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ORIGINAL ARTICLE

Basic Study

Hub genes and their key effects on prognosis of Burkitt lymphoma

Yan-Feng Xu, Guan-Yun Wang, Ming-Yu Zhang, Ji-Gang Yang

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Abstract

BACKGROUND

Burkitt lymphoma (BL) is an exceptionally aggressive malignant neoplasm that arises from either the germinal center or post-germinal center B cells. Patients with BL often present with rapid tumor growth and require high-intensity multi-drug therapy combined with adequate intrathecal chemotherapy prophylaxis, however, a standard treatment program for BL has not yet been established. It is important to identify biomarkers for predicting the prognosis of BLs and discriminating patients who might benefit from the therapy. Microarray data and sequencing information from public databases could offer opportunities for the discovery of new diagnostic or therapeutic targets.

AIM

To identify hub genes and perform gene ontology (GO) and survival analysis in BL.

METHODS

Gene expression profiles and clinical traits of BL patients were collected from the Gene Expression Omnibus database. Weighted gene co-expression network analysis (WGCNA) was applied to construct gene co-expression modules, and the cytoHubba tool was used to find the hub genes. Then, the hub genes were analyzed using GO and Kyoto Encyclopedia of Genes and Genomes analysis. Additionally, a Protein-Protein Interaction network and a Genetic Interaction network were constructed. Prognostic candidate genes were identified through overall survival analysis. Finally, a nomogram was established to assess the predictive value of hub genes, and drug-gene interactions were also constructed.

RESULTS

In this study, we obtained 8 modules through WGCNA analysis, and there was a significant correlation between the yellow module and age. Then we identified 10 hub genes (*SRC*, *TLR4*, *CD40*, *STAT3*, *SELL*, *CXCL10*, *IL2RA*, *IL10RA*, *CCR7* and *FCGR2B*) by cytoHubba tool. Within these hubs, two genes were found to be associated with OS (*CXCL10*, $P = 0.029$ and *IL2RA*, $P = 0.0066$) by survival ana-

lysis. Additionally, we combined these two hub genes and age to build a nomogram. Moreover, the drugs related to *IL2RA* and *CXCL10* might have a potential therapeutic role in relapsed and refractory BL.

CONCLUSION

From WGCNA and survival analysis, we identified *CXCL10* and *IL2RA* that might be prognostic markers for BL.

Key Words: Burkitt lymphoma; Weighted gene co-expression network analysis; Microarray data; Functional enrichment analysis; Prognosis; Therapeutic target

Core Tip: This study represents the pioneering investigation of gene expression in Burkitt lymphoma (BL) using weighted gene co-expression network analysis, coupled with functional enrichment analysis. In this study, we have successfully identified and validated 10 hub genes. Survival analysis has demonstrated that the overexpression of *CXCL10* and *IL2RA* in BL may serve as robust prognostic indicators. Furthermore, an integrated mRNA signature and age nomogram potentially provide valuable prognostic insights for patients with BLs.

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INTRODUCTION

Burkitt lymphoma (BL) is a highly aggressive B-cell non-Hodgkin's lymphoma characterized by the *t* (8; 14) chromosomal translocation involving the *MYC* oncogene and the immunoglobulin heavy chain gene (IGH)[1]. Three distinct clinical subtypes of BL have been identified: Namely endemic (African), sporadic (non-endemic), and immunodeficiency-associated. Notably, chronic Epstein-Barr virus infection plays a pivotal role in the pathogenesis of BL, particularly in the endemic subtype[2]. Endemic BL is primarily found in countries located near the equator in Africa. The estimated annual incidence of endemic BL is 3-6 cases per 100000 children in African countries[3], which is approximately 50 times higher than that in the United States[4]. Sporadic BL predominantly occurs in the United States and Western Europe. The annual incidence of BL in the United States is approximately 3 cases per 1 million individuals, while in Europe it stands at around 2.2 cases per 1 million people[5]. Immunodeficiency-associated BL primarily affects individuals with HIV infection, typically those with relatively high CD4 counts and no opportunistic infections[6].

Patients with BL frequently exhibit rapid tumor growth, spontaneous tumor lysis, and elevated levels of serum lactate dehydrogenase. Currently, patients with BL necessitate a high-intensity multi-drug regimen in conjunction with adequate intrathecal central nervous system prophylaxis. However, the absence of an established standard treatment protocol for BL persists[7]. BL is an aggressive lymphoma, which can potentially be cured; however, patients with refractory and relapsed disease have an extremely poor prognosis[8]. Therefore, it is important to identify robust biomarkers for predicting the prognosis of BLs and discriminating patients who might benefit from therapy. The development of BL depends on the constitutive expression of the MYC gene located on chromosome 8q24, which encodes the transcription factor protein MYC[9]. MYC orchestrates the expression of target genes, regulating a variety of cellular processes, including cell growth, division, apoptosis, metabolism, adhesion, and motility[10]. MYC gene rearrangements are seen in the vast majority of BLs, and factors other than MYC translocation need to be present in the process of BL. However, it is not clear why and how B cells develop genetic alterations that result in increased MYC expression and ultimately lead to BL.

The Gene Expression Omnibus (GEO) is an international public repository constructed and maintained by the National Center for Biotechnology Information[11]. At the time of writing, the GEO database hosts more than 194000 public series. Weighted gene co-expression network analysis (WGCNA) is a widely used systematic biological method for generating gene co-expression networks[12,13]. In this study, WGCNA was first used to analyze genes of BL samples mined from the GEO database. Subsequently, we identified these hub genes and conducted a functional enrichment analysis. Additionally, a survival analysis was conducted to identify an mRNA signature that exhibits a significant association with prognosis. Finally, a prognostic nomogram was established based on the combination of gene signature and clinical characteristics.

MATERIALS AND METHODS

Data collection and preprocessing

The raw gene expressions and the corresponding clinical follow-up data of GSE4475 and GSE69051 were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/. Accessed Jan 20, 2023)[14], and the two datasets were built based on the GPL96 platform (HG-U133A) and GPL14951 platform (Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip) respectively. Analysis was performed on the raw gene expression data of the BL datasets and the corresponding clinical follow-up obtained from GSE4475, which included a total of 36 BL samples. The survival data of the hub genes was verified by downloading another dataset, GSE69051, which included 77 BL samples. The mRNA sequencing data annotation information was used to match the probe with the corresponding gene and transform the gene name into a gene symbol. Probes that corresponded to more than one gene were excluded from the dataset.

Co-expression network construction

WGCNA converts gene expression data into co-expression modules, establishing relationships between genes and focusing on gene modules rather than individual genes[15]. Besides, WGCNA can identify the gene modules related to clinical traits and has been widely used in cancer research. In this study, the top 5000 most variable genes were used to construct a co-expression network by using the package of WGCNA in R[13]. The power value was filtered out during the module construction process using the WGCNA algorithm. The mean connectivity and scale independence of network modules were analyzed using the gradient test under different power values, which ranged from 1 to 20. When the degree of independence was 0.85, the appropriate power value was determined. Then, the soft threshold test was performed. In this study, the soft threshold β was 12, and the network type was "signed". The WGCNA algorithm further identified co-expression modules under these conditions. The minimum size of the gene group was set at 100 to ensure the reliability of the results for this module. Then, the correlation between the characteristics of the module-trait association module and clinical traits was visually expressed. The relationship between the expression profile and traits was analyzed to make a scatter plot between gene significance and module membership.

Hub genes identification and functional analysis

The Protein-Protein Interaction (PPI) network of the interested module was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (available from https://cn.string-db.org/. Accessed 25 Jan 2023)[16] and presented by Cytoscape software. The cytoHubba tool was used to screen the hub genes. Then, the selected hub genes were analyzed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis using Database for Annotation, Visualization, and Integrated Discovery (DAVID v.6.8: available from https://david.ncifcrf.gov/. Accessed Jan 29, 2023)[17]. The possible functions were analyzed by biological processes, cellular components, and molecular functions, while the potential signal pathways were analyzed using KEGG.

Construction of hub genes PPI and genetic interaction network

The PPI network was used to analyze the hub genes at the protein level, and the STRING database (available from https://string-db.org/. Accessed Jan 31, 2023) was used to check and predict the interaction between proteins[16]. The genetic interaction (GI) network, constructed using gene function prediction, aims to understand the complex interactions between genes. We used the Gene Multiple Association Network Integration Algorithm (GeneMANIA, available from https://genemania.org/. Accessed Jan 31, 2023) to analyze the hub genes[18]. The threshold of a collective score of 0.15 was implemented.

Statistical analysis

Based on the 50th percentile cut-off value of each hub gene mRNA, patients were divided into high-expression and low-expression groups. Log-rank test and Kaplan-Meier estimation were performed to obtain log-rank P value and evaluate hub genes in overall survival (OS). Cox regression analysis was conducted to examine the association between the risk score and clinical information, and a nomogram was generated. The survival curve and nomogram were carried out by R version 4.2.1. Additionally, $P < 0.05$ was statistically significant.

Drug-gene interaction

The DGIdb database (available from https://dgidb.org/. Accessed Feb 20, 2023) was used to investigate drug-gene interaction to identify drugs associated with hub genes[19]. The interaction network was visualized *via* Cytoscape.

RESULTS

Construction and screening of BL co-expression module

In this study, we obtained the BL dataset from GSE4475, resulting in a total of 13514 gene expression values. The clinical features of the BL samples are listed in Table 1. Then, we selected a total of 5000 genes with the highest average expression values for cluster analysis. Firstly, the clustering tree of 36 samples of BL was extracted from GSE4475 (Figure 1). Secondly, we calculated the soft threshold (power value), and when the weight was equal to 12, the independence exceeded 0.85, indicating higher average connectivity (Supplementary Figure 1). By utilizing this power value for hierarchical clustering analysis and combining similar analysis results, a total of 8 modules were identified,

Table 1 Clinical features of Burkitt lymphoma patients

Clinical features	Total (*n* = 36)
Age, mean (range)	31.0 (2-90)
Gender	
Male	24
Female	11
Unknown	1
Stage	
I	4
II	10
III	5
IV	6
Unknown	11
Survival status	
Alive	20
Dead	7
Unknown	9
Ki 67	
≤ 75%	4
75%-90%	9
> 90%	22
Unknown	1
CCS	
< 10	29
≥ 10	5
Unknown	4

CCS: Chromosomal Complexity Score.

including black (1073 genes), blue (967 genes), brown (853 genes), green yellow (140 genes), grey (1019 genes), magenta (219 genes), pink (267 genes) and yellow (462 genes) (Figure 2A). Genes in grey were not included in any module, thus we analyzed the interactive relationships underlying the 7 co-expression modules (Figure 2B). Given the well-established association between age and prognosis in BL patients, we opted to investigate the module that exhibited the strongest correlation with age for subsequent analysis[20,21]. A significant correlation between the yellow module and age was discovered (Figure 3). The correlation between modules and samples is shown in Supplementary Figure 2. Finally, we conducted a scatter diagram of the correlation between the yellow module and age (Figure 4).

Hub genes identification

All of the genes from the yellow module were uploaded to the STRING database, and a PPI network was constructed using Cytoscape software (Supplementary Figure 3). And the top 10 hub genes (*SRC, TLR4, CD40, STAT3, SELL, CXCL10, IL2RA, IL10RA, CCR7* and *FCGR2B*) were screened out by cytoHubba tool. GeneMANIA showed the GI network of hub genes interaction at the mRNA expression level (Figure 5A). The STRING database generated the PPI co-expression network by analyzing the hub genes at the protein level (Figure 5B).

Functional and pathway enrichment analysis

Enrichment analyses of GO and KEGG were conducted to explore potential pathways of the hub genes. Forty-five GO-enriched terms were shown in Supplementary Table 1. The top 10 GO terms (Figure 6A) included inflammatory response, external side of plasma membrane, plasma membrane, cellular response to lipopolysaccharide, positive regulation of interleukin-12 production, receptor binding, positive regulation of MAP kinase activity, positive regulation of JNK cascade, immune response and positive regulation of humoral immune response. In the KEGG analysis, 14 pathways enriched by genes in the yellow module were shown in Supplementary Table 2, and the top 10 KEGG terms (Figure 6B)

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Figure 1 Clustering tree of 36 samples of Burkitt lymphoma extracted from GSE4475. Red indicated more gene expression, white less, and grey indicated deletion. CCS: Chromosomal Complexity Score.

included viral protein interaction with cytokine and cytokine receptor, cytokine-cytokine receptor interaction, toxoplasmosis, measles, tuberculosis, chemokine signaling pathway, lipid and atherosclerosis, Toll-like receptor signaling pathway, Hepatitis B and JAK-STAT signaling pathway.

Survival analysis

Additional survival analysis was conducted on the hub genes to evaluate their impact on BL patients' survival. Due to the small sample size of GSE4475, we opted for GSE69051 for survival analysis (Figure 7). Two of the 10 hub genes were significantly associated with OS: *CXCL10* (P = 0.029, Figure 7F) and *IL2RA* (P = 0.0066, Figure 7G).

Establishment of the nomogram and assessment of predictive value

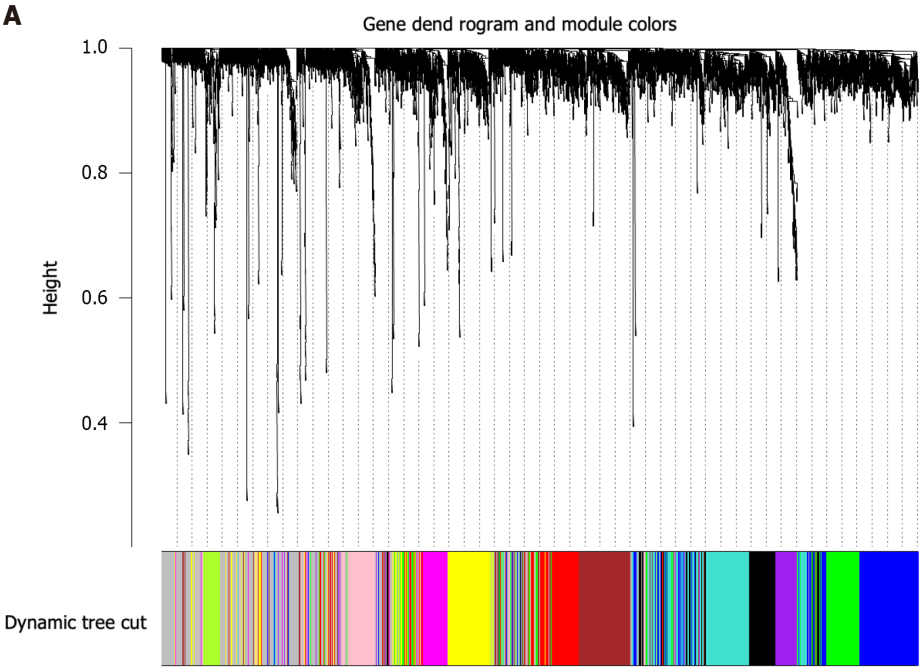
Based on the hub genes and clinical data of the patients, a nomogram was developed to predict the 1- and 3-year OS of BL patients (Figure 8A). The model had a c-index of 0.84, and the calibration curve demonstrated strong agreement between predicted and observed survival times for both 1- and 3-year OS probabilities in the BL cohort (Figure 8B).

Identification of associated drugs

The drugs related to *IL2RA* and *CXCL10* were identified by the DGIdb database, as these were the only significant results from survival analysis (Figure 9). These results may provide new ideas for the treatment of BL with poor prognosis.

DISCUSSION

BL, a highly aggressive lymphoma identified and described by Denis Burkitt in the last century, continues to be the most common childhood malignancy in Africa nowadays[22]. A defining feature of BL is the translocation between the *c-MYC* gene and the *IgH* gene, which is found in 80% of cases [*t* (8; 14)], or between *c-MYC* and either the kappa or lambda light chain gene, which is found in the remaining 20% [*t* (2; 8) or *t* (8; 22)][23]. The proliferation rate and apoptosis rate of BL tumor cells are extremely high, indicating that nearly 100% of the cells are positive for Ki-67. Intensive, short-course combination chemotherapy is recommended for most BL patients. DA-EPOCH (dose-adjusted etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone) + rituximab may be an option for patients who cannot tolerate more aggressive regimens[7]. As the standard of treatment for BL has not yet been established, strictly controlled clinical trials are also recommended. The prognosis of BL patients is associated with both clinical and laboratory characteristics[8,24]. The BL international prognostic index can be used to assess the prognosis of adult patients with disseminated or immunodeficiency-related BL, but it is not currently used for stratifying BL treatment[20]. Previous studies have demonstrated associations between *MYC* rearrangements, *TCF3* mutations or *ID3* alterations (its negative regulator), *TP53* modifications, *CCND3* and *CDKN2A* changes, as well as non-antigen-dependent B cell receptor signaling (tonic B



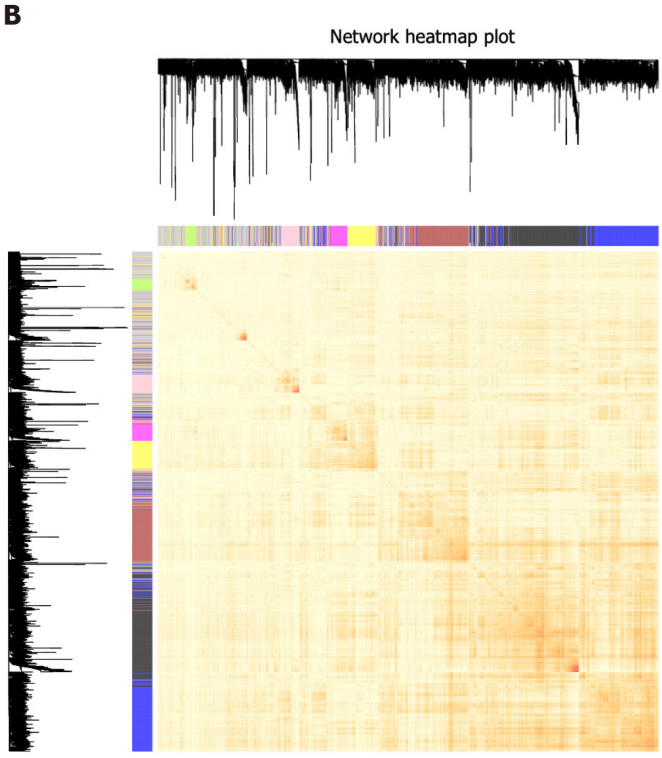


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Figure 2 Sample clustering to detect outliers and construction of co-expression modules. A: The constructed co-expression modules of Burkitt lymphoma genes by weighted gene co-expression network analysis; B: Interaction analysis between gene co-expression modules. The heatmap showed the Topological Overlap Matrix among genes in the analysis. Different colors on the x-axis and y-axis represented different modules. The intensity of inter-module connections was visually represented by the yellow brightness in the central region, gradually transitioning into deeper shades of orange.

cell receptor signaling) with the development and prognosis of BL; however, a comprehensive investigation into the prognostic significance of molecular events associated with BL is lacking[25].

As a bioinformatics algorithm, WGCNA offers numerous advantages over conventional methods for differential expression analysis. It primarily focuses on elucidating co-expression patterns, facilitating the identification of biologically relevant modules comprising interconnected genes, and enabling the detection of pivotal hub genes[26-28]. So far, gene modules related to several cancers have been analyzed and verified using WGCNA[29,30].

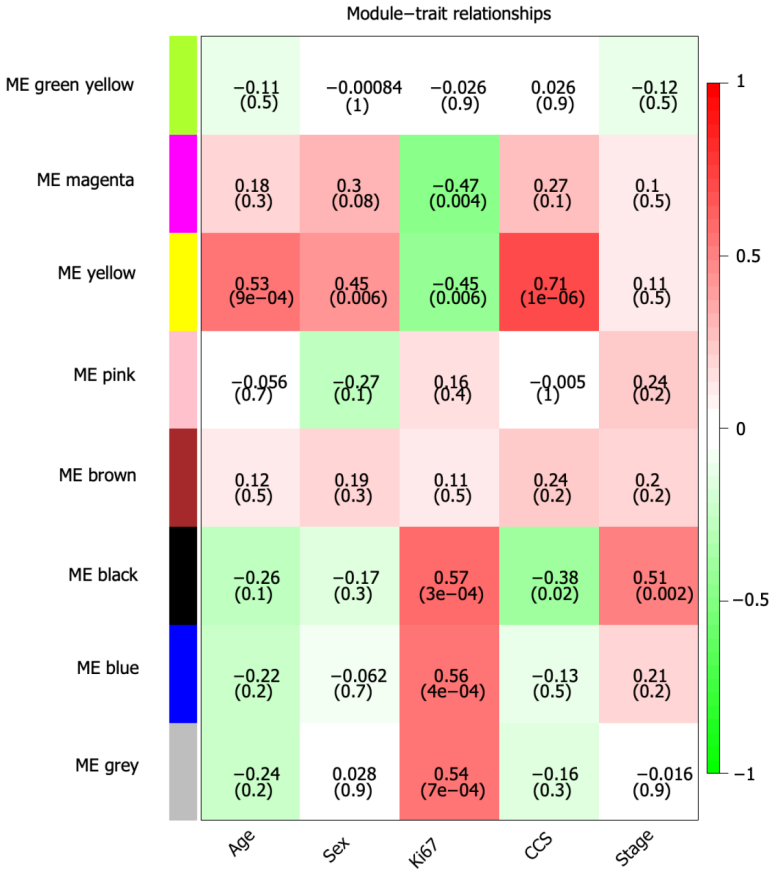

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Figure 3 Module-trait association. Correlation thermography between modular feature genes and clinical features of Burkitt lymphoma. Each row corresponded to a module feature, and the column corresponded to a clinical feature. Each cell contained the correlation and the corresponding *P* value. CCS: Chromosomal Complexity Score; ME: Module membership.

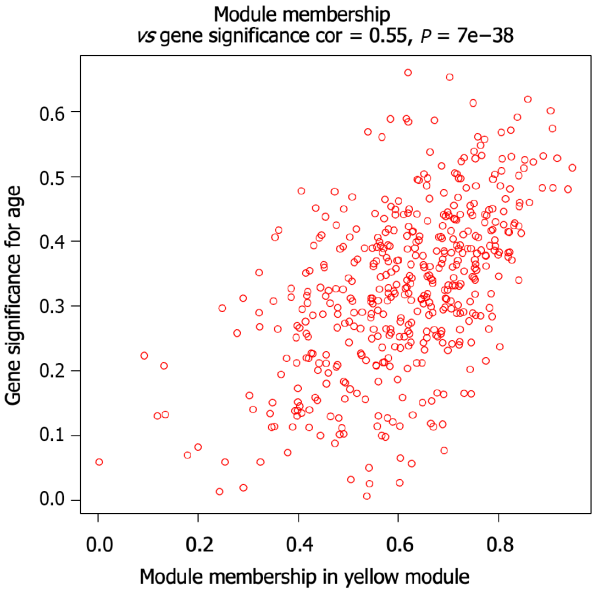

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Figure 4 The scatter plot of the correlation for an age-related gene between module membership and gene significance in the yellow module.

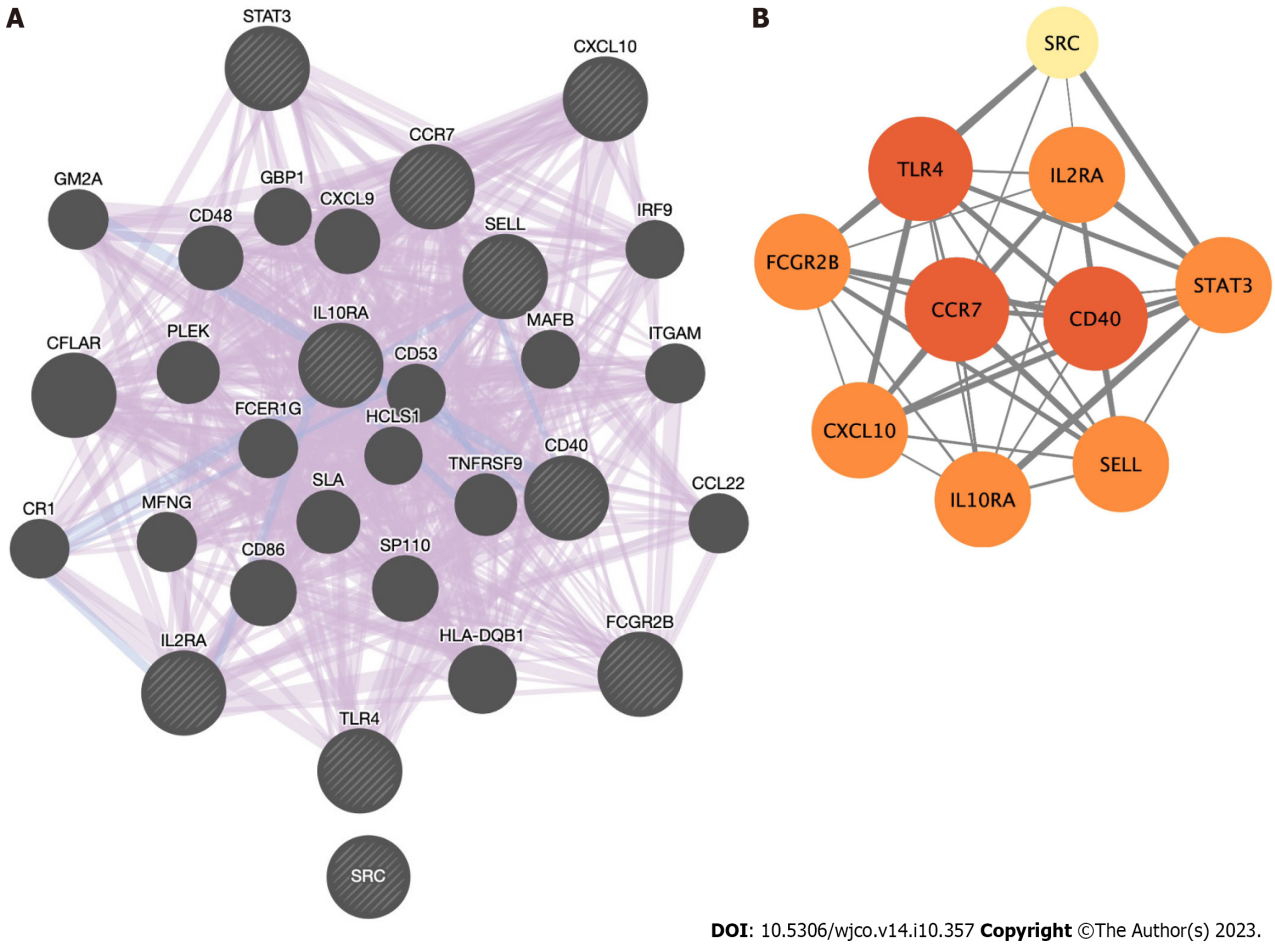

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Figure 5 Genetic and Protein-Protein interaction network of hub genes. A: GeneMANIA was used to construct a genetic interaction network. The black nodes with a slash represent the query gene, while the other nodes represent the predicted genes. The purple edges indicate co-expression, whereas the blue edges signify co-localization; B: A physically and functionally connected Protein-Protein Interaction network implemented common goals through Search Tool for the Retrieval of Interacting Genes/Proteins, where nodes represented proteins and edges represented pairs of interactions between proteins. Node size and color indicated richness, while edge size and color reflected combined scores.

