Identification of hub anoikis-associated genes and risk signature in cutaneous melanoma

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Abstract. – OBJECTIVE: As one of the most lethal and aggressive cutaneous malignancies, cutaneous melanoma (CM) greatly threatens human health and has long challenged clinicians because of its poor therapeutic response. Anoikis is a newly discovered form of apoptosis that was originally identified in the extracellular matrix (ECM). Recent studies have reported that anoikis is central to cancer metastasis. The aim of this study is to explore the role of anoikis-associated genes in CM.

MATERIALS AND METHODS: We identified hub anoikis-associated genes in CM and constructed a risk signature for patients with CM. Gene expression from The Cancer Genome Atlas (TCGA) database was used to screen hub anoikis-associated genes connected with CM, and the Gene Expression Omnibus (GEO) data-set was applied to externally validate the identified genes. Weighted gene co-expression network analysis (WGCNA), differential expression, univariate Cox regression, and least absolute shrinkage and selection operator (LASSO) analyses were used to identify hub genes. Immune cell infiltration in CM was also evaluated to explore the association between hub genes and immune heterogeneity. Finally, an anoikis-associated prognostic model was constructed.

RESULTS: Following complex analysis, FASLG, SOD2, BST2, PIK3R2, IKZF3, CDK2, and RAC3 were identified as hub anoikis-associated genes. Indeed, Kaplan-Meier and receiver operating characteristic analyses suggested that the expression patterns of hub genes can be used as prognostic factors for CM survival. The expression and survival trends of hub genes were verified in the validation cohort. Immune cell infiltration analysis showed that the number of immune cells varied among patients with CM and identified seven genes. Furthermore, functional analyses indicated that the constructed risk signature was significantly associated with patient survival, age, and tumor growth and could also serve as an independent prognostic factor for patients with CM.

CONCLUSIONS: We suggest that the hub genes FASLG, SOD2, BST2, PIK3R2, IKZF3, CDK2, and RAC3 are involved in the anoikis-associated signature. The pattern of hub anoikis-associated genes may have a prognostic potential for CM progression and overall patient survival.

Key Words: Anoikis, Cutaneous melanoma, Risk signature, Immune cell infiltration.

Introduction

Cutaneous melanoma (CM) is a life-threatening, aggressive, malignant tumor. Based on existing investigations, 287,723 people were diagnosed with melanoma worldwide, and 60,712 died of this disease in 2018. Recently, numerous experimental and clinical studies have trialed novel drugs for cancer treatment, such as extracts and cucurbitacin B from Luffa operculata (L.) Cogn, essential oils from Ipomoea L. species, chalcones, methoxylated fractions from Vellozia dasypus Seub, and eleutherin and isoeleutherin from Eleutherine plicata. However, due to the pathogenic complexity of CM and the fact that the precise pathogenesis of the disease is still unknown, there is currently no effective treatment. According to one report, the 10-year overall survival (OS) rates of patients with stage I and II CM are 75-98%. In contrast, only 24-88% of pa-
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Patients with stage IIIA to IIID CM survived after 10 years, suggesting that early CM diagnosis may affect its outcome. Many investigators\(^{10,11}\) have attempted to identify novel biomarkers for the prognostic prediction and personalized therapy of patients with CM; however, only a few biomarkers of clinical significance have been identified\(^{10}\). Therefore, the identification of new biomarkers that can accurately predict the prognosis of patients with CM is urgently needed.

Apoptosis is a central part of organismal protection mechanisms; it can prevent the abnormal proliferation of detached cells by inhibiting re-adherence. Anoikis was originally discovered in endothelial and epithelial cells as a specific form of cell apoptosis. It is caused by cells detached from the extracellular matrix (ECM) and is believed to play a vital role in tissue homoeostasis and development. Recently, studies\(^{11-14}\) have reported that anoikis also participates in the detachment of various cancer cells, including endometrial carcinoma\(^{11}\), lung cancer\(^{12}\), gastric carcinoma\(^{13}\), and breast carcinoma\(^{14}\), from the ECM during metastasis. This indicates that anoikis contributes to the progression of cancer distal metastases and has the potential to be a hallmark of cancer. For example, the anoikis-associated genes KLF5 and FAIM2 are associated with the prognosis of colorectal and lung cancer, respectively, and silencing KLF5 and FAIM2 can significantly inhibit cancer cell anoikis resistance and proliferation\(^{15}\). LICAM, another anoikis-associated gene, can also promote anoikis resistance and influence the prognosis of endometrial carcinoma patients by boosting epithelial-mesenchymal transformation\(^{11}\).

