, which showed potential

value for risk stratification and may aid in predicting patient prognoses. Functional enrichment analyses further indicated that lipid metabolism-related risk patterns were associated with immune and inflammatory pathways, supporting a possible link between lipid metabolism and TIME remodeling. These findings may help clinicians to identify patients at high risk for EwS and provide a basis for future studies on lipid metabolism–targeted and immune-modulating strategies.

Our study specifically focused on lipid metabolism because this metabolic program is positioned at the interface of tumor biology and immune regulation. Lipids serve as structural membrane components, energy substrates, signaling molecules, and precursors of inflammatory mediators.^{8,17} In cancer cells, enhanced fatty acid synthesis and altered lipid uptake can support proliferation, invasion, survival, redox adaptation, and resistance to nutrient-limited microenvironments.¹⁷ In immune cells, lipid availability and lipid-processing pathways influence macrophage polarization, dendritic-cell function, T-cell activation, T-cell exhaustion, and regulatory T-cell differentiation.¹⁸ Thus, lipid metabolism is not merely a biosynthetic pathway but also a microenvironmental regulator.⁸ This dual role may partly explain why LMRG patterns in our study were simultaneously associated with survival, tumor purity, immune scores, and inflammatory pathway enrichment.

Consensus clustering analysis is an unsupervised method commonly employed to explore cancer subtypes within gene expression datasets.¹⁹ In this study, we identified two molecular subtypes with LMRG-based transcriptomic partitions and significantly different overall survival outcomes. It should be noted that the two clusters identified were derived from a preselected set of LMRGs with prognostic relevance, instead of using an unbiased whole-transcriptome analysis, while the relatively small cohort size may also have contributed to the observed separation. This more cautious interpretation is also consistent with recent studies indicating substantial transcriptional heterogeneity and microenvironmental complexity in EwS.²⁰ In the meantime, the Immune analyses revealed that the immune system is significantly affected by lipid metabolism. A growing body of evidence suggests that the TIME plays a critical role in the progression of various sarcomas, thus influencing the patient prognosis.^{19,21} The ESTIMATE algorithm utilizes gene expression signatures to gauge immune and stromal cells and to estimate tumor purity.²² Importantly, the prognostic significance of immune enrichment is highly context-dependent and depends not only on the abundance but also on the composition and functional

state of infiltrating immune cells. Previous investigations have found that patients with increased immune scores and decreased tumor purity may exhibit an immune-evasive or immunosuppressive microenvironment and have a poorer prognosis in EwS.²³ In addition, macrophage-rich immune infiltration has been associated with unfavorable survival in EwS,²⁴ whereas more recent integrative analyses suggest that macrophage-associated immune states may be linked to poor prognosis, while CD8⁺ T-cell infiltration may correlate with more favorable outcomes.¹² These observations are consistent with our findings. Specifically, both TIMER and ssGSEA showed that the abundance of immune cells was notably elevated in cluster 1, in agreement with the ESTIMATE results. However, this immune-enriched phenotype was accompanied by worse survival, suggesting that the increased immune signal in cluster 1 may reflect a myeloid-dominant, dysfunctional, or immunosuppressive inflammatory state rather than effective antitumor immunity. The lower tumor purity observed in this subgroup suggests that part of the prognostic signal may derive from stromal and immune admixture, rather than solely from tumor-intrinsic lipid metabolic programs. Altogether, it could be inferred that aberrant lipid metabolism may reshape the immune microenvironment in ways that contribute to tumor progression and poor prognosis.²⁵

Recently, the significance of dysregulated lipid metabolism in tumors has been increasingly highlighted.^{13,14} Both preclinical and clinical research have demonstrated that various targeted lipid metabolism strategies exhibit antitumor effects. Lipid metabolism dysregulation represents one of the most crucial metabolic alterations observed in cancers.²⁶ There is growing evidence that cancer cells rely on reprogrammed lipid metabolism for unregulated proliferation and survival. However, the mechanisms underlying lipid metabolism dysregulation and its impact on EwS are not clearly understood.²⁶ The cytotoxic effects of inhibitors targeting key enzymes involved in lipid metabolism underscore the essential role of this metabolic pathway for EwS cell survival, such as orlistat targeting fatty acid synthase (FASN) and myriocin against SPTLC1. In addition, targeting lipid metabolism reprogramming may represent a promising therapeutic strategy in EwS; notably, denifanstat (TVB-2640), a clinical FASN inhibitor, has shown antitumor activity in other malignancies by suppressing *de novo* fatty acid synthesis and thereby limiting tumor cell growth and proliferation. Recent reviews support general mechanisms by which lipid metabolism reprogramming shapes the TIME, including suppressing CD8⁺ T-cell function and promoting regulatory T-cell (Treg)-mediated immunosuppression.^{8,27} For example, excess lipids in the TME dampen CD8⁺