In this study, 8 modules were obtained through WGCNA. As many prior studies have shown a strong correlation between age and the prognosis of BL patients[20,21], we chose the yellow module that had the strongest correlation with age for further analysis. Ten hub genes (*SRC*, *TLR4*, *CD40*, *STAT3*, *SELL*, *CXCL10*, *IL2RA*, *IL10RA*, *CCR7* and *FCGR2B*) were identified using cytoHubba. GO and KEGG functional analyses were conducted on hub genes, and the PPI and GI analysis of these hub genes revealed their related biological functions. Based on survival analysis, CXCL10 and IL2RA have been identified as genes that affect survival. Afterward, we used a nomogram to develop a new risk assessment system for BL patients based on the aforementioned genes and age, aiming to aid in identifying high-risk groups for this disease.

CXCL10 is one of the three ligands for *CXCR3*, which is a chemokine receptor[31]. Various studies have demonstrated that in addition to attracting CD8+ and CD4+ effector T cells to tumor sites and sites of inflammation, *CXCL10* also governs the polarization and enhances the biological functionality of these cells. This makes *CXCL10* a key chemokine driver and a valid target for the therapy of autoimmune diseases such as Inflammatory Bowel Disease, Multiple Sclerosis, Rheumatoid arthritis, and others. Previous studies have also found that chemokines and their receptors are involved in supporting tumor development and metastatic spread[32-35]. In addition to inducing effector TH1 cells, *CXCL10* has recently been proven to be associated with the recruitment of CXCR3+ CD8+ T cells to the tumor site and the induction of Granzyme B production by these cells, thereby enhancing their anti-tumor activities[36]. Barreira da Silva *et al*[37] used Dipeptidyl peptidase 4 inhibitors to increase the endogenous level of *CXCL10*, thereby suppressing experimental melanoma. It has also been demonstrated that the combination of *CXCL10* gene therapy and radiotherapy improves therapeutic efficacy in cervical cancer using a HeLa cell murine xenograft tumor model[38]. Numerous studies have demonstrated a positive correlation between increased expression of *CXCL10* at the tumor site and improved prognosis in various human cancers[39-41]. However, the biological functions of *CXCL10* in BL have not been addressed so far. Our study initially discovered that the high expression of *CXCL10* appeared to be associated with a better prognosis. In our prognostic model, *CXCL10* outperforms age, which is an accepted prognostic factor for BL. Further studies are required to investigate and validate the mechanism of *CXCL10* in BL.

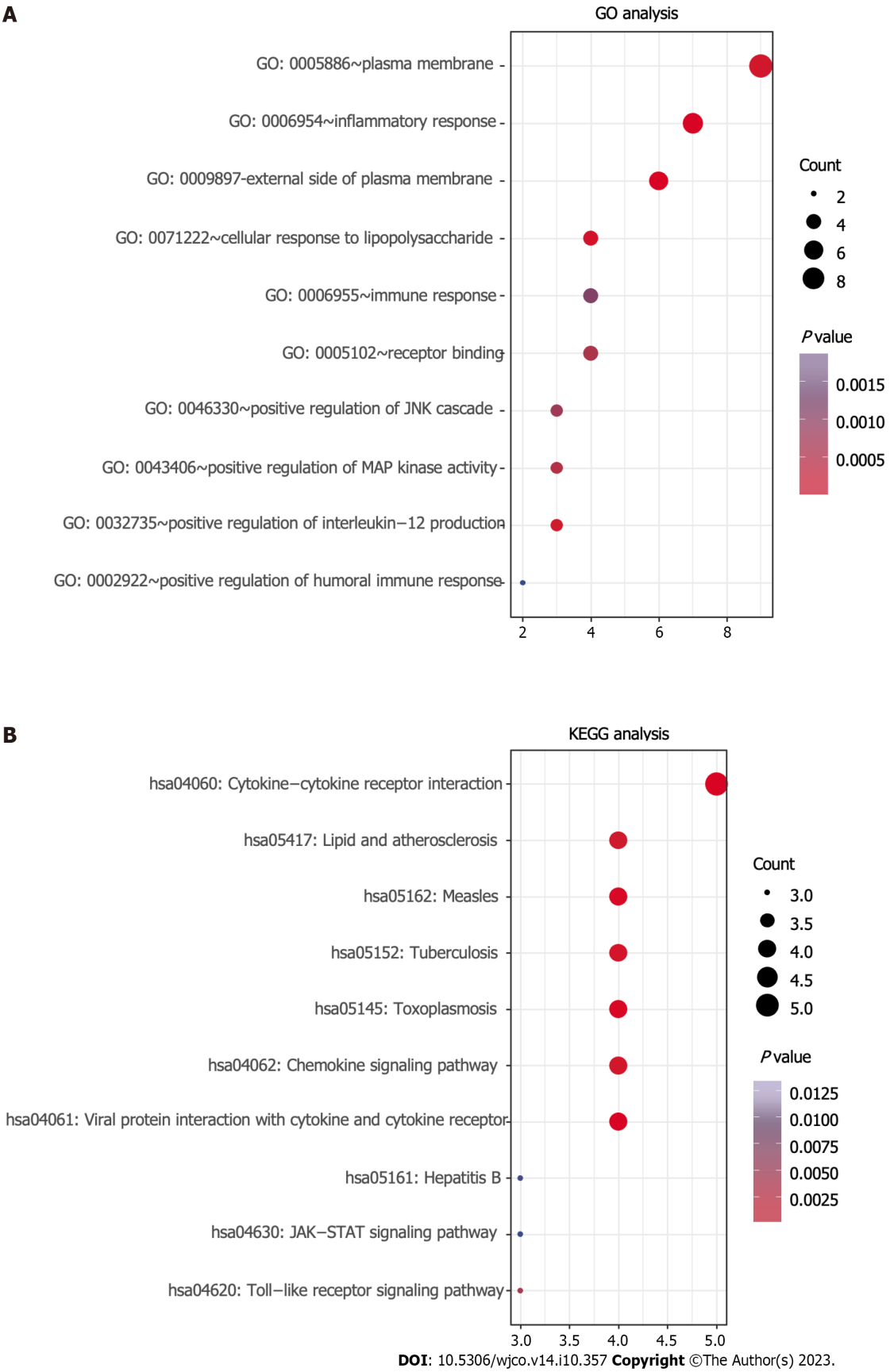

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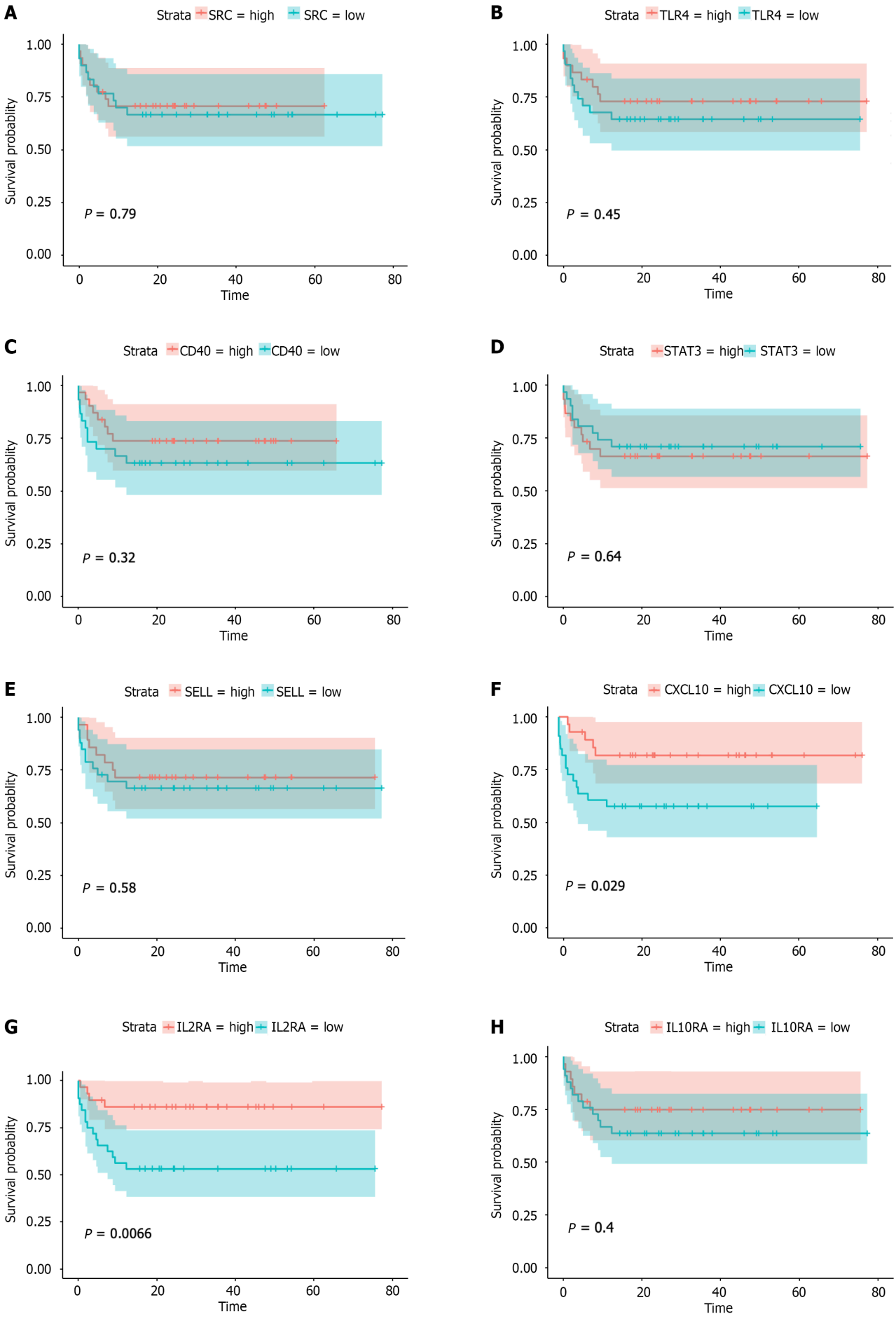
Figure 6 Functional enrichment analysis results of hub genes. A: The top 10 gene ontology terms of hub genes; B: The top 10 Kyoto Encyclopedia of Genes and Genomes pathways of hub genes. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.


A
Strata SRC = high SRC = low
Survival probability
1.00
0.75
0.50
0.25
0.00
P = 0.79
0 20 40 60 80
Time
B
Strata TLR4 = high TLR4 = low
Survival probability
P = 0.45
Time
C
Strata CD40 = high CD40 = low
Survival probability
P = 0.32
Time
D
Strata STAT3 = high STAT3 = low
Survival probability
P = 0.64
Time
E
Strata SELL = high SELL = low
Survival probability
P = 0.58
Time
F
Strata CXCL10 = high CXCL10 = low
Survival probability
P = 0.029
Time
G
Strata IL2RA = high IL2RA = low
Survival probability
P = 0.0066
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P = 0.4
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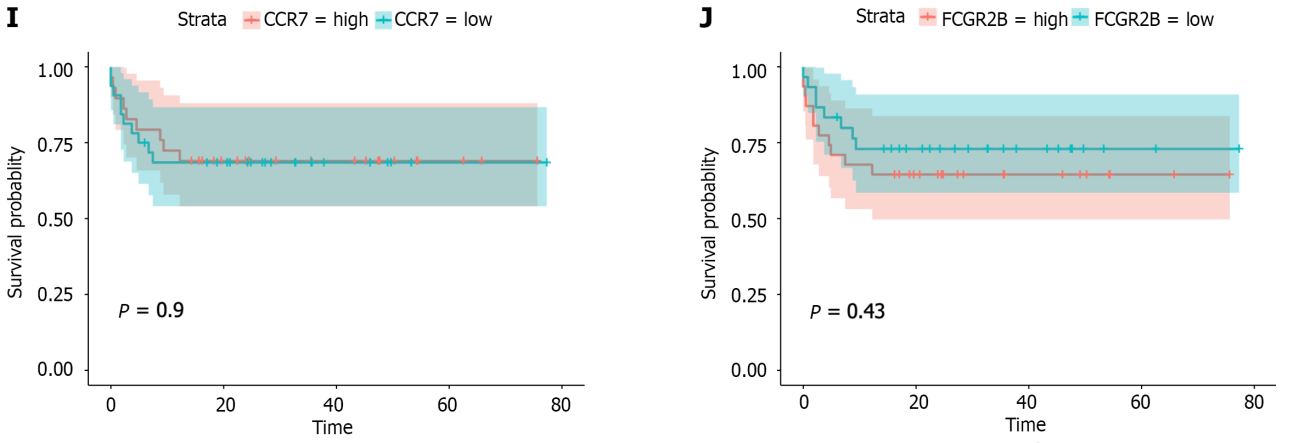

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Figure 7 Kaplan–Meier survival curve. A to J: Kaplan–Meier survival curve of identified hub genes in GSE69051.

IL2RA (CD25) is a low-affinity receptor for its ligand interleukin 2 (IL2), but when combined with *IL2RB* (CD122) and *IL2RG* (CD133), it forms the high-affinity IL2 receptor[42]. The binding of IL2 to IL2 receptor activates JAK1 and JAK3, which in turn activate several pathways that regulate cell survival and proliferation, including the PI3K/AKT, RAS/RAF/MEK/ERK, and STAT5 pathways[43]. *IL2RA* expression is elevated in a variety of cancers, especially hematologic tumors[44-46]. Fell *et al*[47] studied 69 patients with leukemia, lymphoma, or multiple myeloma and found that the expression of *IL2RA* in T cells was associated with frailty independent of age. This means that patients with high *IL2RA* expression showed better tolerance to chemotherapy and thus might have a superior prognosis. However, another study demonstrated that high *IL2RA* mRNA expression was an independent and adverse prognostic factor in acute myeloid leukemia, specifically stratifying patients into a worse prognosis[48], while reports on *IL2RA* in chronic myelogenous leukemia (CML) were controversial, they described it as either a promoter or an inhibitor of CML cell proliferation and disease aggressiveness[45,46]. This study demonstrated that BL patients with high expression of *IL2RA* exhibited a better prognosis. Due to the controversial reports on the function of *IL2RA* and the lack of research on BL, further studies are required to validate the prognostic value of *IL2RA* in BL.

As a predictive statistical tool, a nomogram visually displays the significant factors that impact outcomes in multifactor regression analyses and simplifies survival probability prediction through an easy-to-understand graphical representation[49]. The construction of the nomogram model in this study is based on age, *IL2RA*, and *CXCL10*. The nomogram effectively visualizes the impact of identified hub genes and facilitates survival prediction, with the multivariate regression analysis serving as the fundamental component of this model. However, the nomogram would benefit from a validation cohort to enhance its current model. Therefore, it is recommended that additional patients with long-term follow-up be included in future studies.

Based on *CXCL10* and *IL2RA*, we have also identified some drugs that may potentially play a therapeutic role in relapsed and refractory BL, which require further research on pharmacology and treatment protocols. There are also some limitations of the present study. Firstly, the sample size may not be sufficient and could result in selection bias. Secondly, the three different clinical types of BL have the same histological features and similar clinical behavior but differ in epidemiology, clinical presentation, and genetic characteristics, which might need to be classified and analyzed separately. What's more, additional genetic and experimental studies are required to explain the mechanism and the function of these hub genes in the carcinogenesis and progression of BLs. Due to the limited experimental conditions, our study exclusively utilized data sourced from publicly available databases. However, further validation is needed in larger samples or more external datasets.

CONCLUSION

In conclusion, this study is the first to investigate gene expression in BL using WGCNA. These findings provide a framework for identifying co-expression gene modules and discriminating key pathways and hub genes in BL. In the present study, we identified and verified 10 hub genes. Survival analysis showed that overexpression of *CXCL10* and *IL2RA* in BL may serve as superior prognostic indicators. Additionally, an integrated mRNA signature and age nomogram potentially offer prognostic value for patients with BLs.

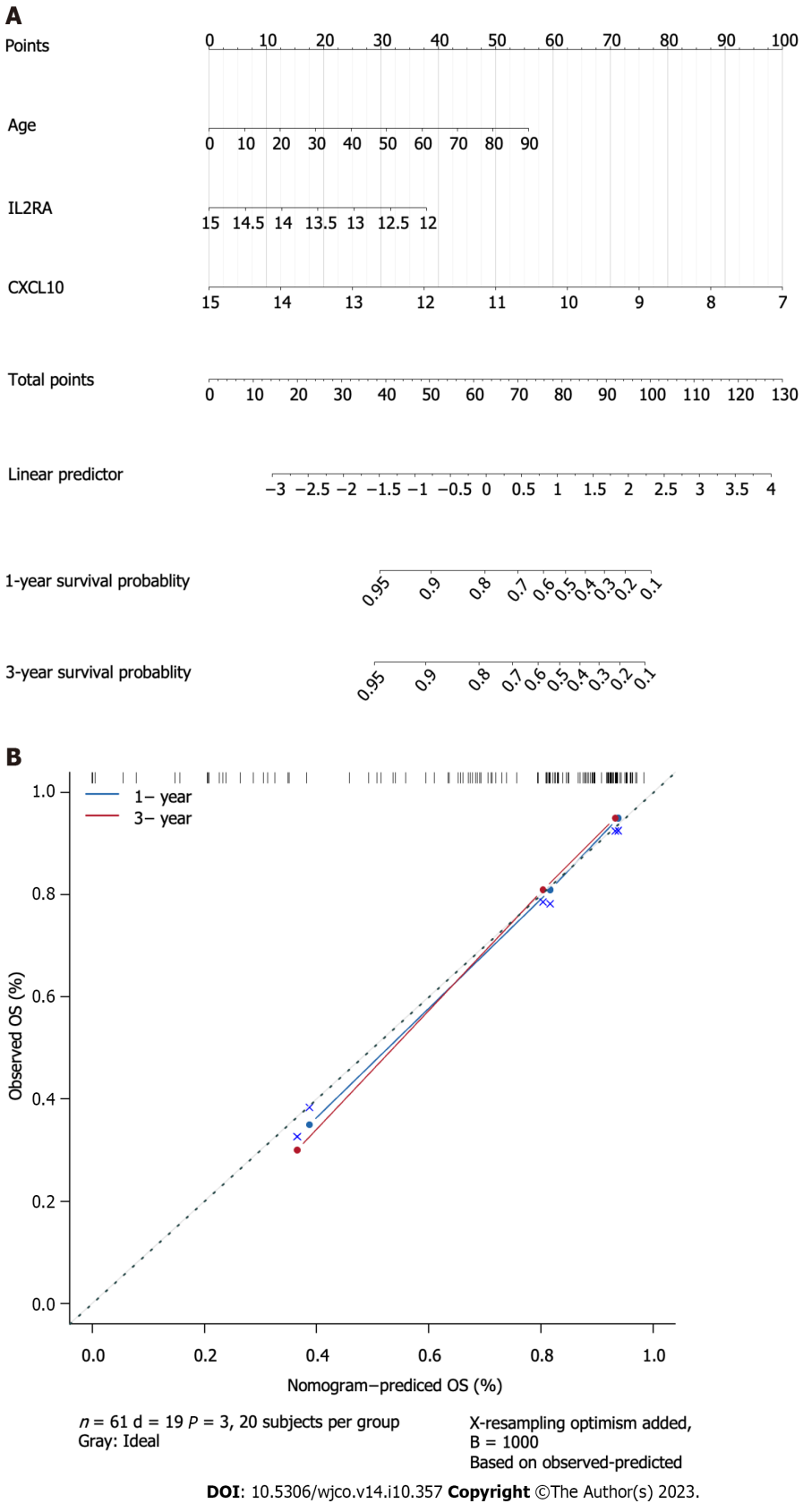

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Figure 8 Nomogram and calibration plot for GSE69051 cohort. A: The nomogram was constructed to predicting1, 3-year survival rate of Burkitt lymphoma patients; B: The calibration curves for predicting patient survival at 1 and 3 years in the cohort. OS: Overall survival.

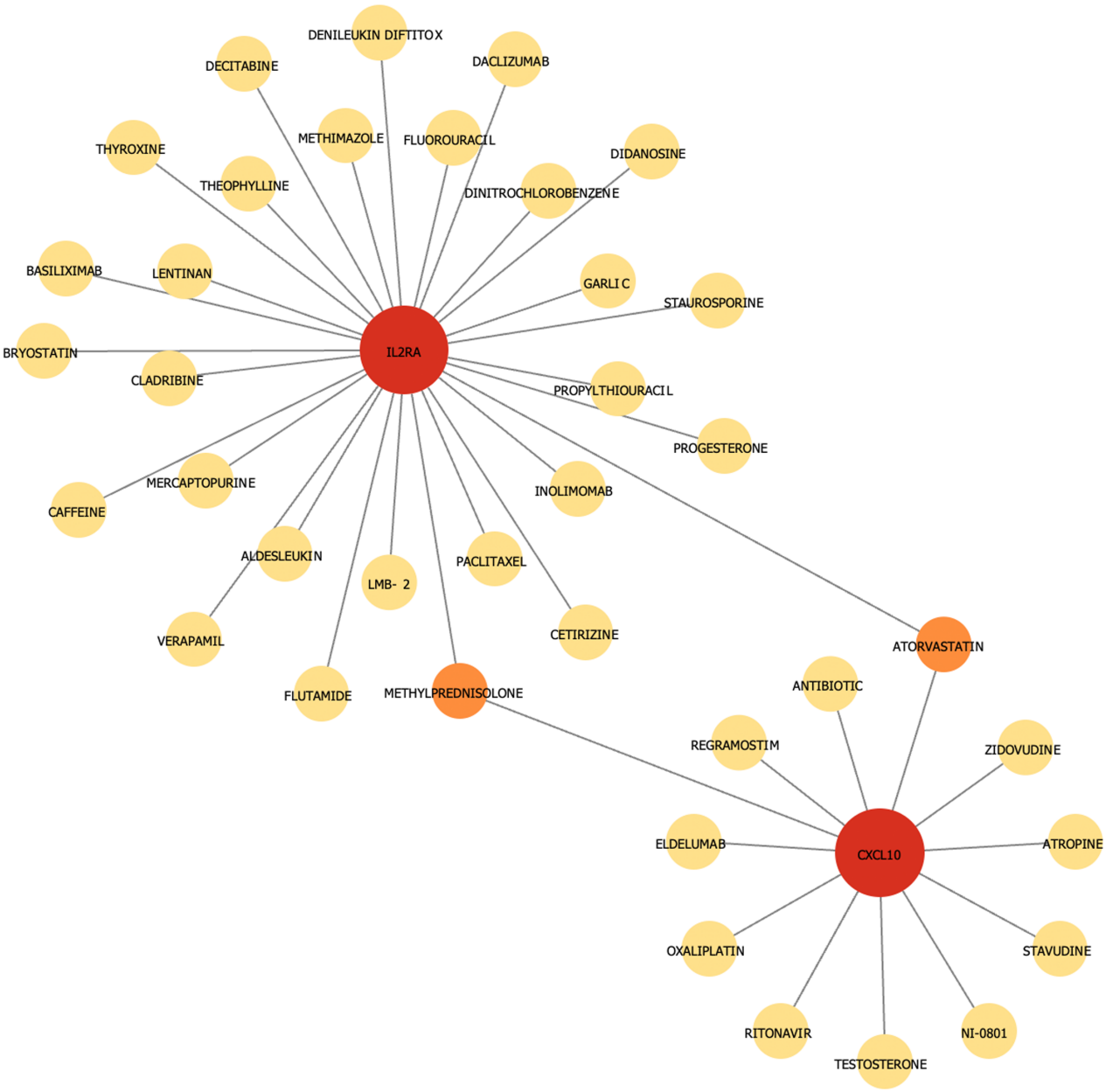

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Figure 9 Drugs related to *IL2RA* and *CXCL10*.

ARTICLE HIGHLIGHTS

Research background

Burkitt lymphoma (BL) is an exceptionally aggressive malignant neoplasm originating from either the germinal center or post-germinal center B cells. However, a standardized treatment regimen for BL has yet to be established. The utilization of microarray data and sequencing information retrieved from public databases presents promising prospects for the identification of novel diagnostic or therapeutic targets.

Research motivation

It is crucial to identify biomarkers that can predict the prognosis of BLs and distinguish patients who would benefit from specific therapies.

Research objectives

The aim of our study was to identify hub genes and conduct gene ontology analysis specifically in BL, as well as perform functional enrichment analysis. Additionally, we performed survival analysis and developed a novel prognostic model incorporating candidate genes along with clinical features.

Research methods
The gene expression profiles and clinical traits of BL patients were obtained from the Gene Expression Omnibus database. Weighted gene co-expression network analysis (WGCNA) was employed to construct gene co-expression modules, while the cytoHubba tool was utilized to identify hub genes. Prognostic candidate genes were identified through overall survival (OS) analysis. A nomogram was developed to evaluate the predictive value of the hub genes.

Research results
In this study, we identified 8 modules through WGCNA analysis and found a significant correlation between the yellow module and age. By using the cytoHubba tool, we identified 10 hub genes (*SRC*, *TLR4*, *CD40*, *STAT3*, *SELL*, *CXCL10*, *IL2RA*, *IL10RA*, *CCR7*, and *FCGR2B*). Among these hubs, two genes (*CXCL10* with $P = 0.029$ and *IL2RA* with $P = 0.0066$) were associated with OS based on our survival analysis.

Research conclusions
This study is the first to investigate gene expression in BL using WGCNA. We have identified and validated 10 hub genes, demonstrating that the overexpression of *CXCL10* and *IL2RA* in BL can serve as robust prognostic indicators. Furthermore, the integration of an mRNA signature with age nomogram holds promising potential for predicting patient outcomes in BLs.

Research perspectives
Further genetic and experimental investigations are imperative to elucidate the underlying mechanism and functional significance of these hub genes in the carcinogenesis and progression of BLs.

FOOTNOTES

Author contributions: Xu YF and Yang JG designed the research; Xu YF, Wang GY and Zhang MY performed the research; Xu YF, Wang GY contributed new reagents/analytic tools; Xu YF analyzed the data; Xu YF, Zhang MY, Wang GY and Yang JG wrote the paper.

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ORIGINAL ARTICLE

Basic Study

Comprehensive analysis of disulfidptosis related genes and prognosis of gastric cancer

Qian Li, Long-Kuan Yin

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Peer-review report's scientific quality classification

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Abstract

BACKGROUND

Gastric cancer (GC) is a common malignant tumor of the digestive system. Disulfidptosis is a new programmed cell death mechanism, although its specific mechanism in GC is incompletely understood.

AIM

In this study, we used bioinformatics analysis to explore a disulfidptosis-based predictive model related to GC prognosis and to identify potential therapeutic targets and sensitive drugs for GC.

METHODS

We extracted GC-related data from The Cancer Genome Atlas and Gene Expression Omnibus databases. R software (version 4.2.1) was used for correlation analysis.

RESULTS

Through the above analysis, we found that the disulfidptosis related gene may be related to the prognosis of GC. Six genes, namely, *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, and *VAMP7*, were found to constitute a predictive model for GC prognosis. *APOD* is a potential therapeutic target for treating GC. Bosutinib and other drugs are sensitive for the treatment of GC.

CONCLUSION

The results of this study indicate that disulfidptosis is related to the prognosis and treatment of GC, while *APOD* represents a potential therapeutic target for GC.