Although anoikis is demonstrably relevant to tumor prognosis and progression, its specific value in CM has not been closely analyzed. In this study, weighted gene co-expression network analysis (WGCNA), differential expression, univariate Cox regression, and least absolute shrinkage and selection operator (LASSO) analyses were comprehensively applied to identify anoikis-associated genes in patients with CM and enhance the discriminatory ability of highly connected genes. Based on the hub anoikis-associated genes, immune infiltration, functional enrichment, and transcription factor regulatory network analyses were performed in CM, and a risk signature was constructed to explore the value of anoikis-associated genes in prognostic prediction, aiming to provide a novel predictive prognostic tool for patients with CM.

Materials and Methods

Data Collection and Processing

Normal skin and CM RNA sequencing datasets TCGA-SKCM (CM samples = 471, normal samples = 1), GSE65904 (CM samples = 214), and GTEx-skin (normal samples = 812) with reliable sample sources were collected from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/), Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/), and the University of California Santa Cruz Xena (UCSC, https://xena.ucsc.edu) databases. All samples were obtained from humans and GSE65904 was used for external validation. Gene expression data from different platforms were normalized and converted into a single matrix using the R package “limma”. Subsequently, the R package “sva” was used to remove the batch effects. A total of 501 anoikis-associated genes (AGs) with a relevance score > 0.4 were extracted from the GeneCards database (https://www.genecards.org/). All public databases in this study were searched following the relevant guidelines, and no ethical approval was required from the Ethics Committee of the First People’s Hospital of Linping District.

Screening for Overlapping Candidate AGs

To improve the discriminatory capacity of highly connected genes, three different bioinformatics tools, WGCNA, differential expression analysis, and univariate Cox regression analysis, were used to screen differentially expressed genes associated with anoikis and were significantly related to CM prognosis. Differential expression analysis for AGs in control skin and CM samples were conducted using the R package “limma” with the criteria |log fold change (FC)| > 1 and p < 0.05. The packages “ggplot2” and “pheatmap” were applied to generate a volcano plot and heatmap, respectively. Meanwhile, the cut-off criterion of p < 0.01 was applied to univariate Cox regression analysis to identify prognosis-related AGs. The co-expression network between AGs and sample modules was constructed with the R package “WGCNA”. The best soft threshold was determined to be five as it met the minimum power value with a scale-free topological criterion of > 0.85. Thereafter, a topological overlap matrix (TOM) transformed the weighted adjacency matrix, and dendrograms of TOM were constructed using the hierarchical clustering method. To avoid the generation of
excessive modules, the major parameters were set as a minModuleSize of 50, DeepSplit of two, and height cut-off of 0.25. Finally, AGs with high interconnections were classified into different patterns. Module eigengenes (MEs) and gene significance (GS) were calculated for each module. AGs overlapping between WGCN, differential expression, and univariate Cox regression analyses were visualized using the R package “VennDiagram” and considered as candidate AGs for further analysis. The associations between candidate AGs were visualized with the R package “Corrplot”. To discover biological systems and advanced functions related to the candidate AGs, Gene Ontology (GO) includes biological process (BP), cellular component (CC), and molecular function (MF) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the R package “ClusterProfiler” with thresholds set at \( p < 0.05 \).

**Hub AG Identification and Clinical Analyses**

The overlapping candidate AGs were subsequently integrated into a LASSO regression analysis, and genes were identified as hub AGs. The clinical value of hub AGs for CM, as well as their specificity and sensitivity, were determined using Kaplan-Meier curves, differential expression, and receiver operating characteristic (ROC) curve methods in both TCGA and GSE65904 validation cohorts using various R packages. Statistical significance was set at \( p < 0.05 \).