T-cell cytotoxicity,¹⁸ while lipid-laden environments support immune evasion and immunosuppressive cell phenotypes.^{28,29}

To gain a clearer insight into how lipid metabolism disorders impact the TIME and to evaluate the prognostic significance of LMRGs, we developed and confirmed a prognostic risk signature composed of five LMRGs (TXNRD1, FABP5, ORMDL1, RAB5A and DBI). TXNRD1 and FABP5 showed positive coefficients and were therefore defined as risk factors, whereas ORMDL1, RAB5A, and DBI showed negative coefficients and were considered protective factors. Notably, patients classified as the high-risk group also exhibited elevated immune infiltration scores in the prognostic model, supporting a close association between lipid metabolism and immune regulation in EwS.

Each identified gene has a role in tumor biology and regulation of the immune system. TXNRD1 encodes thioredoxin reductase 1, a selenoenzyme that reduces oxidized thioredoxin and thereby maintains cellular redox balance under oxidative stress. In EwS, inhibition of TXNRD1 elevates reactive oxygen species levels and suppresses protein synthesis, indicating that TXNRD1 activity contributes to tumor cell survival under oxidative challenge.³⁰

As a critical regulator of lipid metabolism, FABP5 was identified in our study as a risk factor with a positive coefficient and was more highly expressed in the high-risk group, suggesting that its upregulation may contribute to the unfavorable prognosis observed in this subgroup. This interpretation is supported by a previous EwS study, in which FABP5 was incorporated into a metabolism-related prognostic signature, further supporting its adverse prognostic relevance in EwS.⁴ Functionally, FABP5 contributes to EwS pathogenesis by enhancing fatty acid oxidation and maintaining redox balance, while its overexpression correlates with metastatic potential and resistance to therapy.³¹ Beyond its tumor-intrinsic metabolic role, increasing evidence suggests that elevated FABP5 expression is associated with increased Treg infiltration and an immunosuppressive tumor microenvironment, which may contribute to disease progression in EwS.⁴ Moreover, FABP5 has been shown to regulate lipid uptake and mitochondrial fatty acid oxidation in T cells and to be upregulated in partially exhausted CD8⁺ T cells, which are enriched for PD-1 positivity and display features of dysfunctional immune activation.³² This dual role underscores its significance as both a driver of tumor progression and a modulator of immune dysfunction within the EwS microenvironment.²⁸ Although our findings remain correlative and direct

mechanistic evidence in EwS is still limited, they support the possibility that FABP5 represents a molecular link between lipid metabolic reprogramming and immune dysfunction in EwS.

By contrast, evidence for ORMDL1 in EwS remains limited. The *ORMDL1* gene encodes an endoplasmic reticulum-resident protein that functions as a tumor suppressor by maintaining endoplasmic reticulum homeostasis and attenuating stress-induced apoptosis, with higher expression linked to favorable outcomes in colorectal cancer.³³ In our model, ORMDL1 behaved as a protective factor, but its specific functional significance in EwS requires further investigation.

Similarly, the interpretation of RAB5A as a protective factor in the present study is based largely on cross-cancer evidence. RAB5A is a small GTPase involved in clathrin-mediated endocytosis, where it regulates the fusion of endocytic vesicles to early endosomes. In specific cancer contexts, such as HER2-positive breast cancer, elevated RAB5A expression has been associated with improved sensitivity to antibody–drug conjugates and more favorable clinical outcomes; however, its precise role in EwS remains unclear.^{34,35} In addition, RAB5A-related pathways have been implicated in macrophage reshaping, complement receptor trafficking, chemotaxis, and chemokine secretion, suggesting that altered RAB5A expression may influence tumor–immune communication and stromal–immune signaling.³⁶