Key Words: Gastric cancer; Disulfidptosis; Drugs; Prognosis; Targets

Core Tip: Gastric cancer (GC) is a common malignant tumor of the digestive system. Disulfidptosis is a new programmed cell death mechanism. The specific mechanism of disulfidptosis in GC is not fully understood. This study found that the disulfidptosis related gene may be related to the prognosis of gastric cancer. *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, *VAMP7*, these six genes constitute a predictive model for gastric cancer prognosis. *APOD* is a potential therapeutic target. Bosutinib and other drugs are sensitive for the treatment of gastric cancer.

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INTRODUCTION

Gastric cancer (GC) is a common cause of cancer-related death worldwide, with a particularly high incidence in East Asia, such as South Korea, China, and Japan[1-9]. The early clinical symptoms of GC are not obvious and lack specificity[10-14], which leads to a low rate of early diagnosis[15-26]. Most patients with GC are diagnosed late and have a poor prognosis[27-40]. Although the diagnosis and treatment strategies for GC have gradually increased in recent decades, the prognosis of advanced GC remains poor[41-47]. Therefore, there is an urgent need to find more biomarkers as novel therapeutic targets and to develop new drugs to improve diagnosis and treatment measures and, consequently, patient survival and prognosis.

GC is a heterogeneous disease[48], with previous studies suggesting that various cell programmed death mechanisms, including ferroptosis[49-54] and cuproptosis[55-58], represent novel research directions for GC. In recent years, it has been found that disulfidptosis[59], a novel and poorly studied mechanism of programmed cell death, represents a previously uncharacterized form of cell death induced by abnormal accumulation of disulfide in cells under glucose starvation, which is different from copper death and iron death. However, its role in GC and its related mechanisms are still unclear and need to be further explored.

In this study, we analyzed the sequencing data of tumor tissues from databases such as The Cancer Genome Atlas (TCGA)[60] and Gene Expression Omnibus (GEO) (Supplementary material)[61] and 14 disulfidptosis-related gene (DRGs)[59] (*ACTN4, ACTB, CD2AP, CAPZB, DSTN, FLNA, FLNB, INF2, IQGAP1, MYH10, MYL6, MYH9, PDLIM1,* and *TLN1*). We conducted differential analysis of DRGs, as well as analyses of the tumor mutation burden (TMB)[62,63], copy variations, gene ontology (GO)[64], and the kyoto encyclopedia of genes and genomes (KEGG)[65], among others. In this paper, the mechanism of DRGs involved in the occurrence and development of GC is discussed, and new therapeutic targets and drugs that may be related to the prognosis of GC are preliminarily analyzed and screened from a new perspective.

MATERIALS AND METHODS

Data downloading and processing

Expression data, clinical data, mutation data, and copy data related to GC were downloaded and organized from TCGA database. The GSE84433 and GSE26253 datasets and their platform annotation files were downloaded from the GEO database. Data were analyzed and processed using R software (version 4.2.1) and Perl software (version 5.30.0).

Differential and prognostic analyses

GC-related data were extracted from TCGA database and analyzed in combination with the disulfidptosis-related gene. Differential analysis, mutation load analysis, copy number variation frequency analysis, and survival analysis were performed using R software.

Disulfidptosis subtype analysis

R software was used to classify all samples related to the disulfidptosis-related gene in TCGA and GEO databases for survival analysis, heatmap clustering, gene set variation analysis (GSVA), immune cell differential analysis, subtype differential analysis, and GO and KEGG enrichment analyses.

Significant differential gene subtyping, prediction model construction, and analysis

We continued to perform survival analysis, heatmap clustering, and differential analysis of the DRGs on the samples classified by differential gene subtyping. Then, we randomly divided the significant differential samples into groups and performed least absolute shrinkage and selection operator (LASSO) regression analysis and univariate and multivariate

Cox regression analyses and constructed a prognostic model. Using the prognostic model, we calculated the risk score for each patient sample using the following formula:.where $Coef_i$ is the coefficient, and X_i is the expression level of the gene. We constructed a prognostic evaluation model for overall survival based on the risk score. We then constructed a Sankey diagram and analyzed the differences in risk scores between subtypes and the differential risk of the DRGs.

Prognostic model validation

The reliability of the prognostic model was verified by survival analysis, receiver operating characteristic (ROC) curve mapping, risk curve mapping, survival state map, and clustering heatmap of model genes in each subgroup.

Nomogram construction and analysis of the correlation between risk score and immunity, as well as drug susceptibility

Next, the independent prognostic factors of GC and potential therapeutic targets were sought by constructing the column diagram, and survival analysis of potential prognostic genes was performed by Gene Expression Profiling Interactive Analysis (GEPIA). Subsequently, immune cell correlation analysis, tumor microenvironment (TME) difference analysis, waterfall map construction, tumor mutation load analysis, microsatellite instability (MSI), stem cell correlation analysis, and drug sensitivity analysis were performed for the risk score.

Immunohistochemical analysis

We conducted immunohistochemical analysis of *APOD* using the human protein atlas (HPA) network database, comparing the differences in protein expression between GC tissues and adjacent normal tissues.

Statistical analysis

All statistical analyses were performed using R software (version 4.2.1). A P-value < 0.05 was considered statistically significant.

RESULTS

Difference analysis and prognosis analysis of DRGs

Difference analysis revealed that 10 DRGs, namely, *ACTN4*, *ACTB*, *CD2AP*, *CAPZB*, *FLNB*, *INF2*, *IQGAP1*, *MYH10*, *MYH9* and *PDLIM1*, were significantly different in GC samples and adjacent normal tissue samples (Figure 1A). Through mutation load analysis, copy number variation frequency analysis, and a genosphere map, we found that *CAPZB* and *MYL6* were not mutated, while *MYH10* had the most mutations. It was also found that *CAPZB* had the most deletion mutations, while *IQGAP1* had the most insertion mutations. Cyclic analysis led to the identification of disulfidptosis mutations in 14 chromosomes (Figure 1B-D). Moreover, survival analysis showed that patients with high expression of *TLN1*, *MYL6*, *MYH10*, *MYH9*, *IQGAP1*, *INF2*, *FLNA*, *DSTN*, and *ACTB* had a reduced survival time, while those with high expression of *PDLIM1* had an increased survival time (Figure 2A–J). Prognostic network diagram analysis showed that disulfidptosis-related genes, including *PDLIM1*, *FLNA*, *MYH10*, *MYL6*, and *DSTN*, were significantly correlated with the prognosis of GC (P < 0.05), and *DSTN*, *FLNA*, *MYH10*, and *MYL6* were risk factors for the prognosis of GC, while *PDLIM1* was a favorable factor for the prognosis of GC (Figure 2K).

Subtyping of the DRGs and analysis through GSVA, single-sample gene set enrichment analysis, GO, and KEGG analyses

Through clustering analysis of the DRG samples, we found that the best way to divide the samples was into two subtypes, A and B (Figure 3A-D). Through survival analysis of the two subtypes, we found significant differences between the groups, P < 0.05 (Figure 3E), and through clustering heatmap analysis, we found that most DRGs were upregulated in cluster A and downregulated in cluster B (Figure 3F). Using the GSVA package in R software, we performed KEGG pathway enrichment analysis on the DRG subtyping samples and found that the significantly different pathways enriched in the two subtypes included glutamate and glutamine metabolism, extracellular matrix receptor interaction, the transforming growth factor-beta (TGF-β) signaling pathway, and the pentose phosphate pathway (Figure 4A). Through GO functional enrichment analysis of the DRG subtyping samples with the GSVA package in R, we found that the main enrichment was in the positive regulation of the transforming growth factor receptor and Wnt signaling pathways (Figure 4B). We also found significant differences in immune cells, such as activated CD4 T cells, and activated CD8 T cells, between subtypes A and B, according to the analysis of the differences in immune cells between the subtypes (Figure 5A). Subtype differential analysis led to the identification of 282 significantly different co-expressed genes between subtypes A and B (Figure 5B and C). Moreover, GO analysis of these differentially expressed genes revealed that the enriched functions of these differentially expressed genes were mainly in the extracellular matrix tissue and negative regulation of the typical Wnt signaling pathway (Figure 5D). KEGG analysis of these differentially expressed genes revealed that these genes were enriched in the TGF-β, Wnt, and MAPK signaling pathways, as well as in other pathways (Figure 5E).

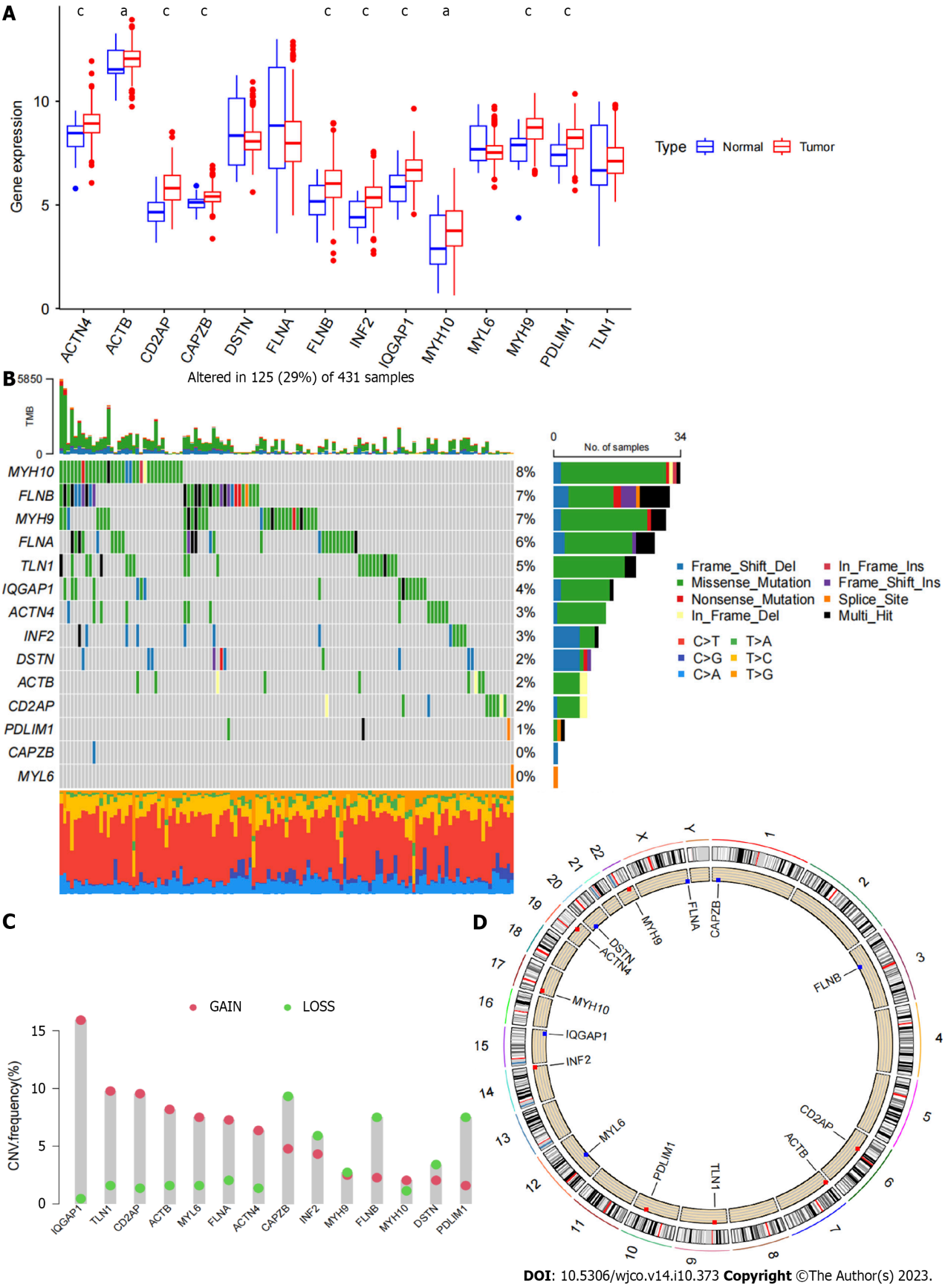

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Figure 1 The results of the differential expression analysis of disulfidptosis related genes in gastric cancer and adjacent normal tissues are presented. A: Shows the difference analysis of disulfidptosis related genes in gastric cancer tissue samples and adjacent normal tissue samples; B: Shows the waterfall plot of disulfidptosis related genes mutations; C: Presents the mutation frequency of disulfidptosis related genes; D: Shows the mutation sites of disulfidptosis related genes. CNV: Copy number variation.

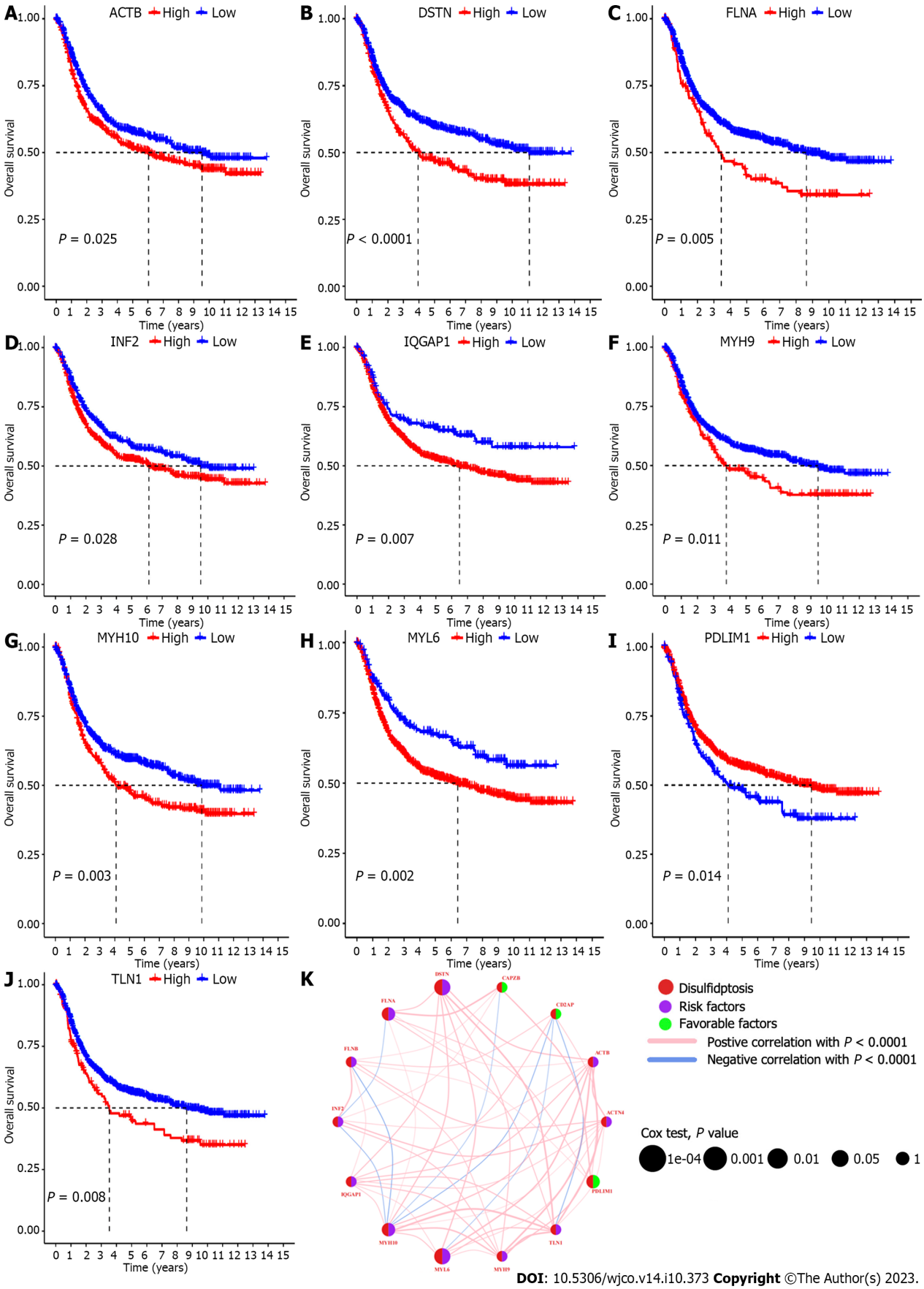

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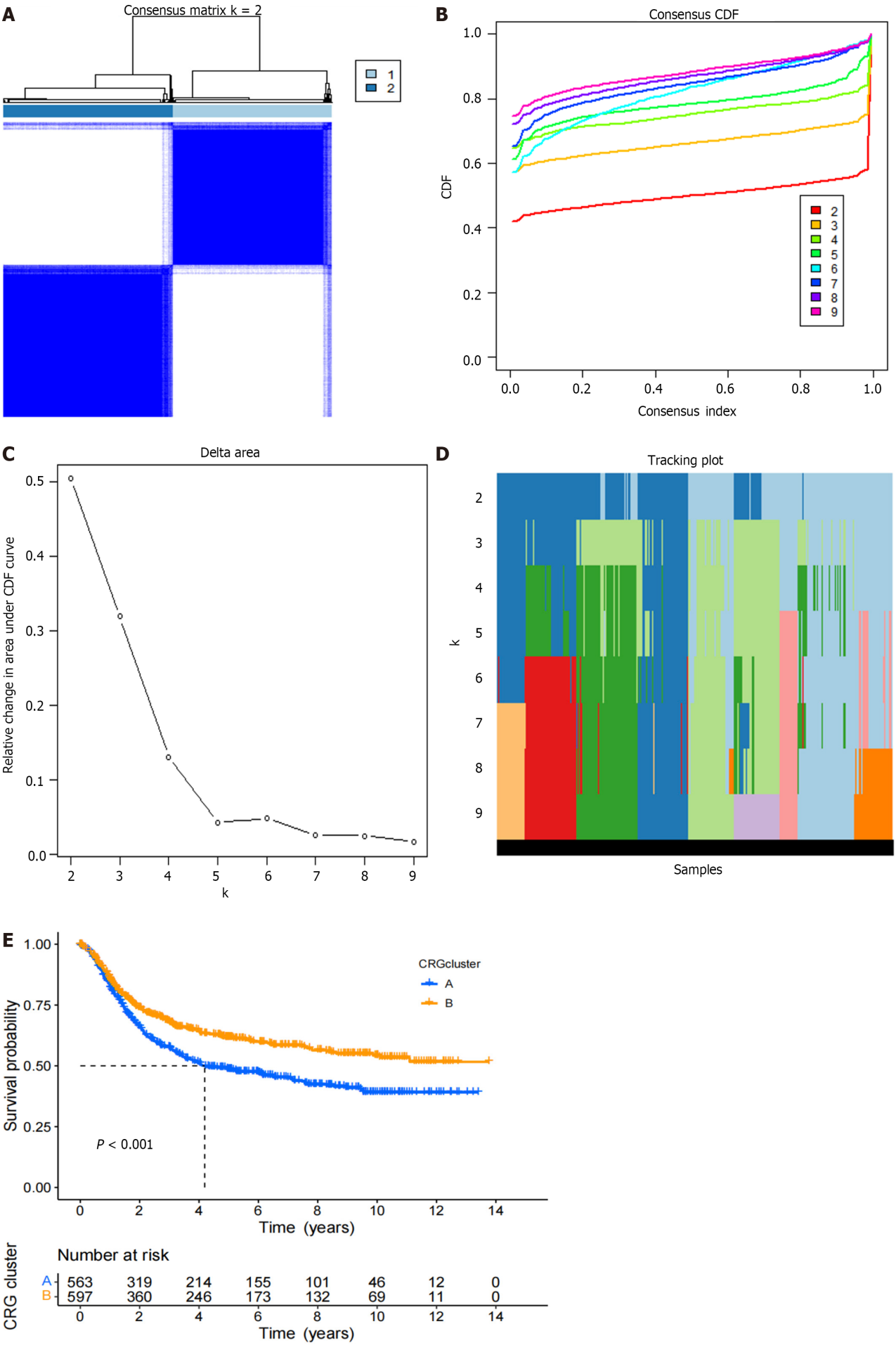
Figure 2 Screening disulfidptosis related genes related to the prognosis of gastric cancer. A-J: Show the Kaplan-Meier analysis of the survival curves of disulfidptosis related genes between high and low expression groups, and 10 disulfidptosis related genes related to gastric cancer prognosis were identified; K: Shows the COX analysis of the disulfidptosis related genes circle plot related to gastric cancer prognosis, and five significantly prognostic disulfidptosis related genes were identified.


A
Consensus matrix k = 2
1
2
B
Consensus CDF
CDF
Consensus index
2
3
4
5
6
7
8
9
C
Delta area
Relative change in area under CDF curve
k
D
Tracking plot
k
Samples
E
CRGcluster
A
B
Survival probability
P < 0.001
Time (years)
Number at risk
CRG cluster
A 563 319 214 155 101 46 12 0
B 597 360 246 173 132 69 11 0
Time (years)

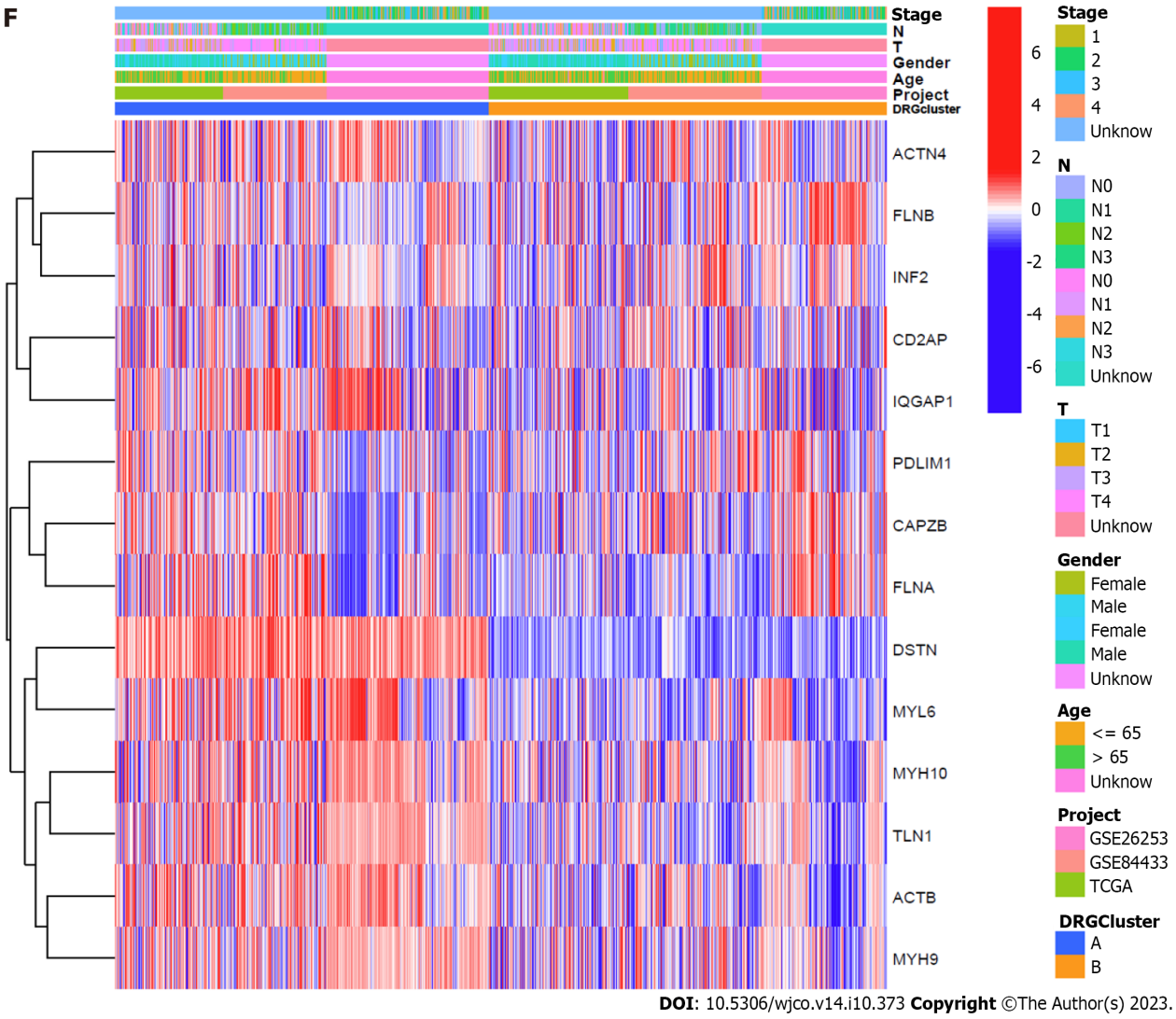


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Figure 3 The sample classification, subgroup survival analysis, and differential gene heatmap related to disulfidptosis related genes are presented. A: Shows the clustering matrix plot of disulfidptosis related genes-related samples; B: Shows the clustering index plot of disulfidptosis related genes-related samples; C: Presents the relative change area under the cumulative distribution function curve; D: Shows the tracking plot of disulfidptosis related genes subgroup samples; E: Presents the survival analysis curves of disulfidptosis related genes subgroups; F: Shows the differential gene clustering heatmap between disulfidptosis related genes subgroups.

Classification and correlation analysis of significant differentially expressed genes obtained from the disulfidptosis subtype samples

The related samples of differentially expressed genes were clustered into three subtypes (Figure 6A–D). Survival analysis showed that the prognosis of subtype C was different from that of subtypes A and B, with better prognosis for subtypes B and C (Figure 6E). Heatmap analysis showed that most samples in subtype C were upregulated, while most samples in subtype B were downregulated (Figure 6F). Differential expression analysis of the DRGs in the different gene subtypes showed that the expression of the DRGs was different in subtypes A, B, and C, with $P < 0.05$ (Figure 6G). We used the create data partition package to randomly divide the samples into two groups of equal size, the training and testing groups. Then, using LASSO regression and Cox regression, we analyzed the training group samples and constructed a six-gene risk model based on the DRG subtype: Risk score = (0.164102181511909* *PLS3* expression) + (0.079055019007862* *GRP* expression) + (0.0649967121599996* *APOD* expression) + (0.0920219139298833* *SGCE* expression) + (0.107438278125729* *COL8A1* expression) + (–0.0723643090076661* *VAMP7* expression) (Figure 6H and I). The results of the risk model are shown in Supplementary Table 1. The Sankey diagram shows the distribution of samples among the different groups (Figure 7A). By evaluating the risk score for each group, we found significant differences in the risk between the groups (Figure 7B and C). By evaluating the risk score for the DRGs, we found that the expression levels of 13 DRGs differed significantly between the high and low risk groups, with nine genes showing higher expression in the high-risk group and four genes showing higher expression in the low-risk group (Figure 7D).