**Gene Set Enrichment Analysis (GSEA) and Gene Set Variation Analysis (GSVA)**

GSEA was applied to the gene expression matrix using the Hallmark and C7 gene sets v7.4. Enriched gene sets were used to detect KEGG pathways. Gene sets with \( p_{\text{adj}} < 0.05 \) was considered significantly enriched after 1,000 substitutions. GSVA was performed for each gene set and scoring. According to the GSVA score matrix, the changes at the gene level were converted into changes at the pathway level by the R package “GSVA”, and the potential biological functions were ultimately evaluated.

**Immune Cell Infiltration Assessment**

Using the CIBERSORT algorithm, the proportion of 22 types of immune cell infiltration was determined on TCGA-CM and GTEx RNA sequencing data, and the R package “vioplot” was used to visualize the difference in immune infiltration between normal skin tissue and CM samples. Furthermore, the correlation between hub AG expression and immune cell infiltration was also quantified based on CIBERSORT analysis, while a correlation heatmap was visualized using the R package “corrplot”.

**MiRNA-MRNA Interactions and Drug Network Construction**

Interactions between hub AGs and miRNAs were predicted using miRWalk3.0 (http://mirwalk.umm.uni-heidelberg.de/). The interactions that were fitted to the TargetScan, miRDB, and miRanda databases were selected to draw the miRNA-mRNA networks. Moreover, the Genomics of Drug Sensitivity in Cancer (GDSC) database, which covers the response and sensitivity of genes to drugs, was used to predict hub AG-targeted drugs. Networks of mRNA-drugs and miRNA-mRNA were constructed and visualized using Cytoscape (version 3.7.1) software.

**Identification of Hub AG-Associated Risk Signature**

According to the LASSO analysis, the risk score of the hub AG-associated risk signature was constructed using the following formula:

\[
\text{risk score} = \sum \text{expgene}_i \times \beta_i
\]

where expgene\( i \) represents the relative expression of hub AGs, and \( i \) and \( \beta \) are regression coefficients.

Patients with CM were separated into low- and high-risk subgroups according to the median value of the constructed risk score. Following this, survival and Cox regression analyses were performed to compare prognostic ability according to risk level. Then, the predictive accuracy of the risk signature was calculated using the R package “timeROC” in both TCGA and validation cohorts. A nomogram, based on the calculated risk score, was built to predict the outcome of patients with CM via the R package “rms”, and its discrimination and accuracy were estimated using calibration curves.

**Statistical Analysis**

The R program 4.0.3 was utilized for statistical assay. K-M curves were utilized for analyzing survival status via the Survminer R package 2.43-3. \( p < 0.05 \) had significance on statistics (*\( p < 0.05 \); **\( p < 0.01 \); ***\( p < 0.001 \)).
Results

Identification of Candidate AGs in CM

By setting the cut-off value at $|\log FC| > 1$ and $p < 0.05$, 205 differentially expressed AGs (DEGs; including 98 downregulated and 107 upregulated AGs) in CM and normal skin samples were selected from the differential expression analysis (Figure 1A). Univariate Cox regression analysis was applied to identify prognostic AGs, and 54 AGs were screened with significant prognostic relevance (Figure 1B). To further evaluate the functional clusters associated with patients with CM, WGCNA was also performed on the expression profile of 501 downloaded AGs. Based on the scale-free $R^2 = 0.85$, the best soft-threshold power was determined as $\beta = 5$ (Figure 2A), and four modules, including brown (79 AGs), blue (129 AGs), turquoise (183 AGs), and gray (8 AGs), were identified in WGCNA (Figure 2B). The blue module showed the closest connection with the two clusters ($r = -0.99$, $p = 0$); therefore, the AGs in the blue module were selected for this analysis. Based on the above analyses, 12 overlapping AGs (including PIK3R2, SOD2, XAF1, ARHGDIB, BST2, FASLG, LCK, IKZF3, DOK2, HAVCR2, CDK2, and RAC3) were identified, and their AG co-expression distribution is shown in Figure 2C. As shown in Figure 3A, the identified candidate AGs, PIK3R2, SOD2, and XAF1, were significantly downregulated in CM samples ($p < 0.05$), whereas ARHGDIB, BST2, FASLG, LCK, IKZF3, DOK2, HAVCR2, CDK2, and RAC3 were significantly upregulated in CM samples ($p < 0.05$). Univariate Cox regressions showed that all these genes were significantly related to patient prognosis in CM ($p < 0.05$; Figure 3B). In addition, we constructed networks to visualize the relationship between these 12 AGs (Figure 3C-D).