Diazepam-binding inhibitor (DBI) links lipid metabolism more directly to immune regulation. Intracellular DBI supports lipid metabolism and cell survival, whereas extracellular DBI has been reported to suppress autophagy and inhibit anticancer immunosurveillance.^{37,38} In addition, DBI plays a critical role in appetite stimulation and lipogenesis, also functions as an endogenous immune suppressor, with its neutralization demonstrating enhanced efficacy of immunotherapy in breast cancer, non-small-cell lung cancer, and sarcoma.³⁹ Taken together, these findings suggest that RAB5A and DBI may participate in lipid–immune crosstalk through vesicular trafficking, immune-cell signaling, and metabolic regulation, although their specific roles in EwS require further experimental validation.

In both the training and validation cohorts, survival analysis indicated that the risk model had prognostic value for patients with EwS. Although the model showed relatively good short-term predictive performance, its long-term discriminatory ability in the external validation cohort was more modest, suggesting that further refinement and validation in larger independent cohorts are needed. Poor survival was associated with elevated ESTIMATE scores

and immune scores, together with reduced tumor purity. Moreover, the integrated nomogram that combined the risk score with clinical variables demonstrated promising predictive performance and may provide a useful framework for individualized prognostic assessment in EwS. By incorporating the LMRG-based risk score together with clinicopathologic variables, the nomogram may offer a more comprehensive prognostic evaluation than the gene signature alone. However, because the model was developed and evaluated in retrospective cohorts, it should be considered hypothesis-generating and requires prospective validation in larger independent populations before clinical application. Nevertheless, this approach may still provide additional prognostic information for identifying high-risk patients and guiding treatment strategies accordingly.

Functional enrichment analyses indicated that DEGs between the high- and low-risk groups were closely associated with immune-related pathways. Immune cells play dual roles in tumor development by suppressing tumor growth or, under certain microenvironmental conditions, promoting tumor progression.⁴⁰ In our study, GO and KEGG analyses suggested that dysregulated immune and inflammatory responses, together with neural development-related programs, were associated with the transcriptomic differences observed between the two risk groups. Notably, the PPAR signaling pathway emerged from KEGG analysis as a potentially important link between lipid metabolism reprogramming and immune microenvironmental alterations. PPARs are well-recognized lipid sensors that regulate fatty acid metabolism and have also been implicated in immune-cell differentiation, activation, and metabolic fitness.^{9,41} GSEA is a widely used method for evaluating coordinated changes in predefined gene sets and provides clear insights into the expression patterns of gene sets in diverse categories. In addition, the GSEA analysis supports these findings. In this research, the GSEA showed that gene sets related to immune cell proliferation and antigen presentation were relatively high in the high-risk cluster. These findings suggest that decreased lipid breakdown and increased lipid hydroxylation may be associated with impaired immune function and inadequate bone remodeling. However, further mechanistic studies are required to determine whether and how lipid metabolic dysregulation directly contributes to these biological processes.

Our study has several limitations that need to be taken into account. First, the study was based on retrospective datasets and requires prospective validation in larger independent cohorts. Second, as this study relied exclusively on transcriptomic data, the functional

roles of the identified genes in lipid metabolism and immune regulation in EwS remain to be further validated. External biological validation at the protein and functional levels, such as immunohistochemistry, proteomics, and mechanistic assays, will be important in future work. Finally, while our findings suggest potential therapeutic implications of targeting lipid metabolism, preclinical and clinical trials are required to evaluate combined strategies with immunotherapies.

5. Conclusion

This study indicates that dysregulated lipid metabolism is intricately linked to immune microenvironment and prognosis in patients with EwS. The identified LMRG-based risk model may provide a potentially useful tool for prognostic stratification and highlights potential therapeutic avenues targeting lipid metabolism and immune modulation.

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Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Kailuo Xie, Jin Hu

Data curation: Wenbin Liu

Formal analysis: All authors

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Writing—original draft: Kailuo Xie

Writing—review & editing: Kailuo Xie, Jin Hu

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The datasets analyzed in this study are publicly available. The GEO dataset can be accessed from the Gene Expression Omnibus under accession number GSE17679. The ICGC dataset can be obtained from the International Cancer

Genome Consortium (ICGC) data portal. All data used in this study are available from the corresponding public repositories.

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