Validation results of the risk model

We next used the risk model to score the differential gene-related samples mentioned above and then divided them into

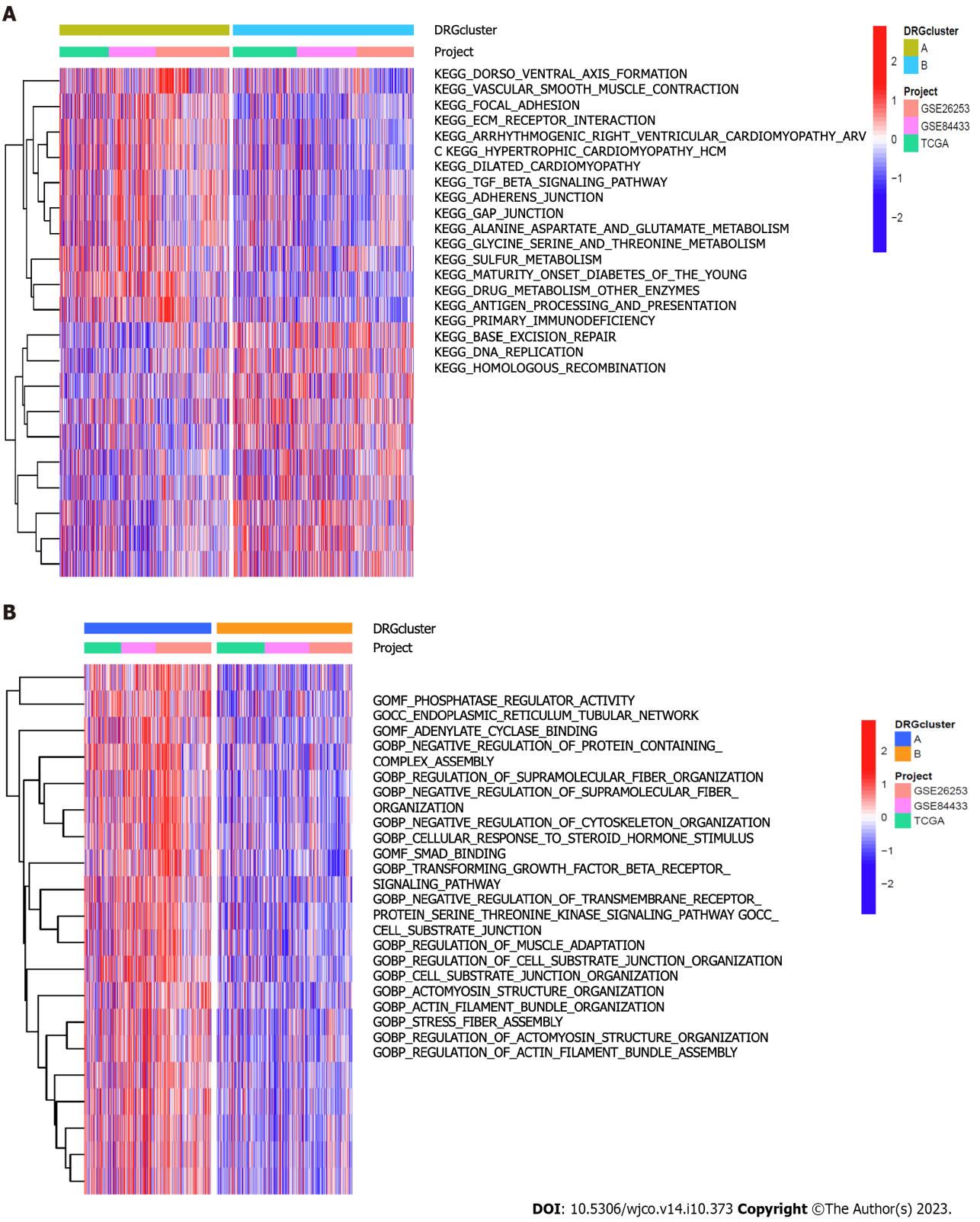

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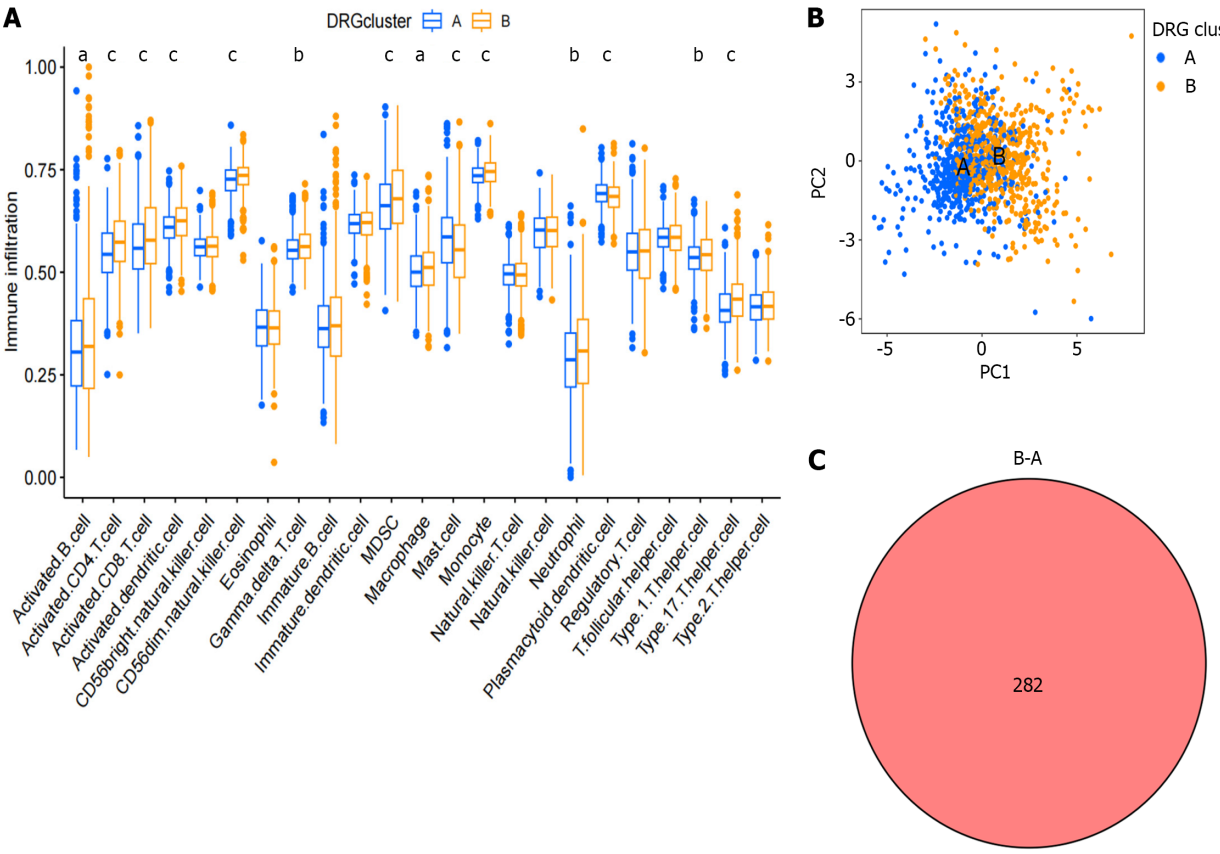
Figure 4 The significantly different kyoto encyclopedia of genes and genomes pathways and gene ontology functional analysis between disulfidptosis related genes subgroups are presented. A: Shows the significantly different kyoto encyclopedia of genes and genomes pathway enrichment analysis between disulfidptosis related genes subgroups; B: Shows the significantly different gene ontology pathway enrichment analysis between disulfidptosis related genes subgroups.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0.50
0.25
0.00
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Activated.CD8.T.cell
Activated.dendritic.cell
CD56bright.natural.killer.cell
CD56dim.natural.killer.cell
Eosinophil
Gamma.delta.T.cell
Immature.B.cell
Immature.dendritic.cell
MDSC
Macrophage
Mast.cell
Monocyte
Natural.killer.T.cell
Natural.killer.cell
Neutrophil
Plasmacytoid.dendritic.cell
Regulatory.T.cell
T.follicular.helper.cell
Type.1.T.helper.cell
Type.17.T.helper.cell
Type.2.T.helper.cell
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PC1
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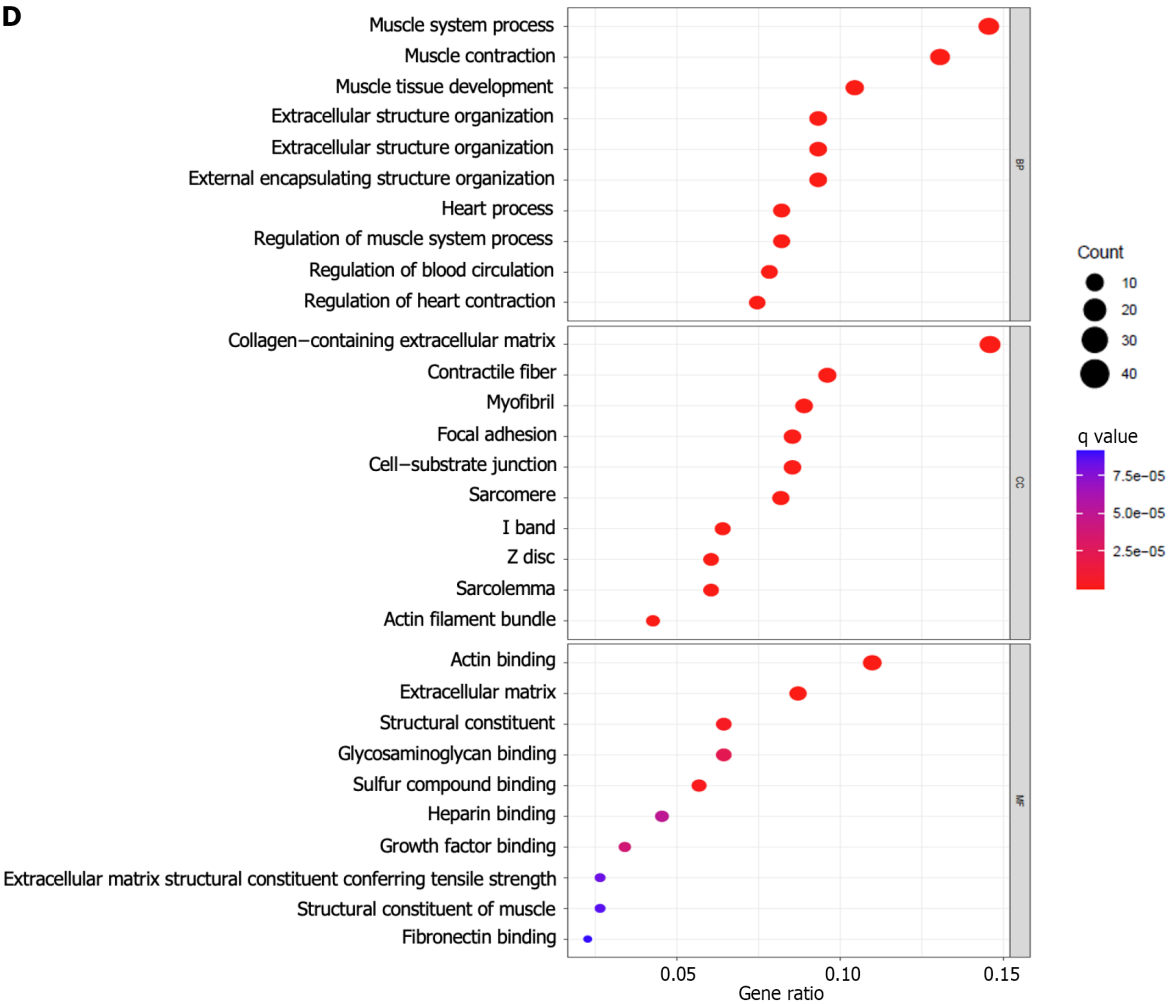
-6
-5
0
5
C
B-A
282


D
Muscle system process
Muscle contraction
Muscle tissue development
Extracellular structure organization
Extracellular structure organization
External encapsulating structure organization
Heart process
Regulation of muscle system process
Regulation of blood circulation
Regulation of heart contraction
Collagen−containing extracellular matrix
Contractile fiber
Myofibril
Focal adhesion
Cell−substrate junction
Sarcomere
I band
Z disc
Sarcolemma
Actin filament bundle
Actin binding
Extracellular matrix
Structural constituent
Glycosaminoglycan binding
Sulfur compound binding
Heparin binding
Growth factor binding
Extracellular matrix structural constituent conferring tensile strength
Structural constituent of muscle
Fibronectin binding
BP
CC
MF
0.05
0.10
0.15
Gene ratio
Count
10
20
30
40
q value
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5.0e−05
2.5e−05

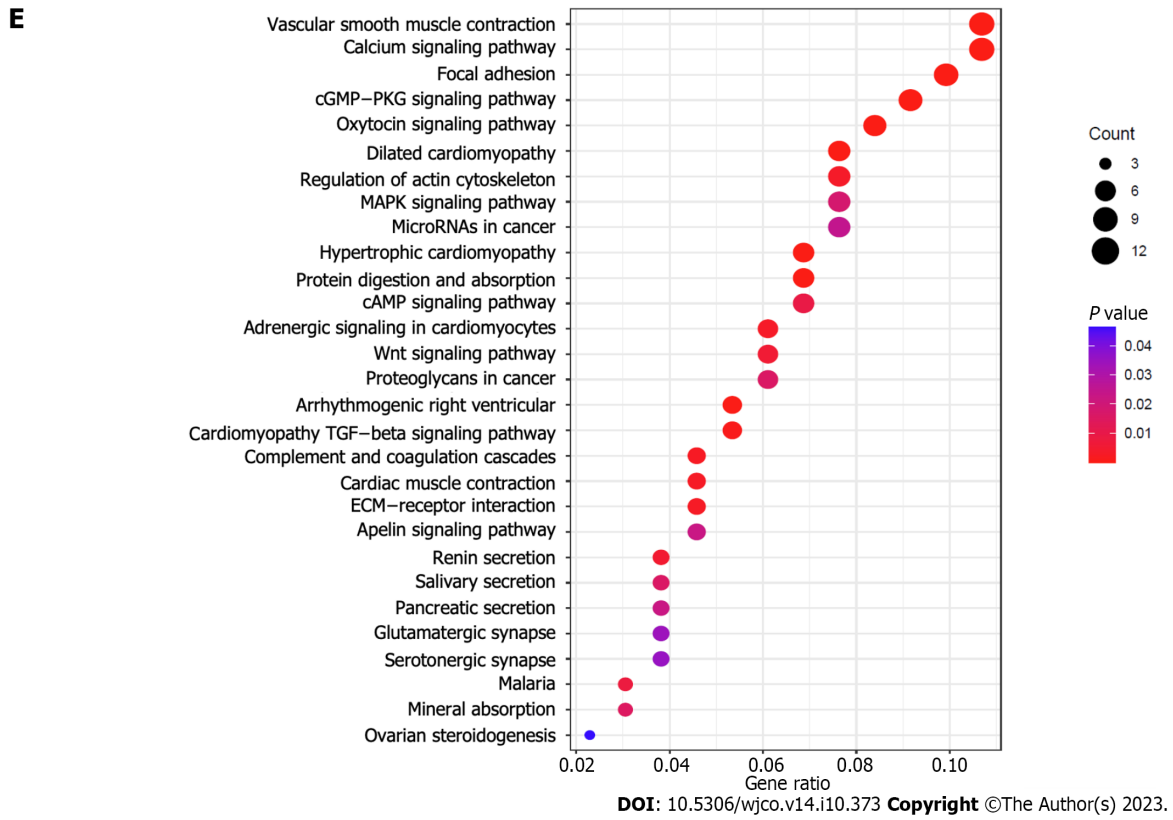

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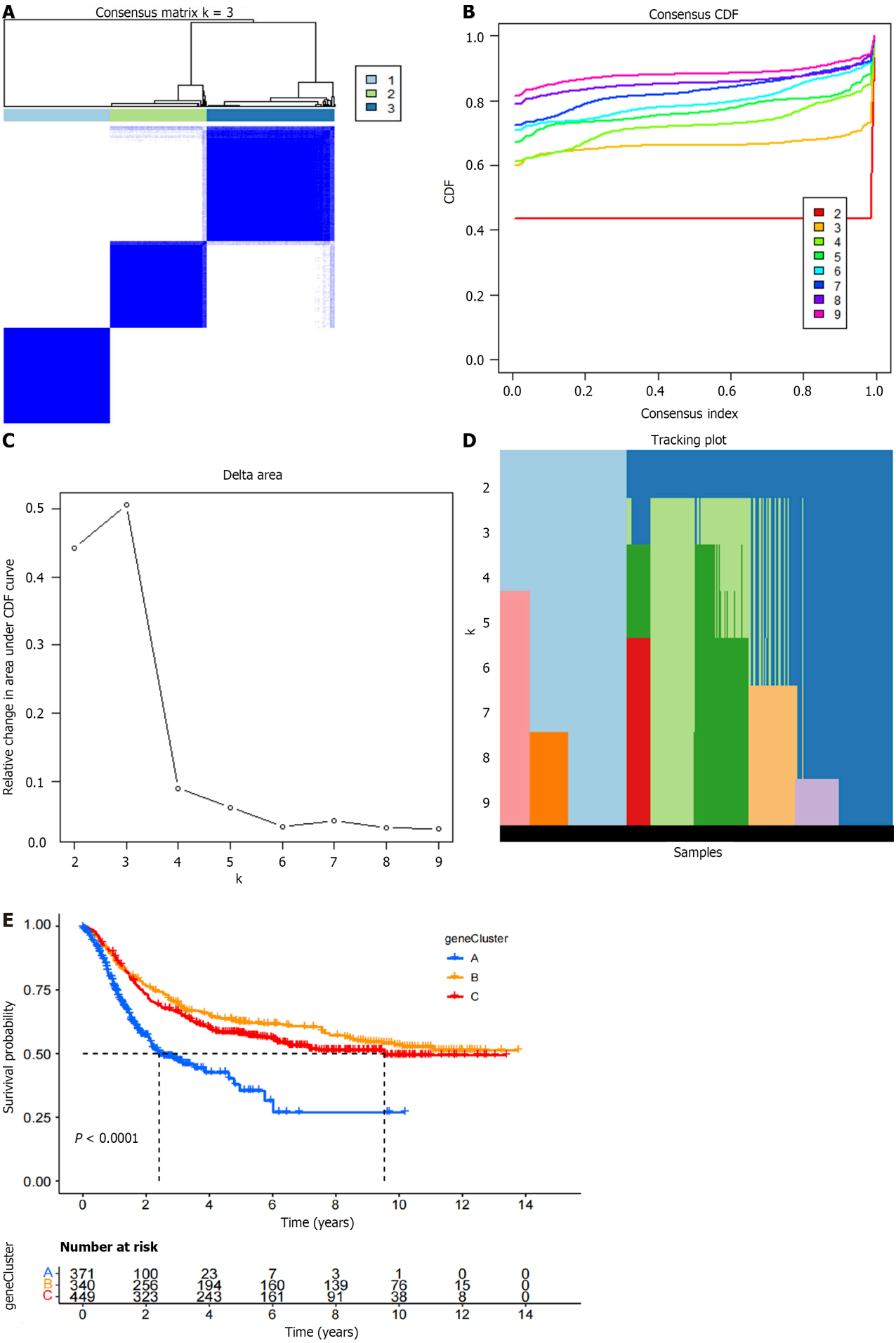
Figure 5 The immune cell differential analysis, principal component analysis analysis, significantly different genes, and gene ontology/kyoto encyclopedia of genes and genomes analysis between disulfidptosis related genes subgroups are presented. A: Shows the immune cell differential analysis between disulfidptosis related genes subgroups; B: Presents the principal component analysis analysis of disulfidptosis related genes subgroups; C: Shows the significantly different genes between disulfidptosis related genes subgroups; D: Presents the gene ontology analysis of significantly different genes between disulfidptosis related genes subgroups; E: Presents the kyoto encyclopedia of genes and genomes analysis of significantly different genes between disulfidptosis related genes subgroups.

high- and low-risk groups for the overall, test set, and training set samples. We then performed survival, ROC, and risk analyses on each group. Survival analysis of each group revealed that the high-risk group had poorer prognosis than the low-risk group (Figure 8A-C). Through ROC curve analysis of each group, we found that the area under the curve values of the overall, training set, and test set samples for 1, 3, and 5 years were all > 0.05, indicating the accuracy of the model in predicting survival prognosis (Figure 8D-F). Risk curve analysis of the total, training set, and test set samples showed an increase in the number of deaths with an increase in the risk score. We also found that *VAMP7* was a low-risk gene, while *PLS3*, *GRP*, *APOD*, *SGCE*, and *COL8A1* were high-risk genes through heatmap analysis (Figure 8G-O). Comparison of the results of survival, ROC, and risk analyses among various groups showed that the results were consistent, indicating the accuracy of this risk model in predicting the prognosis of patients with GC.

Identification of potential therapeutic targets by constructing column line graphs and immune and drug sensitivity analyses

We found that *APOD*, *PLS3*, age, sex, and N staging are independent factors that impact patient prognosis, all of which are risk factors for the prognosis of patients with GC. The odds of patients surviving for 1, 3, and 5 years are 0.806, 0.527, and 0.39, respectively (Figure 9A). The correction curve shows that the predicted value of the model is close to the actual value (Figure 9B). Through immune cell analysis, we found that resting mast cells and *APOD* were significantly positively correlated. Moreover, *PLS3* was significantly positively correlated with resting mast cells (Figure 9C). We also conducted survival analysis on *APOD* and *PLS3* by GEPIA, which were found to have independent effects on the prognosis of GC through column line graph analysis, and found that the survival analysis of *APOD* showed significant differences ($P < 0.05$) (Figure 9D), while the survival analysis of *PLS3* did not indicate the presence of significant differences ($P > 0.05$) (Figure 9E). In the relationship analysis between immune cells and risk scores, we found that 13 types of immune cells were significantly correlated with risk scores (Figure 10). Through TME scoring, we found differences between high and low risk groups in terms of the Stromal Score and ESTIMATE Score, both of which were upregulated in the high-risk group (Figure 11A). The waterfall chart shows that the genes that undergo mutations in the high- and low-risk groups were consistent, while the probability of mutations occurring in the low-risk group was higher than that in the high-risk group (Figure 11B and C). Through TMB analysis, we found significant differences between the high- and low-risk groups, as well as a negative correlation between TMB and risk scores (Figure 11D and E). Through microsatellite instability analysis, we found significant differences between the microsatellite stability and MSI-high (MSI-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Consensus matrix k = 3
1
2
3
B
Consensus CDF
CDF
Consensus index
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3
4
5
6
7
8
9
C
Delta area
Relative change in area under CDF curve
k
D
Tracking plot
k
Samples
E
geneCluster
A
B
C
Surivival probability
P < 0.0001
Time (years)
Number at risk
geneCluster
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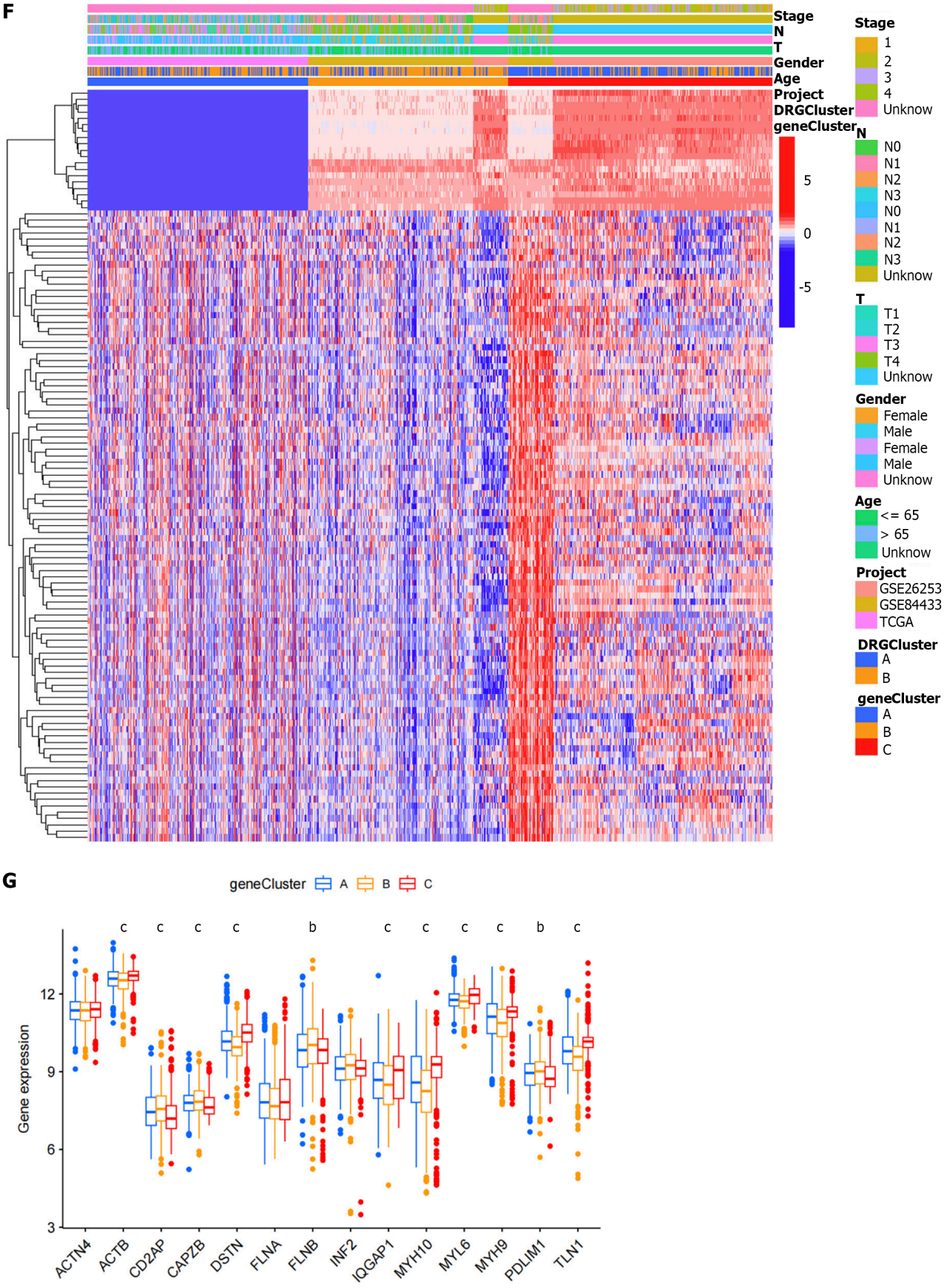
B 340 256 194 160 139 76 15 0
C 449 323 243 161 91 38 8 0
Time (years)


F
Stage
N
T
Gender
Age
Project
DRGCluster
geneCluster
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0
-5
Stage
1
2
3
4
Unknow
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N1
N2
N3
N0
N1
N2
N3
Unknow
T
T1
T2
T3
T4
Unknow
Gender
Female
Male
Female
Male
Unknow
Age
<= 65
> 65
Unknow
Project
GSE26253
GSE84433
TCGA
DRGCluster
A
B
geneCluster
A
B
C
G
geneCluster A B C
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Gene expression
12
9
6
3
ACTN4
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PDLIM1
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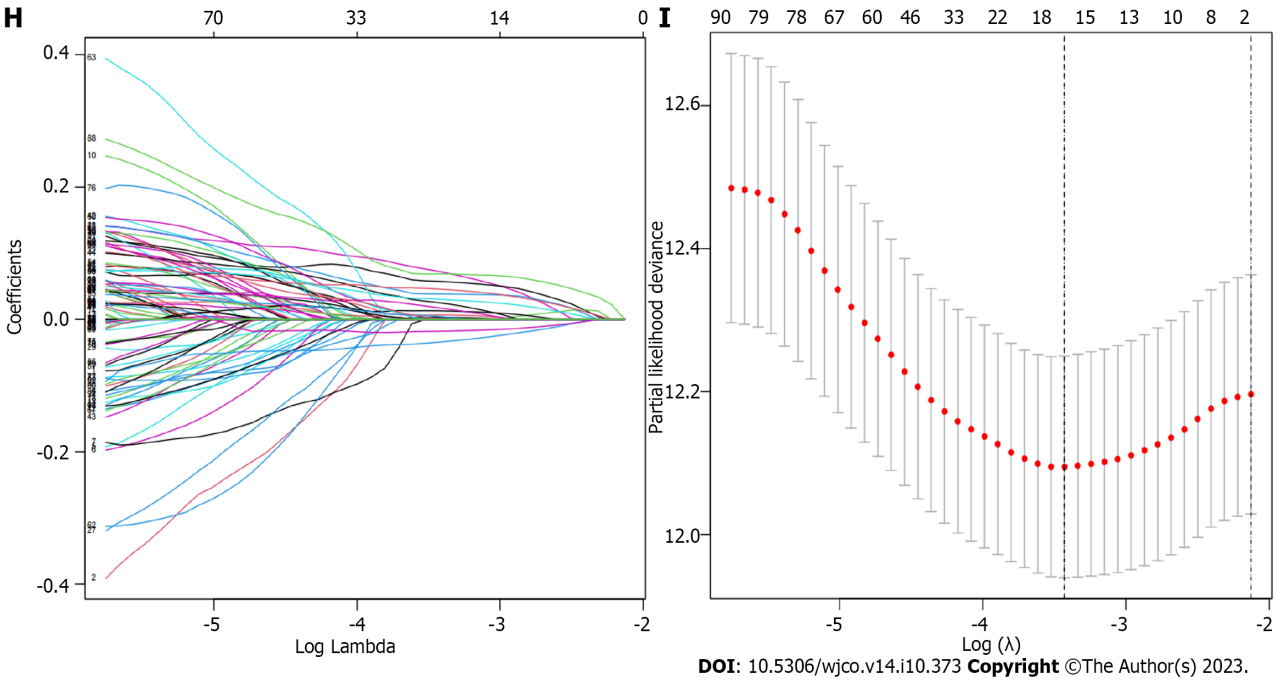

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Figure 6 The differential gene-related sample clustering matrix, clustering index, cumulative distribution function curve, tracking plot, survival curve, heat map, differential analysis of disulfidptosis related genes, lasso regression plot, and cvfit plot are presented. A: Shows the clustering matrix of differential gene-related samples; B: Presents the clustering index of differential gene-related samples; C: Shows the relative change area of the cumulative distribution function curve of differential gene-related samples; D: Presents the tracking plot of differential gene subgroups; E: Shows the survival curve of differential gene-related samples; F: Presents the heat map of differential gene-related samples; G: Presents the differential analysis of disulfidptosis related genes between differential gene-related sample groups; H: Shows the lasso regression plot; I: Presents the cvfit plot of the lasso regression.