Functional analyses were performed based on the intersection of 12 candidate AGs. In terms of BP, candidate AGs were mostly enriched in response to interferon-beta, regulation of leukocyte proliferation, and negative regulation of leukocyte-mediated immunity. In CC, the candidate AGs were linked to immunological synapses, membrane rafts, and membrane microdomains. In terms of MF, the candidate AGs were mainly involved in phosphotyrosine residue binding, protein phosphorylated amino acid binding, and...
Figure 2. Characterization of candidate AGs in CM. A, Soft-thresholding powers scale-free fit index. B, Heatmap showing the correlation between clinical traits and gene module. Each module was assigned with different colors. The correlation coefficient decreased in size from red to blue. C, The Venn diagram of genes among DEGs, prognostic genes, and WGCNA lists.
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Figure 3. Functional analyses of candidate AGs. A, Heatmap of candidate AGs. B, Univariate Cox regression of candidate AGs. C-D, Correlation network of candidate AGs. Red represents a positive correlation, and green represents a negative correlation. The GO enrichment terms of candidate AGs in CC, BP, and MF are shown by a bar plot (E) and loop graph (F). KEGG enrichment terms of candidate AGs are shown by a bar plot (G).
receptor tyrosine kinase binding (Figure 3E). Figure 3F shows a Circos diagram of the GO enrichment analysis. Meanwhile, the KEGG analysis results indicate that the candidate AGs were mostly augmented in the FoxO signaling pathway, natural killer cell-mediated cytotoxicity, and human immunodeficiency virus 1 infection (Figure 3G).

**Hub AG Identification and Prognostic Value Analysis in Patients with CM**

To further reduce the gene size, 12 candidate AGs were subjected to LASSO analysis, and seven genes, namely FASLG, SOD2, BST2, PIK3R2, IKZF3, CDK2, and RAC3, were ultimately identified as hub AGs and used to develop a risk signature in patients with CM (Supplementary Figure 1A-B). As a single diagnostic biomarker, ROC analysis revealed that the area under the ROC curve (AUC) of FASLG was 0.936, SOD2 was 0.947, BST2 was 0.972, PIK3R2 was 0.999, IKZF3 was 0.924, CDK2 was 0.919, and RAC3 was 0.835, indicating that all identified hub AGs had a high predictive accuracy in patients with CM (Supplementary Figure 1C). Meanwhile, when we combined all hub AGs into a prediction model, the ROC analysis showed that its predictive accuracy (AUC value) increased to 1.00 (Supplementary Figure 1D). KM survival analysis was further applied to estimate the correlation between hub AG expression and CM prognosis. The results indicate that the higher expression subgroups of all AGs, BST2 (Supplementary Figure 1E), SOD2 (Supplementary Figure 1F), IKZF3 (Supplementary Figure 1I), and FASLG (Supplementary Figure 1K) had a significantly higher OS of patients with CM ($p < 0.05$), whereas the higher expression of RAC3 (Supplementary Figure 1G), PIK3R2 (Supplementary Figure 1H), and CDK2 (Supplementary Figure 1J) was significantly associated with poor prognosis in patients with CM ($p < 0.05$). These results were confirmed in the GSE65904 validation cohort (Supplementary Figure 1L-R). When evaluating hub gene expression levels in CM, the results show that all hub genes exhibited similar changing trends in TCGA (Supplementary Figure IS-Y) and validation (Supplementary Figure 1Z-AF) cohorts. Hub AGs BST2, RAC3, IKZF3, CDK2, and FASLG were expressed at significantly higher levels in CM tissues ($p < 0.05$), whereas the others were expressed at significantly lower levels in CM tissues than in normal tissues ($p < 0.05$).

**Functional Enrichment Analysis**

GSEA and GSVA analyses were performed to explore the potential role of hub AGs FASLG, SOD2, BST2, PIK3R2, IKZF3, CDK2, and RAC3. GSEA analysis indicated that the hub AGs with the highest enrichment score were significantly connected with pathways related to apoptosis, immune cells, and several types of cancer, such as natural killer cell-mediated cytotoxicity and prostate cancer (Supplementary Figure 2A-G). GSVA analysis also confirmed that the high-expression subgroups of the hub AGs FASLG, SOD2, BST2, PIK3R2, IKZF3, CDK2, and RAC3 were most significantly enriched in various cancer metastatic and immune-associated pathways, including ECM receptor interaction, cell adhesion molecules, and T cell receptor signaling pathways (Supplementary Figure 2H-N), indicating that the activation of these hub biomarkers might participate in modulating cancer progression and metastasis.

**Immune Infiltration Analysis**

The CIBERSORT algorithm was applied to analyze the proportion of immune cells in CM with a threshold of $p < 0.05$. As shown in Supplementary Figure 3A, nearly all immune cells were significantly differential infiltrated between CM and control samples. The results indicated that naïve B cells, memory B cells, plasma cells, CD8 T cells, activated memory CD4 T cells, Tregs regulatory T cells, gamma delta T cells, M0 macrophages, and M1 macrophages were significantly upregulated in CM samples; however, the proportions of naïve CD4 T cells, CD4 memory resting T cells, follicular helper T cells, resting NK cells, activated NK cells, monocytes, M2 macrophages, resting dendritic cells, activated dendritic cells, resting mast cells, activated mast cells, eosinophils, and neutrophils were significantly increased in normal samples. Finally, the correlation between hub AGs and immune infiltration in CM was determined. Supplementary Figure 3B shows that seven hub AGs were strongly associated with the content of immune cells, indicating that hub AGs may be prognostic targets for CM immunotherapy.

**Drug and miRNA-mRNA Interaction Networks**

By constructing a drug network, we found that the hub AGs CDK2, SOD2, FASLG, and PIK3R2 were connected to several drugs, including methotrexate, asunercept, and cyclophosphamide (Figure 4A). To determine the potential underlying mechanisms of hub AGs in CM, we generated a
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Figure 4. Drug and miRNA-mRNA interaction networks construction. **A**, The connection between hub AGs and drugs. **B**, Network of hub AGs and targeted miRNAs. Red represents mRNAs, green represents miRNAs, and yellow represents drugs.
miRNA-mRNA network. The results show that the hub AG \textit{FASLG} might be regulated by miRNAs hsa-miR-1286 and hsa-miR-1266 together, and the AG \textit{SOD2} significantly interacted with miRNA hsa-let-7b-3p (Figure 4B).

\textbf{Risk Signature Construction and Clinical Characteristic Analysis}

Based on the expression levels of seven hub AGs from LASSO analysis, a risk signature was constructed in both TCGA and validation cohorts, and patients with CM were separated into low- and high-risk subgroups based on the median calculated risk score. ROC analysis showed that the AUCs in the TCGA-CM cohort were 0.621, 0.657, 0.675, and 0.686 for 1-, 3-, 5-, and 7-year survival, respectively (Figure 5A). In the validation cohort, the AUCs were 0.608, 0.674, 0.681, and 0.729 for 1-, 3-, 5-, and 7-year survival, respectively (Figure 5B), which indicated that

![Figure 5](image_url)

\textbf{Figure 5}. Prognostic value of the constructed model. TimeROC curves to forecast the overall survival in TCGA (A) and GSE65904 (B) cohorts. Survival curve of the TCGA cohort (C) and GSE65904 cohorts (D). Univariate (E) and multivariate (F) Cox regressions of clinicopathological features.
the AG risk signature had moderate predictive reliability in patients with CM. K-M survival analysis showed that the high-risk subgroup had significantly worse clinical outcomes in both the TCGA (Figure 5C) and validation (Figure 5D) cohorts \((p < 0.05)\). Meanwhile, multivariate and univariate Cox regressions showed that the risk signature was not only significantly associated with patient prognosis but also served as an independent prognostic factor in CM (Figure 5E-F). When exploring the correlation between the risk signature and CM clinical features, there was a significant association between lower T stages and lower risk scores \((p < 0.05; \text{Figure 6A})\). Furthermore, patients with metastatic CM cancers (Figure 6B) and those aged \(\leq 60\) years (Figure 6C) also had significantly lower risk scores \((p < 0.05)\). Heatmaps containing the gene expression of hub AGs confirmed that there were significant differences in the subgroups regarding CM metastatic status, T stage, and patient age \((p < 0.05; \text{Figure 6D})\). This indicates that the constructed risk signature is related to the prognosis and development of CM.