H) groups, as well as between the MSI-H and MSI-low groups. The risk value of the MSI-H group is the lowest, and the proportion of stable samples in the high-risk group is as high as 71% (Figure 11F and G). Stem cell correlation analysis shows that RNA stemness scores (RNAss) is negatively correlated with risk scores (Figure 11H). Finally, drug analysis showed significant differences in the sensitivity of 89 drugs, including Bosutinib and Bryostatin (Figure 11I and J), between high- and low-risk groups.

Immunohistochemical analysis

Through the HPA network database, immunohistochemical analysis of *APOD* revealed that the protein expression level of *APOD* in GC tissues was significantly higher than that in normal tissues adjacent to GC (Figure 12).

DISCUSSION

The occurrence and development of GC are complex pathological processes involving the activation and alteration of multiple genes and signaling pathways[66]. Previous studies have shown that the expression of certain genes in GC tissue and normal gastric tissue can vary[67]. In this study, we analyzed the differential expression of 10 DRGs between GC tissue and adjacent normal tissue and found significant differences between the two. By analyzing the mutation waterfall plot and mutation frequency plot of DRGs, we observed that most DRGs were mutated in GC tissue, further indicating that DRGs are differentially expressed in cancer tissue. Previous studies have found that high expression of the disulfidptosis-related gene *PDLIM1* may inhibit the proliferation, invasion, and migration of GC cells, promote apoptosis, and enhance their sensitivity to cisplatin[68]. It has also been found that the high expression of *FLNA* can lead to low survival rate and migration and invasion energy of GC cells[69]. Additionally, the disulfidptosis-related gene *MYH10* may be related to the occurrence, development, and drug resistance of ovarian cancer[70], but its role in GC requires further exploration. Moreover, previous studies have revealed that *DSTN* increases the colony formation and migration ability of tumor cells when highly expressed[71], although its relationship with GC prognosis requires further study. Study on *MYL6* revealed possible impacts on the migration of melanoma cells[72], but its relationship with GC needs further study. In this study, we found that *PDLIM1*, *FLNA*, *MYH10*, *MYL6*, and *DSTN* are significantly associated with GC prognosis ($P < 0.05$), among which, *DSTN*, *FLNA*, *MYH10*, and *MYL6* are risk factors for GC prognosis, while *PDLIM1* is a protective factor for GC prognosis. Our study further demonstrates the impact of DRGs on GC prognosis.

Our results revealed significant pathway and functional differences, as well as significantly different KEGG and GO pathways and functions between the two subtypes of disulfidptosis, mainly enriched in amino acid metabolism, TGF-β signaling pathway, pentose phosphate pathway, Wnt signaling pathway, and MAPK signaling pathway, among others. These functions and pathways may be related to the presence of GC. Indeed, previous studies have found that the TGF-β

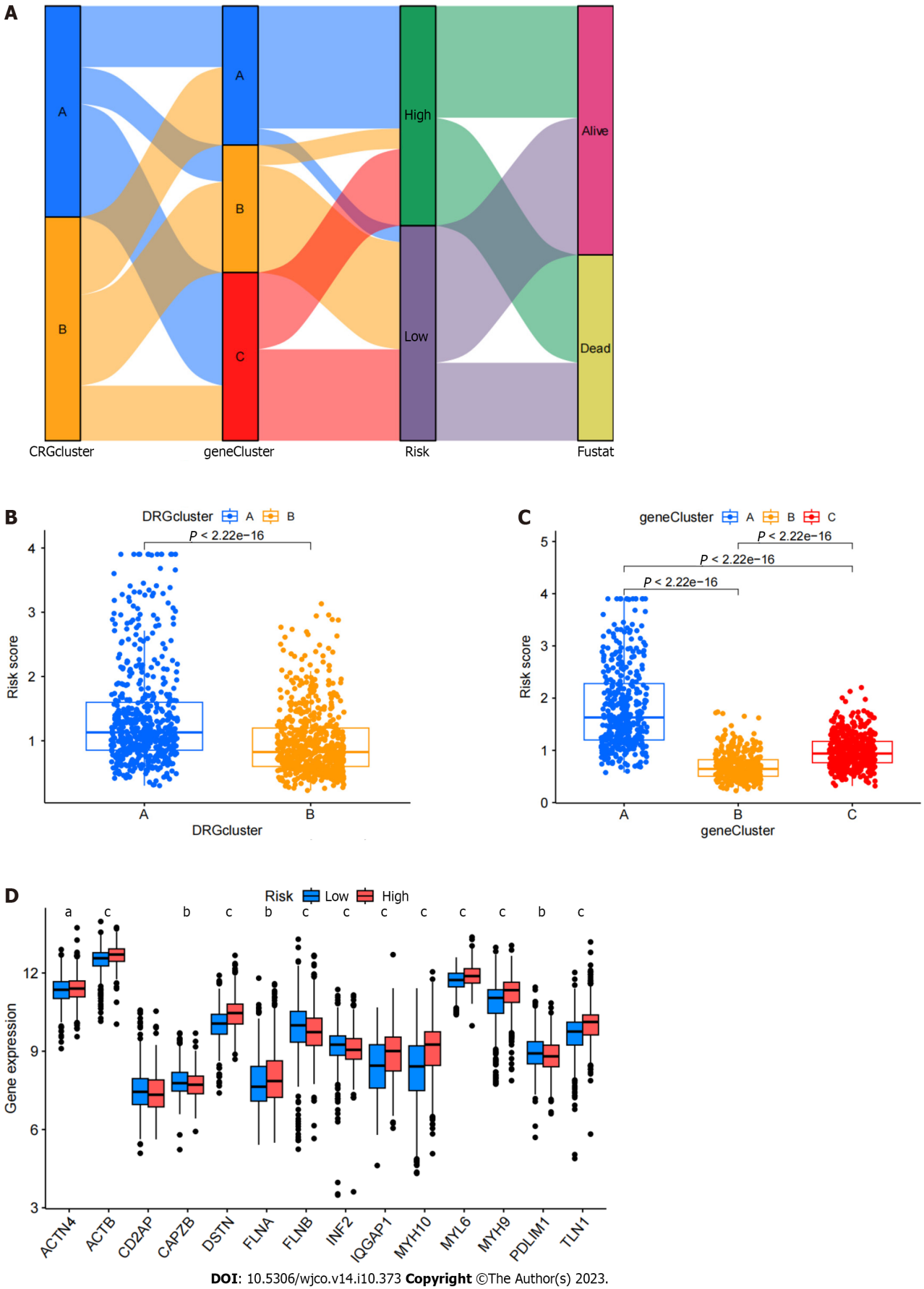

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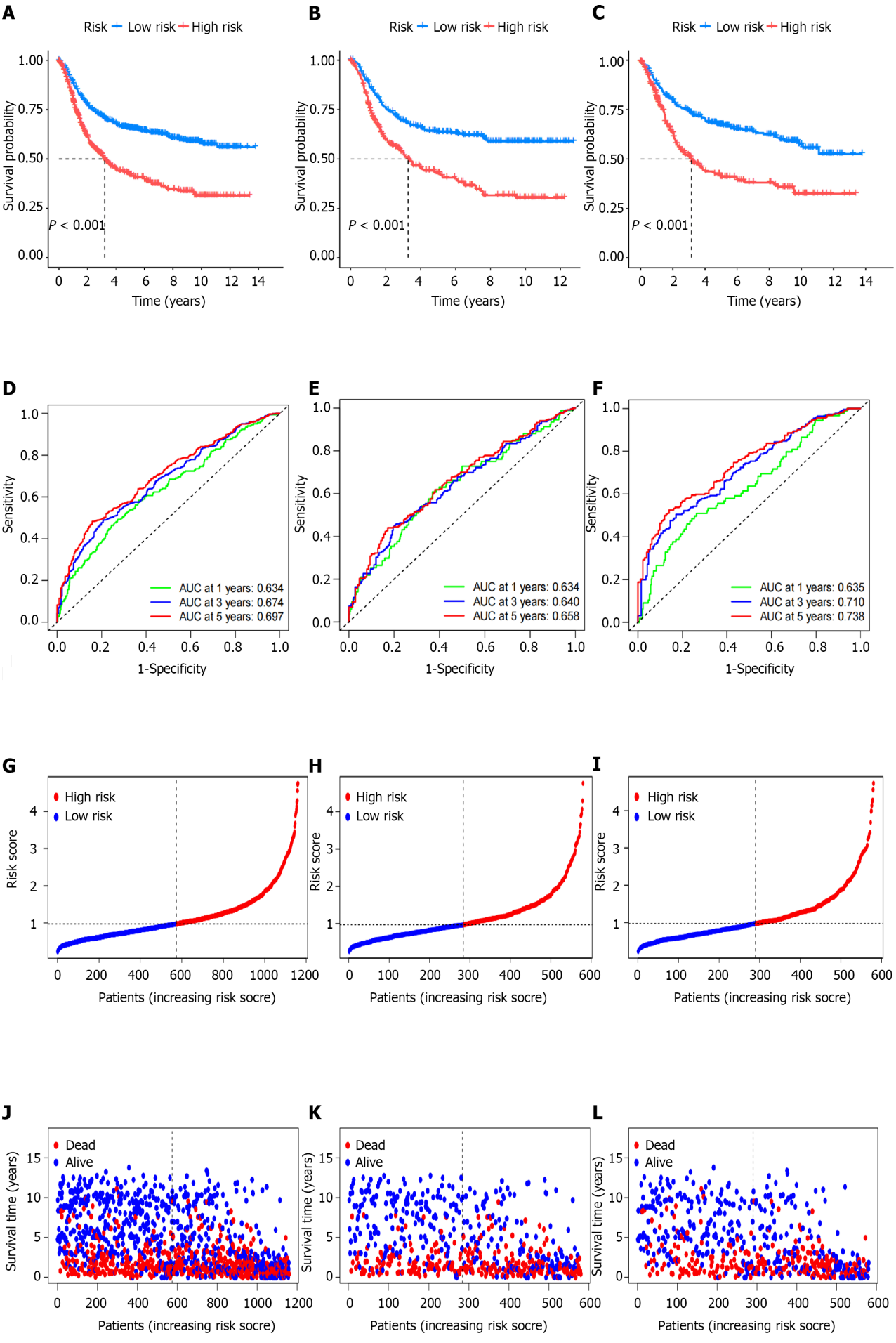
Figure 7 Testing the reliability of prognostic models. A: A Sankey diagram of the relationships between various data is presented; B: Shows a box plot of the disulfidptosis subtype; C: Presents a box plot of gene subtypes; D: Shows the differential analysis of disulfidptosis related genes between high and low-risk groups.


A
Risk Low risk High risk
Survival probability
P < 0.001
Time (years)
B
Risk Low risk High risk
Survival probability
P < 0.001
Time (years)
C
Risk Low risk High risk
Survival probability
P < 0.001
Time (years)
D
Sensitivity
AUC at 1 years: 0.634
AUC at 3 years: 0.674
AUC at 5 years: 0.697
1-Specificity
E
Sensitivity
AUC at 1 years: 0.634
AUC at 3 years: 0.640
AUC at 5 years: 0.658
1-Specificity
F
Sensitivity
AUC at 1 years: 0.635
AUC at 3 years: 0.710
AUC at 5 years: 0.738
1-Specificity
G
High risk
Low risk
Risk score
Patients (increasing risk socre)
H
High risk
Low risk
Risk score
Patients (increasing risk socre)
I
High risk
Low risk
Risk score
Patients (increasing risk socre)
J
Dead
Alive
Survival time (years)
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Survival time (years)
Patients (increasing risk socre)

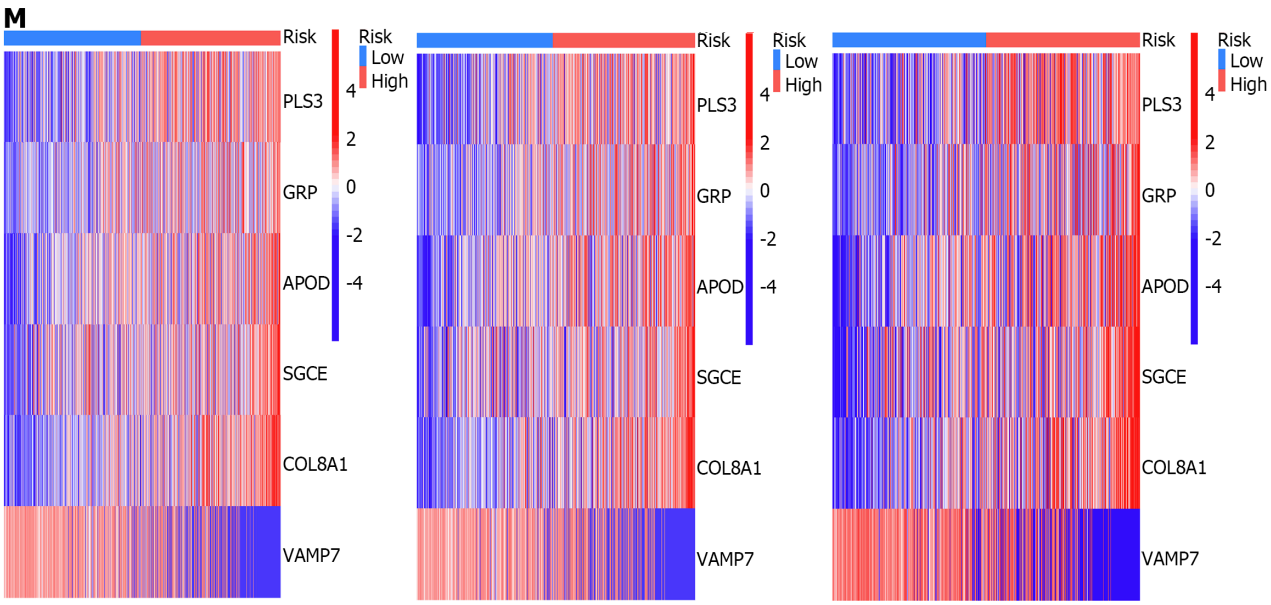

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Figure 8 The accuracy of the prognostic model was verified by subgroup analysis. A-C: Survival curves between different groups are presented in panels; D-F: Show receiver operating characteristic curves between different groups; G-I: Risk curves for each group are presented; J-L: Show survival status plots for each group; M-O: Risk heat maps for each group are presented.

signaling pathway may be involved in the occurrence, invasion, proliferation, and metastasis of GC, affecting the prognosis of patients with GC[73-77]. Furthermore, some studies have suggested that the pentose phosphate pathway may be related to the proliferation of GC cells[78]. Previous studies have also found that the MAPK signaling pathway may also be involved in the occurrence, invasion, proliferation, and metastasis of GC, affecting the prognosis of GC[79-87]. Additionally, some studies have found that the Wnt signaling pathway may be involved in the metastasis, migration, invasion, and progression of GC, affecting the prognosis of GC[88-94]. In the current study, we also found differences in immune cell infiltration between the subgroups of disulfidptosis gene typing. Taken together, these findings and research suggest that DRGs affect various aspects of patients with GC, including amino acid metabolism, various signaling pathways, and immune cell infiltration, all of which may affect the survival or prognosis of patients with GC; however, the specific mechanisms and functions need to be further explored.

In this study, we used a risk model to score differentially expressed genes in overall, training set, and testing set samples, dividing them into high- and low-risk groups. Survival, ROC, and risk analyses were conducted for each group. The results showed that the high-risk group had a poorer prognosis than the low-risk group in all groups, and the result trend was consistent, further demonstrating the reliability of the model.

Through column line chart analysis, we revealed that *APOD*, *PLS3*, age, sex, and N stage represent independent risk factors affecting patient prognosis, all of which are risk factors for GC prognosis. Through column line chart analysis, we observed that the survival rates of patients at 1, 3, and 5 years gradually decreased, with rates of 0.806, 0.527, and 0.39, respectively; this is consistent with the trend of 1-, 3-, and 5-year survival rates in previous studies on GC, further confirming the reliability of the prognostic model[95]. Additionally, we found that *APOD* represents an independent prognostic factor for GC in this model ($P < 0.001$). Previous studies on *APOD* have found that it may be involved in the construction of multiple GC prognostic and immune prediction models[96-103], which may be related to GC prognosis. In this study, we further analyzed the protein encoded by *APOD* in the HPA network database through immunohistochemical analysis and found that its protein expression level in GC tissues was significantly higher than that in adjacent normal tissues, further indicating significant differences in *APOD* between GC tissues and adjacent normal tissues. Overall, our results suggest that *APOD* may play an important role in the occurrence and development of GC, while its expression level may be related to the prognosis of patients with GC, further suggesting that *APOD* represents a potential therapeutic target for GC.

We also found that the genes constituting the GC prognosis model were related to various immune cells, indicating that the DRGs may affect the immunity of patients with GC. The heatmap of the correlation between the model genes and immune cells in this study showed a significant positive correlation between disulfidptosis *PLS3* and resting mast cells ($P < 0.001$). Indeed, previous studies have found a correlation between resting mast cells and GC[104,105], and it has been suggested that *PLS3*[106] may also be related to GC. Through the analysis of TME differences in the prognosis model, we found differences in the Stromal Score and ESTIMATE Score in the high- and low-risk groups, with both scores found to be upregulated in the high-risk group, indicating that the risk score of the prognosis model is related to the TME of GC. Through the analysis of the relationship between the risk score of the prognosis model and TMB, MSI, and stem cell correlation, we found that the risk score of the prognosis model was correlated with TMB, MSI, and RNAss. These results further indicate that the DRGs may be related to the immunity or immune therapy targets of TMB, MSI, and RNAss in patients with GC, which may affect the immune therapy effect and prognosis of patients with GC. Among them, MSI, an

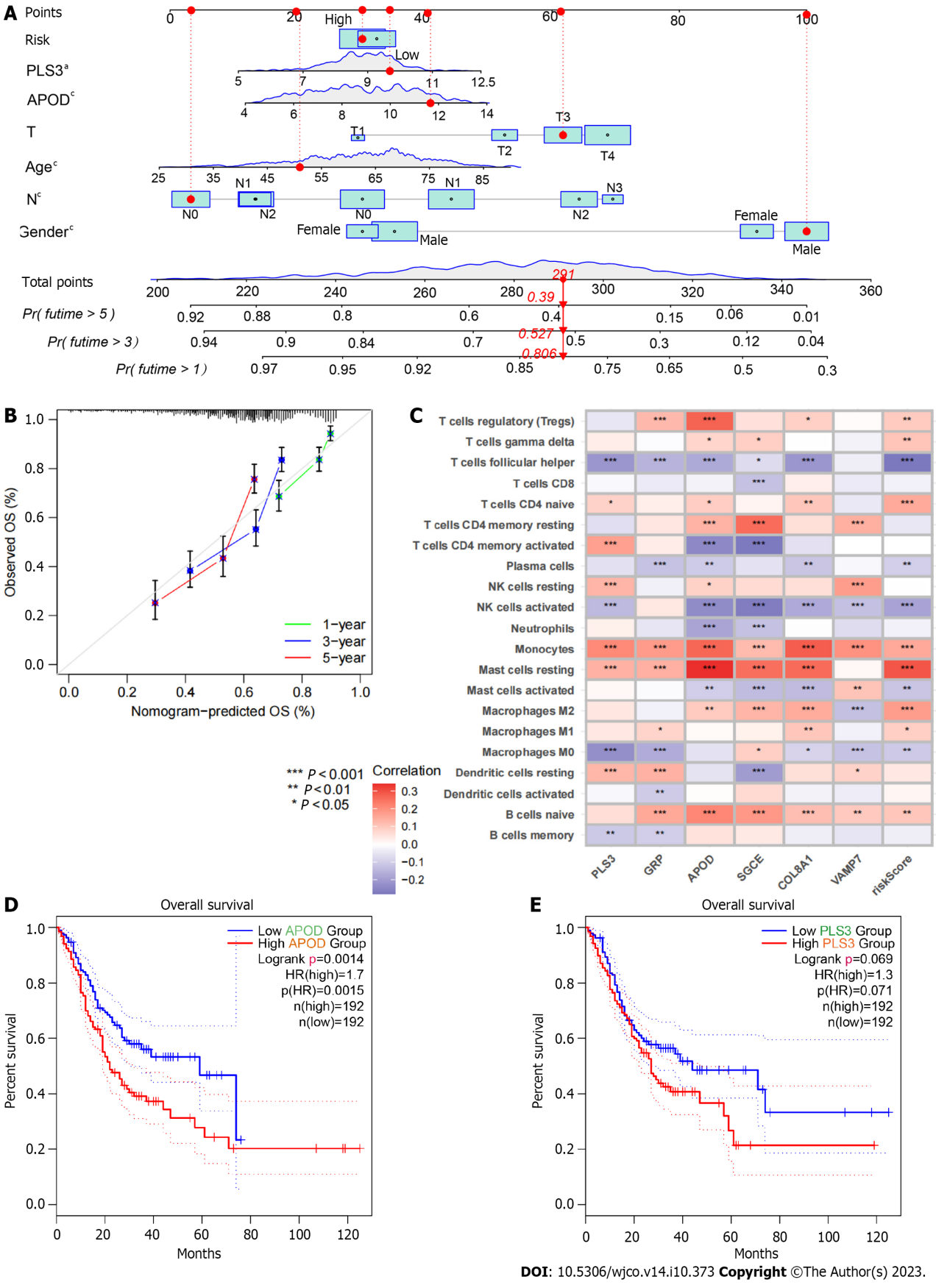

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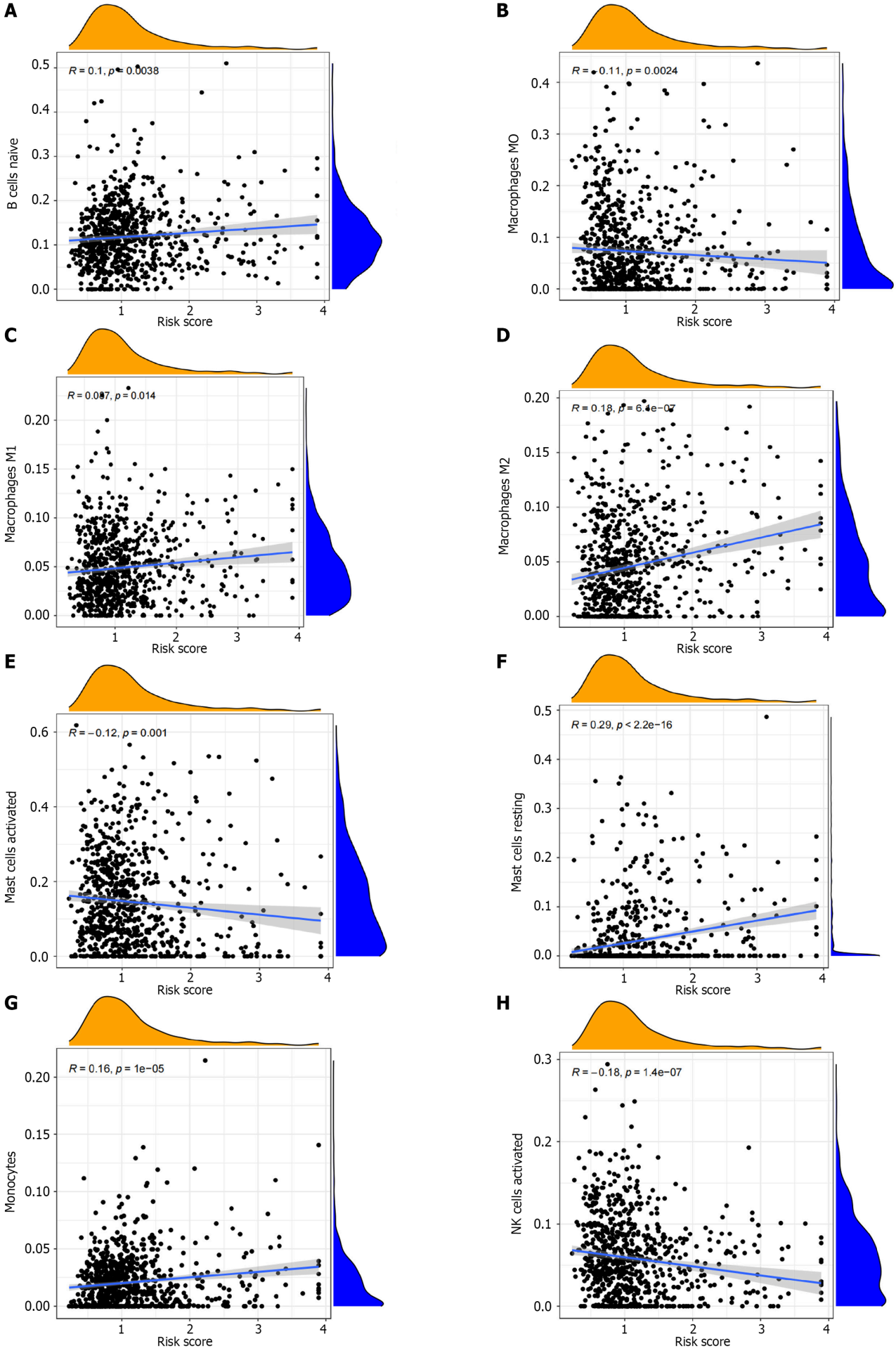
Figure 9 Further analysis of prognostic models to screen potential therapeutic targets. A: Presents a column line chart; B: Shows a calibration curve; C: Presents a heat map of the correlation between model genes and immune cells; D:The survival curve of *APOD* in gastric cancer was significantly different between high and low risk groups ($P < 0.05$); E: The survival curve of *PLS3* in gastric cancer was shown between high and low risk groups, and the results suggested that the difference was not significant ($P > 0.05$).