Finally, a nomogram plot was established by combining the risk signature and clinical features to exploit the prognostic value of the risk signature (Figure 7A). Afterward, the calibration curves at 1-, 3-, and 5-year follow-ups showed that our nomogram had a substantial agreement

**Figure 6.** Clinical value of the constructed model. Correlations between risk scores and T stage (A), metastatic ability (B), and age (C) in the TCGA cohort. D, Heatmap depicts the expression of hub AGs and clinicopathological features according to risk subgroups.
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(Figure 7B), which confirmed that our constructed nomogram could potentially be applied for CM prognosis forecasting.

Discussion

The 5-year survival rate of patients with advanced-stage CM is close to 23%16, and it is difficult to diagnose at early stages owing to a lack of reliable prognosis-associated biomarkers. Thus, there is an urgent need to identify reliable prognostic indicators to enhance the predictive accuracy of CM. As a specific form of cell apoptosis, AGs reportedly modulate the biological behaviors of various tumors, such as cell viability and metastasis17,18, they have been reported to serve as prospective treatment markers and prognostic targets for cancer.

In this study, we identified specific AGs in CM based on the TCGA-CM project. After performing stepwise LASSO analysis, we generated a robust anoikis-associated risk signature for CM. Patients with CM were classified into low- and high-risk subgroups. There was a significant difference in prognosis between these subgroups, and the accuracy of the risk signature was confirmed in the GSE65904 validation cohort. When exploring the relationship between signature and CM clinical features, the results indicate that our signature was dramatically correlated with the growth, metastasis, and age of the CM samples. To expand the performance of the risk signature, a nomogram combining risk score, age, and tumor node metastasis (TNM) stage were generated for patients with CM. Calibration plots showed that our constructed nomogram had a good fit for predicting overall survival.