A
R = 0.1, p = 0.0038
B cells naive
Risk score
B
R = 0.11, p = 0.0024
Macrophages M0
Risk score
C
R = 0.087, p = 0.014
Macrophages M1
Risk score
D
R = 0.18, p = 6.4e−07
Macrophages M2
Risk score
E
R = −0.12, p = 0.001
Mast cells activated
Risk score
F
R = 0.29, p < 2.2e−16
Mast cells resting
Risk score
G
R = 0.16, p = 1e−05
Monocytes
Risk score
H
R = −0.18, p = 1.4e−07
NK cells activated
Risk score

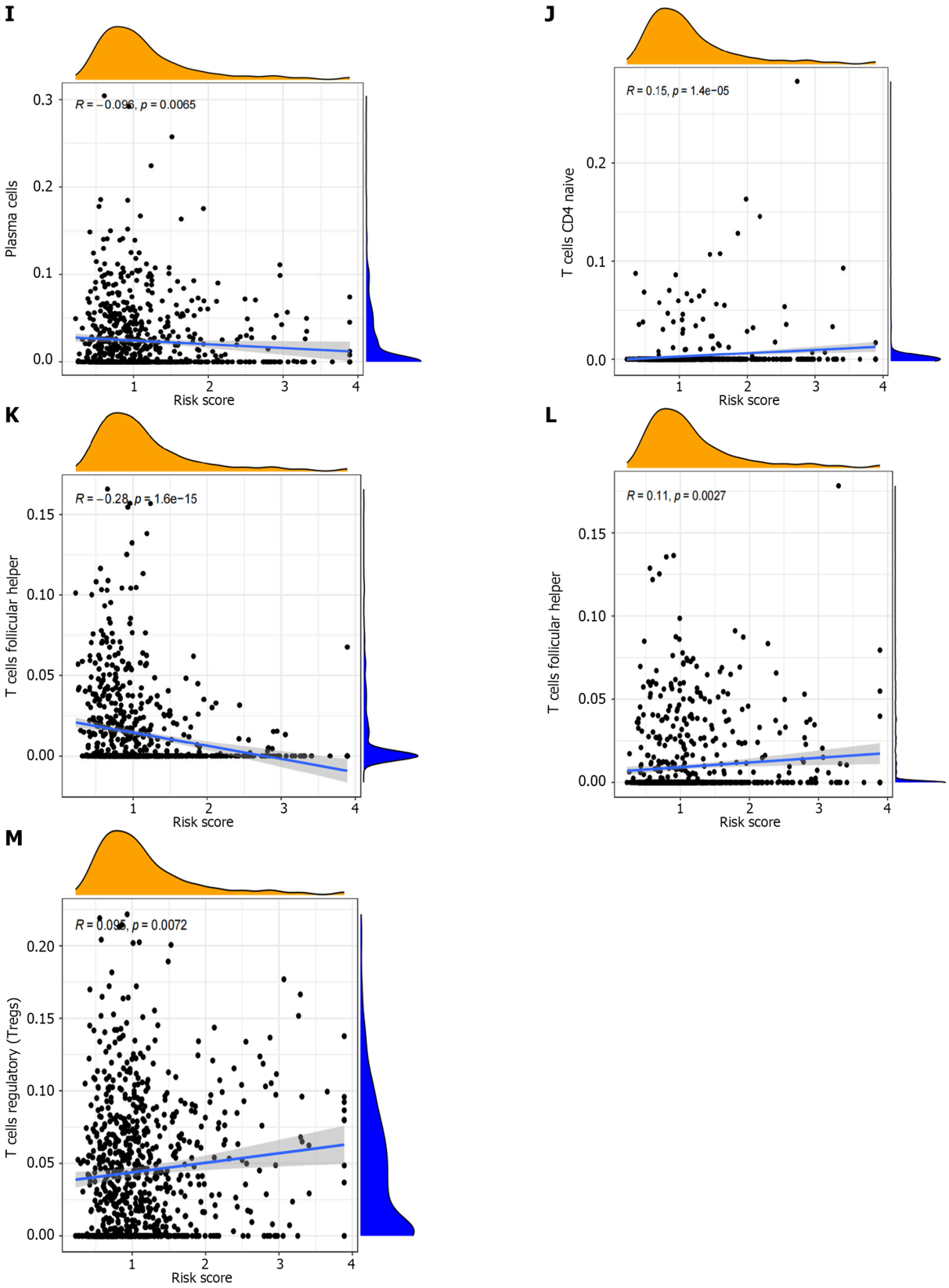


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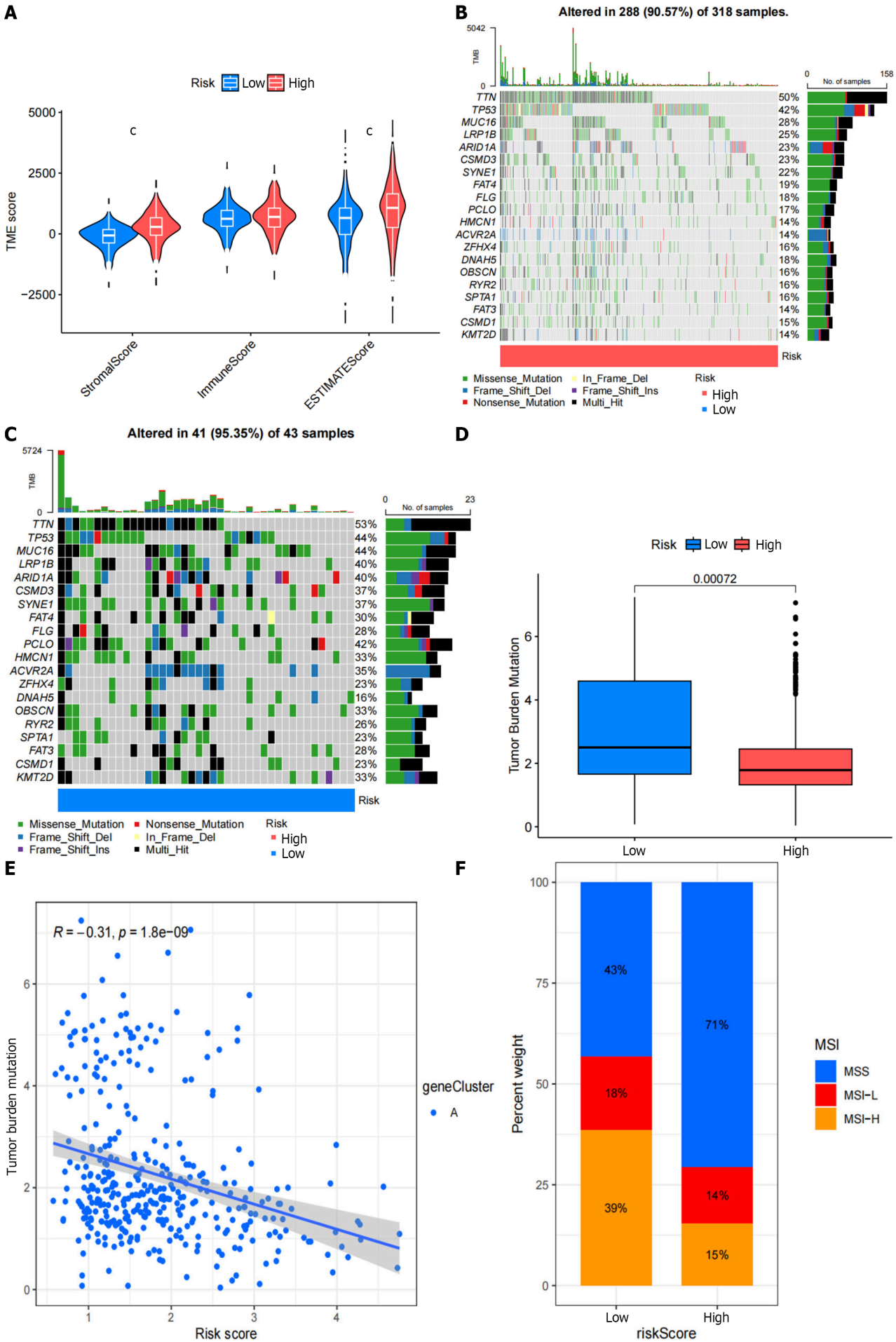
Figure 10 The correlation between immune cells and risk scores is analyzed. A: There was a positive correlation between B cells naive and risk score; B: The result shows that macrophages M0 is negatively correlated with risk score; C: The results showed that macrophages M1 was positively correlated with risk score; D: The results showed that macrophages M2 was positively correlated with risk score; E: There was a negative correlation between mast cells activated and risk score; F: The results showed that mast cells reting was positively correlated with risk score; G: There was a positive correlation between monocytes and risk score; H: There was a negative correlation between natural killer cells activated and risk score; I: There was a negative correlation between plasma cells and risk score; J: There was a positive correlation between T cells CD4 naive and risk score; K: It showed that T cells follicular helper was negatively correlated with risk score; L: There was a positive correlation between T cells gamma delta and risk score; M: There was a positive correlation between T cells regulation and risk score.


A
Risk Low High
TME score
5000
2500
0
−2500
c
c
StromalScore
ImmuneScore
ESTIMATEScore
B
Altered in 288 (90.57%) of 318 samples.
TMB
5042
0
No. of samples
0
158
TTN 50%
TP53 42%
MUC16 28%
LRP1B 25%
ARID1A 23%
CSMD3 23%
SYNE1 22%
FAT4 19%
FLG 18%
PCLO 17%
HMCN1 14%
ACVR2A 14%
ZFHX4 16%
DNAH5 18%
OBSCN 16%
RYR2 16%
SPTA1 16%
FAT3 14%
CSMD1 15%
KMT2D 14%
Risk
Missense_Mutation
Frame_Shift_Del
Nonsense_Mutation
In_Frame_Del
Frame_Shift_Ins
Multi_Hit
Risk
High
Low
C
Altered in 41 (95.35%) of 43 samples
TMB
5724
0
No. of samples
0
23
TTN 53%
TP53 44%
MUC16 44%
LRP1B 40%
ARID1A 40%
CSMD3 37%
SYNE1 37%
FAT4 30%
FLG 28%
PCLO 42%
HMCN1 33%
ACVR2A 35%
ZFHX4 23%
DNAH5 16%
OBSCN 33%
RYR2 26%
SPTA1 23%
FAT3 28%
CSMD1 23%
KMT2D 33%
Risk
Missense_Mutation
Frame_Shift_Del
Frame_Shift_Ins
Nonsense_Mutation
In_Frame_Del
Multi_Hit
Risk
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Risk Low High
0.00072
Tumor Burden Mutation
0
2
4
6
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High
E
R = −0.31, p = 1.8e−09
Tumor burden mutation
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2
4
6
1
2
3
4
Risk score
geneCluster
A
F
Percent weight
0
25
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75
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15%
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High
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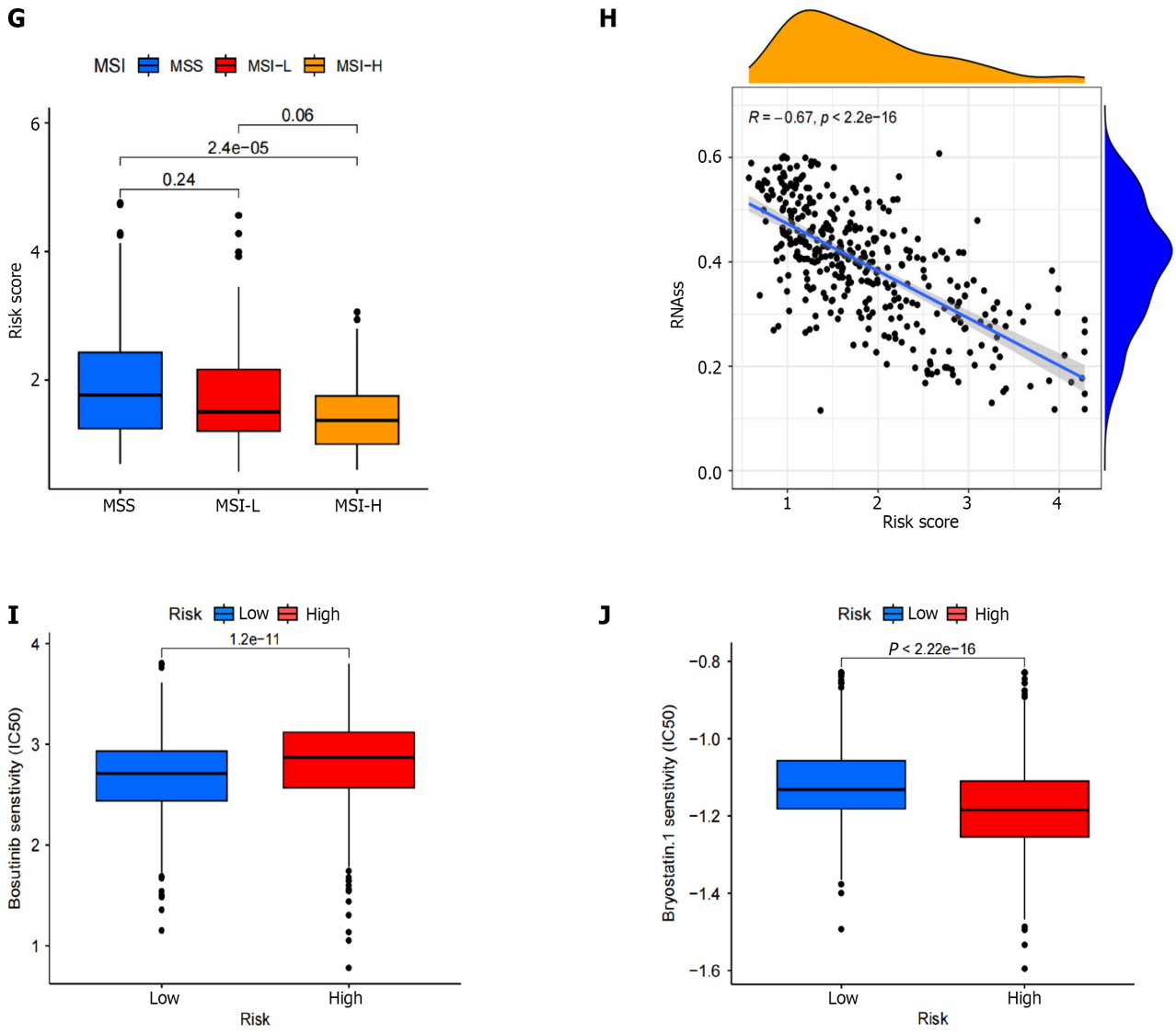

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Figure 11 The correlation between the prognostic scoring model and tumor microenvironment, microsatellite instability, and RNAss, as well as drug sensitivity analysis, is presented. A: Shows the tumor microenvironment score for high and low-risk groups; B and C: Present waterfall plots of mutations for high and low-risk groups; D: Analyzes the differences in tumor mutation burden between high and low-risk groups; E: Shows the relationship between tumor mutation burden and risk score; F and G: Present microsatellite instability analysis for high and low-risk groups; H: Analyzes the correlation between stem cells and risk score; I and J: Present drug sensitivity analysis for drugs such as Bosutinib and Bryostatin.

immune therapy target, has been found to affect the treatment and prognosis of patients with GC in previous studies[107-111], while the significant correlation between the GC risk prediction model established in this study and MSI indicates that MSI-targeted treatment may be meaningful for the treatment and prognosis of patients with GC. This further indicates the correlation between disulfidptosis and the immunity or immune therapy targets of patients with GC.

The results of our drug sensitivity analysis revealed that 89 drugs, including Bosutinib and Bryostatin, were significantly correlated with the sensitivity of GC treatment. Previous studies have found that Bryostatin can enhance the effect of paclitaxel in the treatment of GC[112], while others have found that Bosutinib may inhibit the migration of GC cells[113]. These results suggest that Bosutinib may have therapeutic effects on GC. The high sensitivity of Bosutinib and Bryostatin to GC found in this study suggests that they may be useful drugs for the treatment of GC. Therefore, the 89 drugs represented by Bosutinib in this study may be potential drugs for the treatment of GC.

CONCLUSION

In conclusion, our findings suggest that the DRGs and their submolecules may have an impact on immunity, immunotherapy targets, signaling pathways, and drug sensitivity in patients with GC. DRGs, including *PDLIM1*, *FLNA*, *MYH10*, *MYL6*, and *DSTN*, may be related to the prognosis of GC. Six genes, namely, *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, and *VAMP7*, constituted a prognostic model of GC associated with DRG. *APOD* may be a potential target for the treatment of

GC, while 89 drugs, including Bosutinib and Bryostatin, may be potential drugs for the treatment of GC.

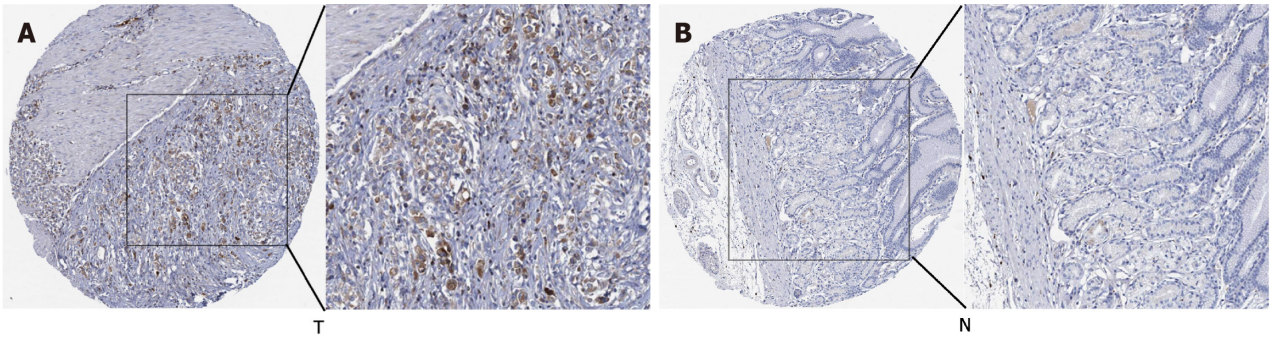

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Figure 12 The immunohistochemical analysis of the *APOD* gene based on human protein atlas is presented. A: Tumor tissue; B: Normal tissue. T: Tumor tissue; B: Normal tissue.

ARTICLE HIGHLIGHTS

Research background

Gastric cancer (GC) is one of the most common malignant tumors, although its pathogenesis remains unclear.

Research motivation

For the first time, in the current study, we constructed a new GC prognostic model based on the sub-group analysis of disulfidptosis-related genes (DRGs) and explored treatment targets and sensitive drugs.

Research objectives

The aims of this study were to explore a new GC prognostic model based on the sub-group analysis of DRGs and explore treatment targets and sensitive drugs.

Research methods

In this study, a bioinformatics strategy was used to extract GC-related data from The Cancer Genome Atlas and Gene Expression Omnibus databases, while R software (version 4.2.1) was used for correlation analysis.

Research results

Through the above analysis, we found that the didisulfidptosis-related gene may be related to the prognosis of GC. Six genes, namely, *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, and *VAMP7*, constitute a predictive model for GC prognosis. *APOD* is a potential therapeutic target. Bosutinib and other drugs are suitable for the treatment of GC.

Research conclusions

The results of this study indicate that didisulfidptosis is related to the prognosis and treatment of GC. Additionally, *APOD* can be used as a potential therapeutic target for GC.

Research perspectives

Six genes, namely, *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, and *VAMP7*, constitute a predictive model for GC prognosis. *APOD* is a potential therapeutic target for treating GC. Bosutinib and other drugs are suitable for the treatment of GC, although this requires further confirmation through molecular biology and clinical experiments.

FOOTNOTES

Author contributions: Li Q contributed to this work; Yin LK and Li Q prepared for the figures and tables; and all authors have approved the final manuscript.

Institutional review board statement: The data supporting the results of this study are available from Gene Expression Omnibus database (GSE84433andGSE26253) and the expression data, clinical data, mutation data, and copy data related to gastric cancer from The Cancer Genome Atlas database.

Conflict-of-interest statement: The authors deny any conflict of interest.

Data sharing statement: No additional data are available.

Country/Territory of origin: China

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S-Editor: Qu XL
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P-Editor: Zhang XD

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ORIGINAL ARTICLE

Retrospective Study

Treatment of patients with multiple brain metastases by isolated radiosurgery: Toxicity and survival

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Abstract

BACKGROUND

Radiosurgery for multiple brain metastases has been more reported recently without using whole-brain radiotherapy. Nevertheless, the sparsity of the data still claims more information about toxicity and survival and their association with both dosimetric and geometric aspects of this treatment.

AIM

To assess the toxicity and survival outcome of radiosurgery in patients with multiple (four or more lesions) brain metastases.

METHODS

In a single institution, data were collected retrospectively from patients who underwent radiosurgery to treat brain metastases from diverse primary sites. Patients with 4-21 brain metastases were treated with a single fraction with a dose of 18 Gy or 20 Gy. The clinical variables collected were relevant to toxicity, survival, treatment response, planning, and dosimetric variables. The Spearman's rank correlation coefficients, Mann-Whitney test, Kruskal-Wallis test, and Log-

rank test were used according to the type of variable and outcomes.

RESULTS

From August 2017 to February 2020, 55 patients were evaluated. Headache was the most common complaint (38.2%). The median overall survival (OS) for patients with karnofsky performance status (KPS) > 70 was 8.9 mo, and this was 3.6 mo for those with KPS ≤ 70 (P = 0.047). Patients with treated lesions had a median progression-free survival of 7.6 mo. There were no differences in OS (19.7 *vs* 9.5 mo) or progression-free survival (10.6 *vs* 6.3 mo) based on prior irradiation. There was no correlation found between reported toxicities and planning, dosimetric, and geometric variables, implying that no additional significant toxicity risks appear to be added to the treatment of multiple (four or more) lesions.

CONCLUSION

No associations were found between the evaluated toxicities and the planning dosimetric parameters, and no differences in survival rates were detected based on previous treatment status.

Key Words: Radiosurgery; Brain metastases; Radiotherapy; Survival; Toxicity; Cancer

Core Tip: Toxicity and survival outcome of radiosurgery in patients with multiple brain metastases (≥ 4) were evaluated. A total of 55 patients were evaluated; headache was the most common complaint, but no associations were found between the evaluated toxicities and the planning and dosimetric parameters. The median overall survival found was 10 mo and the survival of the group that did not undergo irradiation before radiosurgery was 9.5 mo. The results are equivalent to those found by authors who evaluated patients with up to four lesions. Our data demonstrate the safe use of isolated stereotactic radiosurgery to treat patients with four or more brain metastases.

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INTRODUCTION

It is estimated that 19.3 million new cancer cases and 10 million deaths occurred in 2020. Breast (11.7%) and lung (11.4%) cancers are among the most common cancer cases, causing 2.5 million deaths (24.9% of all cancer deaths)[1]. Besides being the most prevalent in the population, they are the most prevalent cancer types that evolve into brain metastasis due to their favorable microenvironment for brain metastases development[2,3].

The main radiotherapy technique used in brain metastasis is stereotactic radiosurgery (SRS) performed in a linear accelerator (LA). Thus, it is necessary to determine whether the treatment of multiple brain metastases by isolated radiotherapy is safe and non-inferior to the treatment of one or few lesions, regarding toxicity and survival[4-7] and if previous treatment, such as whole brain radiotherapy (WBRT), is beneficial before radiosurgery[8,9]. Moreover, to determine which therapy is appropriate for each patient's prognosis, it is also important to estimate the survival rate of patients with brain metastasis through prognostic factors such as Karnofsky performance status (KPS), diagnosis-specific graded prognostic assessment (DS-GPA), score index for radiosurgery (SIR), and recursive partitioning analyses (RPA), to determine which therapy is adequate for the prognosis of each patient[10-14].

This work aimed to evaluate the toxicity of isolated radiosurgery in patients with multiple brain metastases (≥ 4 lesions). In addition, overall survival and progression-free survival were evaluated, and survival was correlated with the prognostic index.

MATERIALS AND METHODS

Retrospective data were collected from 55 patients who underwent radiosurgery at Barretos Cancer Hospital from August 2017 to February 2020. Patients who presented with 4-21 brain metastases delineated with the aid of magnetic resonance (MR) were treated in a single fraction with a dose of 18 Gy or 20 Gy. Patients who met the inclusion criteria were included regardless of previous systemic and primary local treatment since all of them received radiation therapy for four or more brain lesions in palliative manners, and the main outcomes were either local or systemic toxicity. A frameless immobilization system was used for simulation and treatment. Simulation computed tomography with a slice thickness of 1.25 mm was used for all plannings.

All lesions were treated on a Varian TrueBeam® ™ STX Varian Medical Systems LA with high-definition mulitleaf (120-leaf) collimator and planned with an Eclipse® treatment planning system (Varian Medical System Inc, version 13.6). The calculation algorithm used was the anisotropic analytical algorithm with a 1.25 mm calculation grid and heterogeneity correction. VMAT (RapidArc®, Varian Medical System, Inc.) treatment technique was used for all cases with a planning target volume (PTV) margin of 1 mm from the gross target volume contour. Before treatment, a cone-beam computed tomography scan was performed. Planning was carried out by the Department of Radiation Oncology with many physicists and radiation oncologists in who followed the institutional protocol of dose constraints in the organs at risk and of coverage of targets.

The following toxicities were collected: Headache, convulsion, focal deficit, drop in the level of consciousness, fatigue, nausea or vomiting, and mental confusion. They were based on the Common Terminology Criteria for Adverse Events. The patient's first complaint after radiosurgery was selected.

Prognostic factors were also collected: Initial KPS and that at the first follow-up after radiosurgery, DS-GPA, SIR, and RPA. In addition, age, gender, and the International Classification of Disease of the primary tumor were also surveyed.

Dosimetric variables included were V5Gy, V8Gy, V10Gy, V12Gy, V14Gy, conformity index (CI), heterogeneity index (HI), dmax, and 50% isodose CI (CI_R50). The VxGy represents the volume of the "x" Gy dose that the normal brain minus PTV received. The CI was calculated by the ratio between the volume of the prescription isodose and the volume of the PTVs: $V_{presc\_isodose}/V_{PTVs.}$ The HI was calculated as $(D_{2\%}-D_{98\%})/D_{50\%}$[15]. Dmax is the maximum point dose of the plan and the CI_R50 is the ratio between the volume of the 50% isodose line and the volume of the PTVs.

The geometric variables collected were the number of lesions, total target volumes, the smallest and largest target volumes, and the distance between the isocenter and the most distant lesion. The distance between the isocenter and the most distant lesion was determined using the coordinates of the lesion center and its respective isocenter. The calculation was according to equation 1. In cases where there was more than one isocenter, the distance was measured between the isocenter and the most distant lesion that its arcs was treated, as demonstrated in the Supplementary material.

Technical variables included were the total number of arcs, coplanar or non-coplanar arcs, number of non-coplanar arcs if used, and number of isocenters. The correlations between the dosimetric, geometric, and technical variables collected for this work were previously published by our group[16].

We reported the response in treated lesions as complete response, partial response, stable disease, progressive disease, or radionecrosis. Complete response indicated complete remission of all lesions; partial response indicated that some lesions entered complete remission, while others remained stable; stable disease indicated that all lesions remained the same size; progressive disease indicated that at least one of the lesions enlarged in size; and radionecrosis indicated that at least one lesion went through necrosis due to radiation. Information on the location of new lesions was also reported as either parenchymal or meningeal.

The initial date of treatment was used for estimating the overall and progression-free survival rates and for the outcomes, respectively, the date of death or the date of the last information obtained in the medical records after the treatment, and the date of the MR in which the progression of the treated lesions was detected or the date of the MR in which the appearance of new lesions was detected were used. Survival rates were calculated based on the data of 53 patients with assessable clinical records.

In this study, radiosurgery treatment of multiple brain metastasis (≥ 4) delivered in a isolated manner and at a single dose was referred to as radiosurgery. Whenever the patient underwent radiosurgery in more than one course of treatment, we would consider the first radiosurgery with four or more lesions. To evaluate if the previous treatment influenced survival rates, patients were divided into two groups: No previous irradiation (NP) and irradiation before radiosurgery (P).

Comparisons of toxicities between categories or between different groups of patients were made using chi-square tests or Fisher exact tests, and Mann-Whitney tests for continuous variables, and the relation between prognostic factors and age was evaluated using Mann-Whitney or Kruskal-Wallis tests. The results are presented as proportions or median and interquartile when appropriate. Spearman's correlation coefficient was used to determine if there was a correlation between toxicities and dosimetric and geometric variables. KPS comparison was made using a marginal homogeneity test. Survival was estimated by the Kaplan-Meier method and the curves of each category were compared using the Log-rank test. Statistical relevance was considered if $P < 0.05$. Data were collected and managed using the research electronic data capture platform[17] and analyzed using the software SSPS® (v. 20).

RESULTS

The descriptive characteristics of groups NP and P are displayed in Table 1. Briefly, the most prescribed dosage was 18 Gy (83.6%), and 67.3% of patients were female. Of the 55 patients who underwent radiosurgery, 32 (58.2%) declared feeling some toxicity, with headaches (38.2%) being the most frequent. Incidence rates for each toxicity are shown in Table 2.

The number of reported cases of toxicity as a function of time after treatment is shown in Table 3. It was observed that the highest incidence (40.6%) occurred between the first and third month after treatment. To deal with the heterogeneity of patients who had nervous system irradiation more than once, they were divided into four groups: (1) Patients with ≥ 4 lesions who underwent radiosurgery only; (2) patients with previous either WBRT or SRS with less than four lesions or fractionated stereotactic radiotherapy (SRT); (3) patients that underwent irradiation after radiosurgery and reported side effects after the second irradiation; and (4) patients that were irradiated before and after radiosurgery and reported some toxicities after the last irradiation.

Table 1 Descriptive characteristics of 55 patients treated by radiosurgery for multiple brain metastases (%)

Characteristic	NP (*n* = 35)	*P* (*n* = 20)	*P* value
Age at treatment (yr)[1]	62 (51-67)	53 (42-58)	0.032
Age (yr)			0.162
< 55	15 (42.9)	13 (65)	
≥ 55	20 (57.1)	7 (35)	
Gender			0.391
Female	22 (62.9)	15 (75)	
Male	13 (37.1)	5 (25)	
Number of lesions[1]	5 (4-7)	5 (4-7)	0.89
Target volumes[1]	5.4 (2.2-9.9)	2.9 (1.63-4.3)	0.08
Primary site			0.85
Lung	16 (45.7)	7 (35)	
Breast	8 (22.9)	7 (35)	
Melanoma	8 (22.9)	6 (30)	
Others	3 (8.6)	0	
KPS	*n* = 34	*n* = 20	0.194
≤ 70	6 (17.6)	7 (35)	
> 70	28 (82.4)	13 (65)	
DS-GPA	*n* = 31	*n* = 20	0.7
0-1	11 (35.5)	8 (40)	
1.5-2	14 (45.2)	7 (35)	
2.5-3	4 (12.9)	2 (10)	
3-4	2 (6.5)	3 (15)	
RPA	*n* = 34	*n* = 20	0.751
Class 1	2 (5.9)	1 (5)	
Class 2	28 (82.4)	15 (75)	
Class 3	4 (11.8)	4 (20)	
SIR[1]	*n* = 34	*n* = 20	0.104
	4 (4-6)	5 (4-6.5)	
Prescription dose (Gy)			0.133
20	8 (22.9)	1 (5)	
18	27 (77.1)	19 (95)	

[1]Median and interquartile range (p25-p75).

P < 0.05 was considered significant. P: Previous irradiation before radiosurgery; NP: No previous irradiation; KPS: Karnofsky performance status; DS-GPA: Diagnosis-specific graded prognostic assessment; RPA: Recursive partitioning analyses; SIR: Score index for radiosurgery.

The proportion of patients per technique that had one irradiation before SRS of multiple lesions was ten for WBRT, seven for SRS with less than four lesions, and eight for SRT. Five patients did two previous irradiations before treating multiple brain metastases (≥ 4 lesions) by SRS. The proportion of patients in each group that reported toxicity was 17 of 18 patients (94.4%) in group 1; 7 of 11 (12.7%) in group 2; 3 of 17 (17.6%) in group 3; and 5 of 9 (55.6%) in group 4.

The incidence of toxicities in each patient group is presented in Table 4. Despite the higher incidence in group 1, no statistical relevance was found between the four groups regarding the seven toxicities. There was also no difference in the toxicities among the different categories of DS-GPA, RPA, and SIR.

Regarding the response of treated lesions and the emergence of new lesions, there were no differences observed between the P and NP groups ($P = 0.643$ and $P = 0.412$, respectively). A single patient had a complete response, 17 had partial responses, seven were stable, 15 had progression, and six presented radionecrosis (half in each group).

Table 2 Incidence of toxicities

Toxicity	*n* of patients	Percentage (%)
Headache	21	38.2
Convulsion	4	7.3
Focal deficit	5	9.1
Drop in level of consciousness	3	5.5
Fatigue	8	14.5
Nausea or vomiting	6	10.9
Mental confusion	1	1.8

Table 3 Number of reported cases of toxicity per period after treatment

Period (mo)	*n* of patients	Percentage
$t < 1$	4	12.5
$1 \leq t < 3$	13	40.6
$3 \leq t < 6$	6	18.8
$6 \leq t < 9$	6	18.8
$9 \leq t < 12$	2	6.2
$t \geq 12$	1	3.1

Table 4 Incidence of toxicity in each group of patients, *n* (%)

	Group 1	Group 2	Group 3	Group 4
Headache	10 (58.8)	4 (57.1)	2 (66.7)	5 (100)
Convulsion	1 (5.9)	1 (14.3)	1 (33.3)	1 (20)
Focal deficit	3 (17.6)	1 (14.3)		1 (20)
Drop in level of consciousness	2 (11.8)		1 (33.3)	
Fatigue	6 (35.3)	1 (14.3)	1 (33.3)	
Nausea or vomiting	5 (29.4)	1 (14.3)		
Mental confusion	1 (5.9)			
Total	28	8	5	7

Despite the higher number of patients that presented new lesions in group NP (18) compared to group P (11), there were no differences between the two groups. The number of patients with new lesions was 29, and 17 patients did not develop new lesions. According to location, new parenchymal lesions (26) were more frequent than meningeal ones (3).

Comparing the initial KPS with that evaluated in the first consult after treatment, a relevant difference was observed between them ($P = 0.033$). The percentage of patients whose KPS decreased after treatment was 39.6%, and 60.4% of patients improved or maintained their KPS.

No statistical correlation was observed between dosimetric and geometric variables and toxicities. The descriptive statistics of dosimetric, geometric, and technical variables have previously been published by our group[16].

The average overall survival (OS) was 13.3 mo, and the median was 10 mo. The life expectancy over time can be observed in Figure 1. It is noteworthy that the survival rate at 12 mo was 42%. Of 53 patients, 78% died, and of those, only 10 patients (18.9%) were due to neurological causes.

No differences were observed in the OS rates between groups NP and P. The median survival in group NP was 9.5 mo, and in group P it was 19.7 mo ($P = 0.110$). Considering patients with KPS > 70 and KPS ≤ 70, a difference was observed in OS ($P = 0.047$) (Figure 1B). The median survival of the group with KPS > 70 was 8.9 mo, and in the group with KPS ≤ 70 it was 3.6 mo. No differences were observed in the survival of DS-GPA ($P = 0.547$), RPA ($P = 0.113$), and SIR categories 0 to 4 and 5 to 10 ($P = 0.586$).

The OS of patients was categorized into two groups for each variable for analysis. No difference between them was found: Number of lesions ($P = 0.840$), $n < 6$ (10.5 mo) and $n \geq 6$ (9.3 mo); volume of targets ($P = 0.786$), v < 5 cc (10.5 mo)

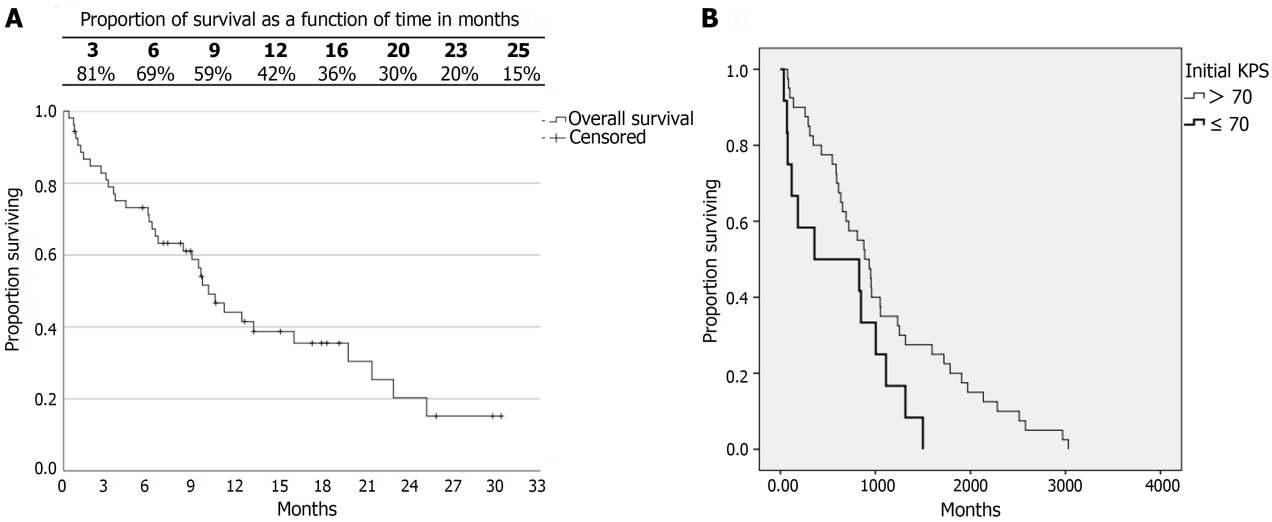

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Figure 1 Kaplan-Meyer curves for overall survival of 53 patients treated by radiosurgery for multiple brain metastases. A: Overall survival (OS); B: OS according to Karnofsky performance status (KPS) > 70 and KPS ≤ 70.

and v ≥ 5 cc (9.5 mo); V12Gy ≤ 10 cc (11.1 mo) and V12Gy > 10 cc (9.6 mo) (P = 0.693); CI_R50 ≤ 8 (13.2 mo) and CI_R50 > 8 (9.6 mo) (P = 0.655).

The median progression-free survival of patients with treated lesions (PFSL) was 7.6 mo. No differences (P = 0.293) were found between groups NP and P. The median PFSL of group NP was 6.3 mo, and in group P it was 10.6 mo. The curves for the PFSL of both groups are displayed in Figure 2A. The median survival free from the appearance of new lesions was 6 mo. No difference was observed between groups NP and P (P = 0.188). The median for group NP was 4.5 mo, and for group P it was 8.9 mo. The curves of survival free from the appearance of new lesions in both groups are displayed in Figure 2B.

DISCUSSION

It was observed that in group 1, a higher proportion (94.4%) of patients reported grievances and a higher number of different toxicities. Nevertheless, no difference was observed between groups when comparing their toxicity incidence. Besides, the toxicities reported varied regarding their start point. One of the patients reported a grievance a year after treatment, thus rendering it difficult to classify it as a side effect of radiosurgery.

Analyzing the responses of treated lesions, six patients developed radionecrosis. As discussed by Blonigen *et al*[18], V10Gy and V12Gy can be predictors of radionecrosis. The median of V10Gy and V12Gy of those six patients was 27.8 cc (9.7-45.5 cc) and 17.6 cc (6.2-27.4 cc), respectively (only a single patient had the dosage of 20Gy as prescription).

The median OS found by Chang *et al*[8] was 9.2 mo and the median survival of patients treated only by SRS was 15.2 mo. Aoyama *et al*[9] obtained a median OS of 8 mo on the arm of patients treated only by SRS. Brown *et al*[19] found a median OS of 13.5 mo and a median survival of 10.4 mo for patients treated only by SRS.

Sahgal *et al*[20] found a median OS of 10 mo for the group that only received SRS. The median time to local failure and development of new lesions was 6.6 mo and 4.7 mo, respectively. This last result matches the PFSL and the development of new lesions in this study. The four aforementioned studies compared patients who underwent SRS alone with patients treated by SRS + WBRT.

Scorsetti *et al*[21] observed a median OS of 16.2 mo and a 12-mo survival rate of 65.3% in the group of patients who underwent only SRS with an LA. They also indicated that 27 of the 130 patients (20.8%) included in that study presented symptomatic radionecrosis. The incidence obtained in that study was 10.9%.

Differently from the studies mentioned before, in which treated patients had up to four lesions, the current study evaluated patients with 4 to 21 lesions and, despite the underestimated OS (group P began treatment of metastasis before the studied SRS), it was observed that survival values are similar to studies with up to four lesions, especially when analyzing the global survival of all patients, and the survival of patients without previous irradiation, whose comparison is possible with the aforementioned studies.

Among the prognostic indexes, despite the predictive power of survival from DS-GPA, RPA, and SIR[11-14,22] being better than KPS for patients with brain metastasis, only KPS showed a difference in OS (P = 0.047) between patients with KPS > 70 and KPS ≤ 70. This likely occurred due to KPS considering only the clinical condition of patients, whereas other indexes also consider specific parameters of patients with brain metastasis that were not discretized in the analysis, such as the primary site of disease, number of lesions, and systemic diseases, among others.

Regarding the number of patients whose KPS decreased, it is important to note that metastatic patients have systemic diseases that worsen the clinical outcome. Therefore, we cannot contribute the decline of KPS to SRS, which is corrob-

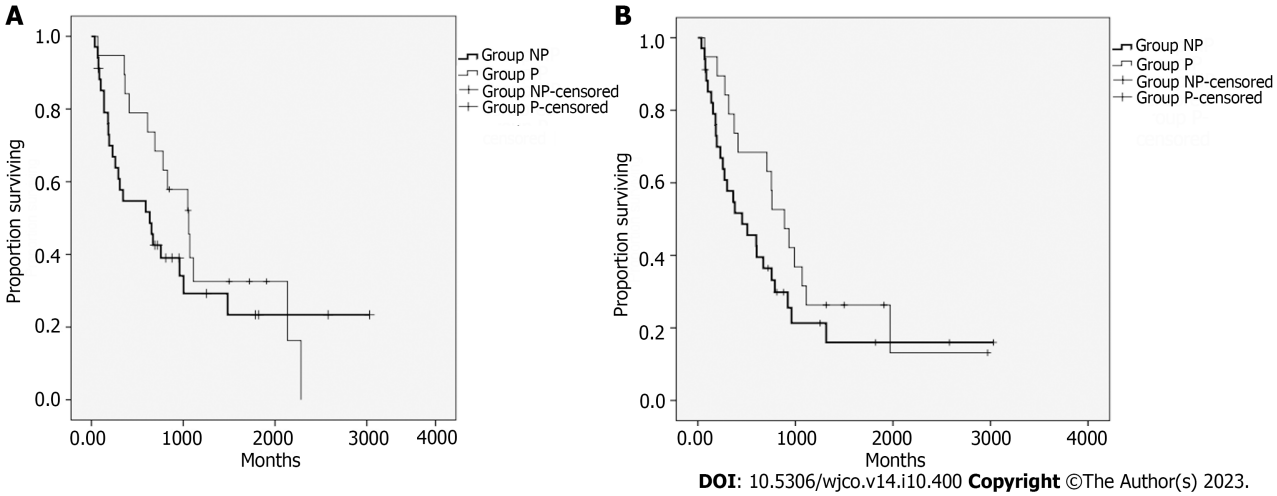

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Figure 2 Kaplan-Meyer curves for progression-free survival. A: Progression-free survival (PFS) of 34 patients with treated lesions who did not undergo previous irradiation (NP group) and 19 patients who underwent prior irradiation (P group); B: PFS addressing the appearance of new lesions in both NP and P groups.

orated by the low death number due to neurological causes.

According to dosimetric, geometric, and technical variables, the lack of correlation with toxicities does not imply they do not impact each other, especially considering dosimetric variables used for planning approval. It is known that the volume of targets, number of lesions, distance between lesions, and the isocenter impact these plan evaluation indexes[16, 23-25]. What can be observed is that the indication of isolated radiosurgery for multiple brain metastases was safe, considering that the technique achieved dosimetric values good enough not to cause collateral effects.

This study has limitations inherent to the retrospective cohort model where selection and information biases cannot be discarded. There were patients subjected to multiple irradiation techniques before the SRS in this study, and many of the patients were also under systemic treatment, which may interfere with the clinical results. In addition, all of them received some or many kinds of local and systemic treatment for many types of tumors, since in our institution the radiosurgery for four or more lesions was reserved for local control in a palliative manner and usually failed for previous treatments. Regarding the toxicities, precise graduation was not possible to obtain and therefore, they were not differentiated. Although our study had some missing data for clinical variables, they represent less than 4%, which seems acceptable for a retrospective study[26]. If factors were significantly associated with outcomes in univariate analysis, and they were not as demonstrated in Table 1, they would be entered into a multivariate analysis, but it was not possible.

The planning was performed by different personnel, with distinct dose prescriptions and, in some patients with one or more lesions (more significant volumes), planned with three fractions but, even in these cases, there were also four or more lesions treated with a single dose. Considering the prescription, we tested the difference between the doses of 18 and 20 Gy regarding geometric, dosimetric, and technical variables, and no differences were observed.

CONCLUSION

Our data demonstrate the safe use of isolated SRS to treat patients with four or more brain metastases, with no significant association between dosimetric, geometric, or clinical parameters and the related toxicities.

ARTICLE HIGHLIGHTS

Research background

Radiosurgery for multiple brain metastases has been more reported recently without using whole-brain radiotherapy, but mainly for oligometastatic scenarios (up to 3-4 lesions). Nevertheless, the sparsity of the data still claims more information about toxicity and survival and their association with both dosimetric and geometric aspects of this treatment, especially for the presence of more lesions or in patients with previous irradiation.

Research motivation

To evaluate the toxicity of treatment offered for patients with four or more lesions.

Research objectives

To assess associations of toxicity and survival outcome of stereotactic radiosurgery (SRS) among patients with four or more brain lesions with or without previous brain irradiation.

Research methods
Retrospective cohort.

Research results
Neither difference in toxicity nor survival was detected when comparing patients who underwent SRS for four or more brain lesions with or without previous brain irradiation.

Research conclusions
This retrospective study did not detect differences in toxicity for this population with or without previous irradiation, suggesting that the use of SRS for four or more brain lesions with or without previous brain irradiation is safe.

Research perspectives
This study claims for more data in larger studies in a prospective manner to better address this question.

FOOTNOTES

Author contributions: de Camargo AV, Borges ABB, Vazquez VL, and Araujo RLC contributed to conceptualization; de Camargo AV, de Mattos MD, Kawasaki MK, Gomes DNS, and Borges ABB contributed to data collection; de Camargo AV and Araujo RLC contributed to data analysis; all authors have read and approved the final manuscript.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Barretos Cancer Hospital.

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

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ORIGINAL ARTICLE

Observational Study

Classification of patients with metastatic colorectal cancer into consensus molecular subtypes into real-world: A pilot study

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Abstract

BACKGROUND

Colorectal cancer is a complex disease with high mortality rates. Over time, the treatment of metastatic colorectal cancer (mCRC) has gradually improved due to the development of modern chemotherapy and targeted therapy regimens. However, due to the inherent heterogeneity of this condition, identifying reliable predictive biomarkers for targeted therapies remains challenging. A recent promising classification system—the consensus molecular subtype (CMS) system—offers the potential to categorize mCRC patients based on their unique biological and molecular characteristics. Four distinct CMS categories have been defined: immune (CMS1), canonical (CMS2), metabolic (CMS3), and mesenchymal (CMS4). Nevertheless, there is currently no standardized protocol for accurately classifying patients into CMS categories. To address this challenge, reverse transcription polymerase chain reaction (RT-qPCR) and next-generation genomic sequencing (NGS) techniques may hold promise for precisely classifying mCRC patients into their CMSs.

AIM

To investigate if mCRC patients can be classified into CMS categories using a standardized molecular biology workflow.

METHODS

This observational study was conducted at the University of Chile Clinical Hospital and included patients with unresectable mCRC who were undergoing systemic treatment with chemotherapy and/or targeted therapy. Molecular

biology techniques were employed to analyse primary tumour samples from these patients. RT-qPCR was utilized to assess the expression of genes associated with fibrosis (TGF-β and β-catenin) and cell growth pathways (c-MYC). NGS using a 25-gene panel (TumorSec) was performed to identify specific genomic mutations. The patients were then classified into one of the four CMS categories according to the clinical consensus of a Tumour Board. Informed consent was obtained from all the patients prior to their participation in this study. All techniques were conducted at University of Chile.

RESULTS

Twenty-six patients were studied with the techniques and then evaluated by the Tumour Board to determine the specific CMS. Among them, 23% (n = 6), 19% (n = 5), 31% (n = 8), and 19% (n = 5) were classified as CMS1, CMS2, CMS3, and CMS4, respectively. Additionally, 8% of patients (n = 2) could not be classified into any of the four CMS categories. The median overall survival of the total sample was 28 mo, and for CMS1, CMS2, CMS3 and CMS4 it was 11, 20, 30 and 45 mo respectively, with no statistically significant differences between groups.

CONCLUSION

A molecular biology workflow and clinical consensus analysis can be used to accurately classify mCRC patients. This classification process, which divides patients into the four CMS categories, holds significant potential for improving research strategies and targeted therapies tailored to the specific characteristics of mCRC.

Key Words: Metastatic colorectal cancer; Targeted therapy; Consensus molecular subtypes; Personalized medicine

Core Tip: Colorectal cancer is molecularly heterogeneous. Consensus molecular subtype classification sheds light on its biology, potentially guiding targeted therapy selection. However, an optimal consensus molecular subtype classification mechanism remains elusive. This workflow, which combines reverse transcription polymerase chain reaction and next-generation sequencing, introduces a novel approach for molecular patient classification. We aim to use these techniques to improve the precision of tumour subtyping.

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INTRODUCTION

Colorectal cancer (CRC) exhibits high incidence and mortality rates. At the time of diagnosis, approximately 25% of patients already present with metastatic disease, while 50% of those initially diagnosed with localized stages later develop disseminated disease[1]. Recent years have seen significant advancements in systemic therapies for metastatic colorectal cancer (mCRC) patients, including diverse combination chemotherapy regimens, targeted therapy, immunotherapy, and multi-kinase inhibitors[2]. Despite these improvements, patients' responses remain variable and unpredictable due to the molecular heterogeneity of this disease. Thus, it is imperative to identify specific mutations for a personalized treatment approach[3].

Numerous efforts have attempted to identify distinct molecular mCRC phenotypes. In 2015, bioinformatic studies revealed a promising classification system with four consensus molecular subtypes (CMS)[4]. This classification system has gained widespread clinical acceptance and is currently guiding various ongoing clinical trials[5]. The four CMS are as follows: CMS1, or immune subtype, primarily affects young patients and exhibits rapid progression and resistance to conventional therapies. This subtype may benefit from aggressive chemotherapy and, potentially, immunotherapy. CMS2, or canonical subtype, is characterized by mutations in specific pathways linked to cellular metabolism. CMS3, or metabolic subtype, is characterized by mutations in pathways responsible for cellular metabolism, with a high prevalence of *KRAS* pathway mutations. Finally, CMS4, or mesenchymal subtype, is associated with mutations in fibrogenesis and epithelial-mesenchymal transition pathways, leading to a poor prognosis and a higher incidence of metastasis[5]. To date, there is no established methodology for effectively classifying patients into CMS categories. However, given that each CMS is linked to distinct patterns of mutations and gene expression, it is plausible that a molecular biology workflow designed to identify specific mutations could help accurately classify patients into different CMS groups[6]. Therefore, the objective of this study was to establish a workflow for assigning mCRC patients to CMS categories using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and next-generation sequencing (NGS) techniques.

MATERIALS AND METHODS

Study design and participants

In this observational study conducted between 2020 and 2023, we analyzed primary tumor tissue samples from mCRC patients who were receiving systemic treatment at the University of Chile Clinical Hospital. Colon or rectal tissue samples were collected through colonoscopy or surgical procedures. The samples were processed and stored according to protocols established by the Biobank of Tissues and Fluids at the University of Chile (http://biobanco.uchile.cl/). Both formalin-fixed paraffin-embedded (FFPE) tissue biopsies and fresh neoplastic tissue (frozen without fixation) were examined.

The inclusion criteria for this study were as follows: Patients diagnosed with unresectable mCRC (colon or rectal cancer) confirmed through histological diagnosis. Undergoing treatment at the University of Chile Clinical Hospital. Receiving systemic therapy in accordance with international clinical guidelines (National Comprehensive Cancer Network[7] and European Society of Medical Oncology)[8]. Treatment regimens included chemotherapy (FOLFOX, CAPOX, or FOLFIRI) and targeted therapy (bevacizumab, aflibercept, cetuximab, panitumumab, regorafenib, and TAS102). Chemotherapy and targeted therapy regimens were selected by the physicians on a case-by-case basis.

The exclusion criteria for this study were as follows: Patients who underwent the removal of metastases (metastasectomy) before enrollment. Any comorbidity leading to a life expectancy of less than six months. Inability to maintain clinical follow-up.