Seven identified hub AGs, including FASLG, SOD2, BST2, PIK3R2, IKZF3, CDK2, and RAC3, were significantly associated with CM prognosis. All these signature AGs are closely associated with cancer progression. As a natural ligand of FAS, FASLG, also named CD95L, mediates cell apoptosis by binding to FAS19. However, FASLG expression is significantly increased in several cancers, such as cutaneous melanoma20, bladder cancer21, and cervical cancer22. This is mainly because apoptosis-resistant tumor cells can express FASLG against antitumor cells. A study concerning the effect of necroptosis-related genes in CM confirmed that FASLG was significantly highly expressed in CM tissues and was associated with the overall survival of patients with CM, which is similar to our results. SOD2 is a key superoxide detoxifying enzyme in cells that catalyzes the conversion of superoxide to hydrogen peroxide, leading to unbalanced redox homeostasis of mitochondrial oxidants and ultimately acting as a suppressor or promoter in cancer cells23,24. In this study, our results indicate that SOD2 could reduce the risk of CM,
which was consistent with Jana et al\textsuperscript{25} analyses that upregulation of SOD2 plays a virtual role in \(O_2\) scavenging and thus inhibits the progression and metastasis of malignant CM. The suppressive effects of SOD2 have also been found in prostate cancer\textsuperscript{26} and breast cancer\textsuperscript{27} cells, although its specific mechanism requires further exploration. BST2 (also known as CD317) is abundantly expressed in several solid tumors and affects the progression and invasion of multiple myeloma\textsuperscript{28}. Owing to the cytotoxicity of multiple myeloma cells, a BST2-associated humanized monoclonal antibody has been applied as immunotherapy for the treatment of multiple myeloma\textsuperscript{29}. In melanoma tissues, BST2 serves as an important networking cooperator of YWHAE and plays a virtual role in melanocyte development\textsuperscript{30}. Meanwhile, a bioinformatic analysis also proved that BST2 was expressed at significantly low levels in a high-risk subgroup and associated with the overall survival of patients with CM\textsuperscript{31}, which is consistent with our study. PIK3R2 is a member of the phosphatidylinositol 3-kinase (PI3K) enzyme family\textsuperscript{32}. As a driver of CM, aberrant PIK3R2 activation is strongly associated with melanoma progression and is characterized as an oncogene in carcinogenesis\textsuperscript{33}. Additionally, reports show > 70\% downregulation of PTEN expression and AKT mutations in CM due to PI3K pathway activation\textsuperscript{34}. Our results confirm that PIK3R2 was negatively correlated with OS. IKZF3, also known as Aiolos, is a member of the Ikaros family (including IKZF1-5). It is expressed by lymphocytes and participates in the modulation of lymphocyte development and differentiation\textsuperscript{35,36}. As our results confirm, Yang et al\textsuperscript{37} also reported that IKZF3 could serve as an independent prognostic factor for CM and was positively associated with OS through immunological status modulation. CDKs are central regulators of the cell cycle and play a critical role in cell proliferation. During cell proliferation, CDK2 combines with cyclin E to initiate DNA replication by transitioning the cell proliferation process from the late G1 phase to the S phase\textsuperscript{38}. Studies\textsuperscript{39} showed that CDK2 overexpression can lead to cancer metastasis by promoting uncontrolled cancer cell proliferation. Meanwhile, inhibition of CDK2 expression can delay cancer cell progression\textsuperscript{40}. These results are consistent with the CDK2 survival analysis in our study, which showed that low CDK2 expression significantly increased CM OS. Ras-related C3 botulinum toxin substrate 3 (RAC3) is a member of the Rho GTPase family that promotes cancer migration and progression\textsuperscript{41,42}. Combination with GTP can effectively activate the gene RAC3 and then stimulate c-Jun amino-terminal kinase signaling pathway activation\textsuperscript{43}. RAC3 is highly expressed in several cancers, including brain tumors\textsuperscript{44} and breast cancer\textsuperscript{45}. However, their role in CM has not been studied. In this study, we confirmed that RAC3 expression was significantly higher in CM samples, and RAC3 overexpression was related to poor prognosis in patients with CM.

Recently, immune treatment has been regarded as a novel therapeutic option for patients with CM, and CIBERSORT analysis is a widely approved method for detecting the relative content of immune cells. In this study, pathway enrichment analyses revealed that hub AGs were significantly enriched in various immune-associated pathways, such as natural killer cell-mediated cytotoxicity, the T cell receptor signaling pathway, and the B cell receptor signaling pathway. When hub AG expression was used to explore the distribution of immune cells, the results confirmed that they were all significantly connected with several immune cells, indicating that these genes might play a virtual role in the immune response in CM and offer a valuable reference for immunotherapy.

**Limitations**

Although this study identified hub AGs in CM and proposed an anoikis-associated signature that displayed a powerful prognostic value in patients with CM, it still had some limitations. First, all gene expression and clinical CM cohorts were obtained from TCGA and GEO public websites, and our conclusions should be validated by additional experimental assays. Second, the results of our retrospective study require further confirmation in prospective studies. In the future, functional studies should be performed to gain mechanistic insights into the role of AGs in CM progression.

**Conclusions**

Our study provides insights into the role of hub AGs and develops a novel anoikis-related signature for patients with CM. All identified AGs could improve the prediction of overall CM survival and reflect the immune conditions of patients with CM. This study provides a novel perspective for therapeutic improvements in patients with CM.
Conflict of Interest
The Authors declare that they have no conflict of interests.

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Authors’ Contribution
Conceived and designed the experiments: Y.-W. He. Performed the experiments: Q.-P. Fan. Analyzed the data: A.-L. Hua. Contributed reagents/materials/analysis tools: Q. Liu. Wrote the paper: Y.-W. He.

Data Availability
The datasets analyzed during the current study are available and sourced from the publicly available TCGA (https://portal.gdc.cancer.gov) and Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database.

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References
14) Buchheit CL, Angarola BL, Steiner A, Weigel KJ, Schafer ZT. Anoikis evasion in inflammatio-
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