RT-qPCR

The expression of TGF-β, β-catenin, and c-MYC was investigated as follows: RNA was extracted from FFPE tissue using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Invitrogen). Subsequently, the concentration of each RNA sample was determined using the Quant-iT™ RiboGreen™ RNA Reagent Kit (Invitrogen) on a Cytation 3 instrument (BioTek). RNA (1000) ng was then used to prepare cDNA with the AffinityScript qPCR cDNA Synthesis Kit (Agilent) according to the manufacturer's instructions. Amplifications by qPCR (real-time PCR) was conducted in triplicate using the Brilliant II SYBR Green qPCR Master Mix kit (Agilent) on an Eco Real-time PCR System (Illumina). The following cycling conditions were used: an initial denaturation step at 95°C for 10 min, then 40 cycles of amplification (each cycle is 10 s at 95°C, 30 s at 60°C and 15s at 72°C). A melting curve for each primer ensured amplification of a single product. Finally, six FFPE non-tumour tissue samples treated in the same manner as the FFPE tumour tissues from each patient were included as controls. The relative expression was calculated using the ΔΔCt method[9] and normalized using expression levels of reference genes: B2M, PPIA, and RPLP0. Table 1 presents a summary of the primers used to conduct the RT-qPCR experiments[10-15].

NGS

The presence of genomic mutations was assessed using a 25-gene panel (TumorSec) as described by our team[16]. The RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE was utilized to extract genomic deoxyribonucleic acid (DNA) from FFPE samples. Briefly, samples were incubated with 1 mL of Histo-Clear at 50°C for 3 min to remove paraffin. The supernatant was then removed, followed by two ethanol washes, and the residual ethanol was evaporated using a SpeedVAC (Thermo Scientific). The samples were then incubated overnight in a digestion solution containing proteases. The next day, the samples were incubated at 80°C for 15 min and an isolation additive was added and centrifuged. Subsequently, the supernatant was transferred to a filter column and centrifuged to isolate the RNA, which was then treated with DNase. The column contained the DNA, which was subsequently treated with RNase. The DNA and RNA were washed with wash buffers and eluted in elution buffer in separate tubes.

Quantification and quality analysis: The purity and quantity of DNA and RNA were determined by measuring absorbance at 260/280 nm with the PicoGreen assay (Quant-iT™ PicoGreen® dsDNA, Invitrogen) and the Quant-iT™ RiboGreen™ RNA Reagent Kit, respectively, on a Cytation 3 instrument (Biotek). Additionally, DNA quality analysis was conducted by measuring fragment size with the HS Genomic DNA Analysis Kit (DNF-488) (Agilent) on a Fragment Analyzer instrument (Agilent). As the extraction of genomic DNA from FFPE samples often results in low yields and degradation ranging from more than 1000 bp to less than 200 bp, fragments less than 200 bp were not used for library preparation due to excessive degradation. To ensure adequate DNA quantity, a minimum of four, 6-μm FFPE sections per patient were used for sequencing. Moreover, each sample needed to contain more than 20% tumour content.

Library preparation: The KAPA HyperPlus Library Preparation Kit (Kapa Biosystems) was utilized to prepare DNA libraries. Library sizes and concentrations were verified for quality control purposes. The 260/280 nm ratio was measured with Cytation equipment and quantification was carried out using the Quant-iT™ PicoGreen™ dsDNA Assay Kit. Furthermore, library sizes were visualized using the HS NGS Analysis Kit in a Fragment Analyzer instrument.

NGS: NGS was conducted following a protocol previously published by our team[9]. For sequencing, an equimolar pool of libraries (4 nM) was prepared, diluted, and denatured to achieve a final concentration of 9.4–9.5 pM according to guidelines in the "MiSeq System Denature and Dilute Libraries Guide" (Illumina). Paired-end sequencing (300 cycles) was performed using the Illumina MiSeq System (MiSeq Reagent Kits v2). Finally, bioinformatics analysis was conducted.

Classification of patients into CMS categories

Given the absence of a singular marker that differentiates each of the four CMS categories on its own, we developed a comprehensive protocol involving analysis by a Tumour Board consisting of experts in Molecular and Medical Oncology.

Table 1 Primers employed for reverse transcription-quantitative polymerase chain reaction experiments to determine the expression of β-catenin, c-Myc and TFG-β, and the genes used as reference genes

Name	Primer	Sequence	Product Lenght	Ref.
TGF-β	Forward	5′- TACCTGAACCCGTGTTGCTCTC-3′	122	[10]
	Reverse	5′- GTTGCTGAGGTATCGCCAGGA-3′		
β-catenin	Forward	5′- CACAAGCAGAGTGCTGAAGGTG-3′	146	[11]
	Reverse	5′- GATTCCTGAGAGTCCAAAGACAG-3′		
c-MYC	Forward	5′-GCCACGTCTCCACACATCAG-3′	132	[12]
	Reverse	5′-TGGTGCATTTTCGGTTGTTG-3′		
B2M	Forward	5′-GTGCTCGCGCTACTCTCTC-3′	150	[13]
	Reverse	5′-GTCAACTTCAATGTCGGAT-3′		
PPIA	Forward	5′-GCAAATGCTGGACCCAACACAAAT-3′	174	[14]
	Reverse	5′-AATGGTGATCTTCTTGCTGGTCTTG-3′		
RPLP0	Forward	5′-GCAATGTTGCCAGTGTCTG-3′	142	[15]
	Reverse	5′-GCCTTGACCTTTTCAGCAA-3′		

Each case was individually assessed and the CMS was determined based on the criteria defined by Guinney *et al*[4]. The Tumour Board relied on patients' clinical characteristics, mismatch repair (MMR) expression, and RT-qPCR and NGS results. Each patient's CMS was determined by consensus among all committee members. Patients for whom a CMS consensus could not be reached were considered unclassifiable.

The Tumour Board employed the following criteria to classify each patient into one of the four CMS categories. It is important to note that none of these elements individually serve as a specific CMS marker; instead, classifications were based on the combination of multiple elements and reached through tumour board consensus. CMS1: presence of *BRAF* mutation; MMR protein deficiency; low TGF-β, β-catenin, and c-MYC mRNA expression; and absence of *APC* or *KRAS* mutations. CMS2 and CMS3: presence of *APC* mutation; absence of *BRAF* mutation (with a predominance of *KRAS* mutations in CMS3); MMR-proficient; low TGF-β and β-catenin mRNA expression; and high c-MYC mRNA expression. CMS4: MMR-proficient; high expression of TGF-β and β-catenin mRNA; low expression of c-MYC mRNA; and presence of non-categorical mutations identified through NGS[6].

Ethics

All procedures conducted in this study were in full compliance with the ethical standards set by the Institutional and National Research Committee, as well as the principles outlined in the 1964 Declaration of Helsinki and its subsequent amendments. Ethical approval for this study was obtained from the Ethics Committee of the University of Chile Clinical Hospital and Faculty of Medicine prior to beginning the research. Informed written consent was obtained from all patients before their participation in the trial.

Statistics

Results are presented as the number and percentage of total patients included in this study. To determine the appropriate sample size, we considered the estimated prevalence of each mCRC CMS. According to previous work[4], the expected prevalence of each CMS is approximately 20%–25%. A sample size of 25 patients was deemed sufficient to analyze the prevalence and distribution of the different CMS categories. Indeed, prior research has utilized sample sizes of 20–30 patients; thus, a sample size of 25 patients is consistent with the literature. For the overall survival analysis of the studied patients, log-rank test was conducted using GraphPad Prism 10.0 software.

RESULTS

Between 2020 and 2023, a total of 26 patients with unresectable mCRC undergoing systemic treatment at the University of Chile Clinical Hospital were included in this study. Table 2 presents the demographic and clinical characteristics of the patients, including age, gender, primary tumour site, and the presence or absence of MMR proteins. Each patient is identified with a number from 1–26.

Molecular studies

Table 2 illustrates the results of an RT-qPCR-based gene expression analysis of TGF-β, β-catenin, and c-MYC in each of the patients studied. It is observed that the expression of these three genes is heterogeneous among patients. Table 3 provides a comprehensive overview of the mutations identified with the 25-gene TumorSec panel. The most frequently observed

Table 2 Clinical characteristics, overall survival, and reverse transcription-quantitative polymerase chain reaction results of the *n* = 26 patients included in the final analysis

Patient number	Age	Gender	Site of primary cancer	Overall survival (mo)	Miss-match repair proteins expression	β-catenin expression (RT-qPCR) (relative expression with respect to reference gene average)	c-MYC expression (RT-qPCR) (relative expression with respect to reference gene average)	TGF-β expression (RT-qPCR) (relative expression with respect to reference gene average)	CMS
1	69	Male	Sigmoid	5	Proficient	0.185	2.864	0.201	CMS2
2	85	Female	Right colon	31	Proficient	0.100	0.352	0.169	CMS1
3	68	Female	Rectal and sigmoid	12	Proficient	0.042	0.384	0.076	CMS3
4	57	Male	Rectal and sigmoid	34	Proficient	2.684	18.817	9.778	CMS3
5	45	Female	Transverse	40	Proficient	1.812	19.445	5.231	CMS2
6	62	Male	Rectum	28	Proficient	0.010	4.401	0.973	CMS3
7	54	Male	Rectum	20	Proficient	0.301	3.234	1.433	CMS2
8	55	Male	Sigmoid	53	Proficient	0.080	1.870	11.718	CMS4
9	73	Male	Sigmoid	62	Proficient	0.038	0.645	0.461	CMS1
10	79	Male	Rectum	40	Proficient	0.121	2.080	3.513	CMS3
11	56	Female	Right colon	29	Proficient	0.235	3.799	14.700	CMS4
12	66	Female	Right colon	10	Proficient	0.351	6.004	76.116	CMS4
13	53	Male	Sigmoid	52	Proficient	0.233	3.863	2.688	CMS4
14	75	Male	Sigmoid	35	Proficient	0.089	0.760	0.205	CMS3
15	63	Male	Right colon	32	Proficient	0.089	0.760	0.466	CMS3
16	48	Female	Sigmoid	28	Proficient	0.038	1.110	0.498	CMS3
17	53	Female	Rectum	20	Proficient	0.084	1.124	0.801	Not classifiable
18	71	Female	Right colon	12	Proficient	0.083	1.540	0.897	CMS1
19	61	Female	Sigmoid	45	Proficient	0.106	9.208	6.820	CMS4
20	71	Male	Rectum	10	Proficient	0.013	0.855	0.065	CMS3
21	49	Female	Sigmoid	6	Deficient	0.063	2.968	1.871	CMS1
22	74	Male	Right colon	11	Deficient	0.047	0.552	0.249	CMS1
23	65	Female	Rectum	39	Proficient	0.059	0.828	0.084	Not classifiable
24	59	Female	Sigmoid	8	Proficient	0.045	1.324	0.152	CMS2
25	54	Male	Sigmoid	5	Deficient	0.036	0.543	0.127	CMS1
26	69	Male	Sigmoid	22	Proficient	0.192	5.025	2.654	CMS2

Each patient is individually identified in the first column on the left with a sequential number ranging from 1 to 26. Additionally, the consensus molecular subtype assigned based on the Tumour Board analysis is provided. RT-qPCR: reverse transcription-quantitative polymerase chain reaction; TGF-β: Transforming growth factor beta; CMS: consensus molecular subtype.

mutations were in *KRAS*, *TP53* and *ARID1A*. All observed mutations were single nucleotide variants (SNVs) and two patients possessed deletions.

Classification of patients into CMS categories

Out of the 26 patients analyzed, a specific CMS could be identified for 24 patients (92%) by clinical consensus by the Tumour Board. Two patients (8%) were found to be unclassifiable. Figure 1 illustrates the distribution of patients across

Table 3 Mutations identified in the *n* = 26 patients included in the final analysis through massive genomic sequencing using the TumorSec panel

Patient number	Mutation	Mutation variant classification	Affected protein	Variant type
1	TSC2	Missense	p.R1729C	SNV
	TP53	Missense	p.R175H	SNV
2	KRAS	Missense	p.G12C	SNV
3	KRAS	Missense	p.G12V	SNV
	TP53	Missense	p.R175H	SNV
4	KRAS	Missense	p.Q61H	SNV
	PIK3CA	Missense	p.E545G	SNV
5	TP53	Missense	p.P152L	SNV
6	KRAS	Missense	p.G12D	SNV
7	BRCA2	Missense	p.K584E	SNV
	ARID1A	Nonsense	p.Q1584	SNV
8	KRAS	Missense	p.N116H	SNV
	TP53	Missense	p.R175H	SNV
	PIK3CA	Missense	p.H1047R	SNV
	BRAF	Missense	p.N581Y	SNV
9	BRCA2	Frameshift (deletion)	p.N863Ifs11	SNV
	ARID1A	Frameshift (deletion)	p.P1326Rfs155	SNV
	PIK3CA	Missense	p.H1047R	SNV
10	PTEN	Nonsense	p.Y225	SNV
	KRAS	Missense	p.G12C	SNV
	TP53	Frameshift (insertion)	p.Q317Pfs20	SNV
11	KRAS	Missense	p.Q61H	SNV
12	KRAS	Missense	p.G12D	SNV
	TP53	Missense	p.R280K	SNV
13	TP53	Missense	p.R273H	SNV
14	KRAS	Missense	p.G12D	SNV
	TP53	Missense	p.P278L	SNV
15	KRAS	Missense	p.K117N	SNV
	TP53	Missense	p.R282W	SNV
16	KRAS	Missense	p.G12D	SNV
	TP53	Frameshift (deletion)	p.S260Qfs3	Deletion
17	KRAS	Missense	p.Q61L	SNV
	BRCA2	Missense	p.S3147Y	SNV
	TP53	Missense	p.R249G	SNV
18	KRAS	Missense	p.G12C	SNV
	ARID1A	Frameshift (deletion)	p.Q611Hfs7	Deletion
19	TP53	Missense	p.Y220C	SNV
20	KRAS	Missense	p.A59G	SNV
	KRAS	Missense	p.G12D	SNV
	TP53	Missense	p.H214R	SNV

21	NRAS	Missense	p.Q61R	SNV
	ARID1A	Frameshift (deletion)	p.K1072Nfs21	SNV
22	TP53	Missense	p.R273C	SNV
23	TP53	Nonsense	p.E51	SNV
	ARID1A	Frameshift (deletion)	p.Q372Sfs19	SNV
24	TP53	Missense	p.R248W	SNV
	PIK3CA	Missense	p.E545K	SNV
25	PTEN	Nonsense	p.Q149	SNV
	KRAS	Missense	p.G13D	SNV
	TSC2	Missense	p.R1713C	SNV
	TP53	Missense	p.R273C	SNV
	TP53	Missense	p.R158H	SNV
	ARID1A	Nonsense	p.R1335	SNV
26	BRCA2	Missense	p.E3002K	SNV
	TP53	Missense	p.C176Y	SNV

SNV: Single nucleotide variant.

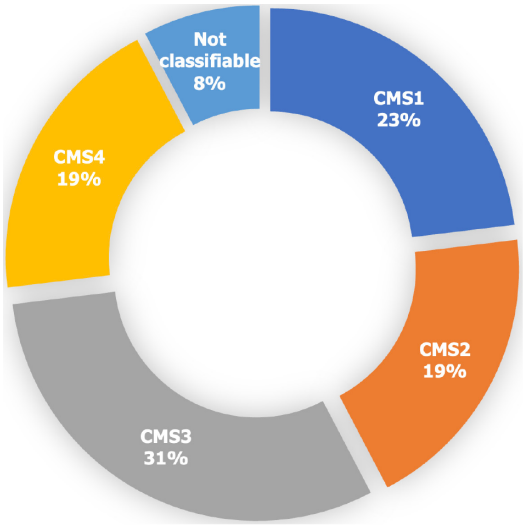

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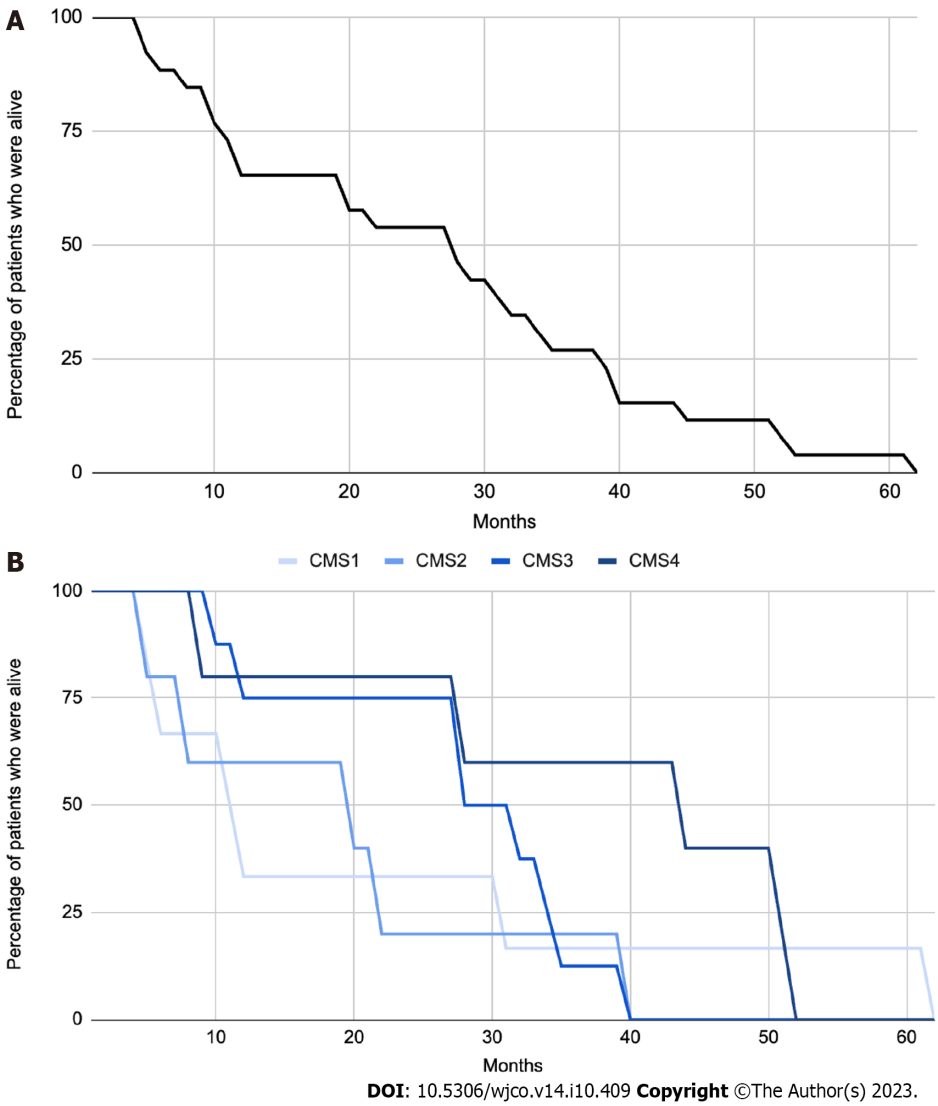
Figure 1 Proportion of patients in each consensus molecular subtype after analysis by the Tumour Board among the 26 patients included on the final analysis. A specific consensus molecular subtype (CMS) was successfully identified in 24 out of the 26 patients. CMS1 *n* = 6. CMS2 *n* = 5. CMS3 *n* = 8. CMS4 *n* = 5. Not classifiable *n* = 2. Each patient underwent an individual assessment by the Tumour Board, and a consensus was reached to determine their molecular subtype. Classification was based on clinical and histological characteristics, as well as the results of RT-qPCR (β-catenin, c-MYC and TGF- β) and NGS (TumorSec panel). CMS: Consensus molecular subtypes.

the four CMS categories. Specifically, 23% (*n* = 6), 19% (*n* = 5), 31% (*n* = 8), and 19% (*n* = 5) were classified as CMS1, CMS2, CMS3 and CMS4, respectively. Remarkably, the percentage of patients classified into each CMS category closely aligns with findings reported by Guinney *et al*[4]. The median overall survival of the total sample was 28 mo (Figure 2A), and for CMS1, CMS2, CMS3 and CMS4 it was 11, 20, 30 and 45 mo respectively, with no statistically significant differences between groups (Figure 2B).

DISCUSSION

The objective of the workflow outlined in this manuscript was to develop an RT-qPCR- and NGS-based method by which to classify mCRC patients into CMS categories. Our results demonstrate that it is possible to classify mCRC patients into a



DOI: 10.5306/wjco.v14.i10.409

Figure 2 Classification of patients into consensus molecular subtype categories. A: Kaplan-Meier Curve with overall survival (OS) of the *n* = 26 patients included on the final analysis. mOS = 28 mo; B: Kaplan-Meier curve which shows OS of patients based on their molecular subtype classification. The median overall survival times were 11, 20, 30, and 45 mo for CMS1, CMS2, CMS3, and CMS4, respectively. There were no statistically significant differences observed among the studied groups (*P* = 0.0968).

specific CMS in approximately 90% of the cases.

To date, there are no validated tools from prospective studies for classifying patients into the four CMS categories. Although genomic platforms such as ColotypeR[17] and CMSCaller[18] have been utilized, they have not significantly impacted clinical practice. Our findings present an alternative protocol for patient classification, leveraging a 25-gene panel (TumorSec) and a three-gene RT-qPCR panel (TGF-β, β-catenin, and c-MYC). The selected genes play vital roles in the epithelial-mesenchymal transition, particularly TGF-β and β-catenin, which are specific to CMS4 (fibrotic)[19]. Additionally, c-MYC was chosen due to its utility for identifying CMS2 (metabolic)[20]. However, distinguishing between CMS2 and CMS3 remains challenging as they share genetic signatures and patterns of gene expression.

The relevance of classifying mCRC patients into CMS categories must be contextualized. Thus far, the selection of targeted therapies and the design of clinical studies have primarily relied on the identification of *KRAS*, *NRAS*, and *BRAF* mutations and MMR expression analyses[7-8]. However, incorporating knowledge of the CMS categories can offer significant advantages in both aspects. First, it can enhance the selection of targeted therapies, enabling a more personalized approach. Additionally, a better understanding of the CMS categories can lead to improved clinical study design, allowing for more tailored and effective treatments for patients with specific CMS profiles[6]. For instance, CMS1, characterized by high lymphocytic infiltration and a worse prognosis, may benefit from aggressive therapeutic strategies such as combination triplet chemotherapy (FOLFOXIRI) and anti-angiogenic agents[21]. Monodrug immunotherapy could also be beneficial for these patients given their high frequency of microsatellite instability-high tumours as demonstrated in the KEYNOTE177 study[22]. Considering the high prevalence of *BRAF* mutations, future studies should examine the efficacy of BRAF inhibitors for these patients[23]. CMS2 and CMS3 share significant features and may respond to similar agents. For example, they may show sensitivity to anti-EGFR therapy, especially in CMS2 cases[24]. However, CMS3 patients frequently develop *KRAS* mutations, primarily in exon 2, leading to constitutive activation of the mitogen-associated protein kinase pathway, associated with a poorer prognosis and response to standard treatment[25]. CMS4,

which carries the worst prognosis, calls for the development of new strategies targeting the epithelial-mesenchymal transition or the TGF-β pathway. CMS4 tumours also show better response to irinotecan-based treatments or anti-angiogenic agents such as bevacizumab[26].

It is important to note that the classification of CMS can also predict the prognosis of patients with mCRC[4]. While this study documented the overall survival of patients, there were no significant differences between groups, likely due to the low number of patients in each CMS category. Therefore, it cannot be established whether patients with different CMSs have different prognoses.

The principal innovation of this exploratory study lies in the establishment of a protocol for the classification of mCRC patients into CMS through RT-qPCR (TGF-β, β-catenin, and c-MYC) and a 25-gene NGS panel (TumorSec). Our results demonstrates that this combined approach has the potential to classify patients with mCRC into one of the four CMS categories in over 90% of cases. As there is currently no gold-standard for conducting this clinical-molecular classification, this approach may represent a significant advancement in the development of an optimal technique that could become the standard for these purposes. In the future, it is important to further explore CMS categories and incorporate this knowledge into clinical practice. While this protocol proposes a CMS classification scheme, prospective and large-scale studies are imperative to assessing whether this methodology truly influences therapeutic decisions for patients[5] and for validating the clinical utility of CMS categories[6].

CONCLUSION

In conclusion, we successfully classified mCRC patients into CMS categories using an RT-qPCR and NGS-based workflow. This approach opens avenues for tailoring therapies according to CMS subtypes, potentially leading to improved patient outcomes.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer is a heterogeneous disease; therefore, it is crucial to progress towards a molecular consensus classification in order to predict prognosis and therapy response.

Research motivation

The primary motivation is to progress towards a consensus molecular classification of metastatic colorectal cancer patients, to better guide targeted therapy.

Research objectives

The aim of this study is to classify a sample of metastatic colorectal cancer patients into consensus molecular subtypes using a reverse transcription -quantitative polymerase chain reaction polymerase chain reaction (RT-qPCR) and next-generation genomic sequencing (NGS) protocol.

Research methods

Patients with unresectable metastatic colorectal cancer who were undergoing systemic treatment with chemotherapy and/or targeted therapy. Molecular biology techniques were employed to analyse primary tumour samples from these patients. RT-qPCR was utilized to assess the expression of genes associated with fibrosis (TGF-β and β-catenin) and cell growth pathways. NGS using a 25-gene panel (TumorSec) was performed to identify specific genomic mutations. The patients were then classified into one of the four CMS categories according to the clinical consensus of a Tumour Board.

Research results

n = 26 metastatic colorectal cancer patients analyzed. 23% (n = 6), 19% (n = 5), 31% (n = 8), and 19% (n = 5) were classified as CMS1, CMS2, CMS3, and CMS4, respectively. Additionally, 8% of patients (n = 2) could not be classified into any of the four CMS categories.

Research conclusions

It is possible to classify patients with metastatic colorectal cancer into consensus molecular subtypes through RT-qPCR and NGS techniques.

Research perspectives

Prospective studies are needed to determine if this classification is useful and if it has an impact on predicting the survival of patients with metastatic colorectal cancer.

FOOTNOTES

Author contributions: González-Montero J led the study, wrote the manuscript, and created the figures and tables; González-Montero J, Burotto M, and Barajas O led the molecular classification of the patients; Valenzuela G, Toro J, and Marcelain K performed molecular biology procedures; Barajas O, Mateluna D, and Buen-Abad F recruited the patients.

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SCIENTOMETRICS

What should be the future direction of development in the field of prostate cancer with lung metastasis?

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Abstract

BACKGROUND

Since the start of the 21st century, prostate cancer with lung metastasis (PCLM) has accumulated significant scientific research output. However, a systematic knowledge framework for PCLM is still lacking.

AIM

To reconstruct the global knowledge system in the field of PCLM, sort out hot research directions, and provide reference for the clinical and mechanism research of PCLM.

METHODS

We retrieved 280 high-quality papers from the Web of Science Core Collection and conducted a bibliometric analysis of keywords, publication volume, and citation frequency. Additionally, we selected differentially expressed genes from global high-throughput datasets and performed enrichment analysis and protein-protein interaction analysis to further summarize and explore the mechanisms of PCLM.

RESULTS

PCLM has received extensive attention over the past 22 years, but there is an uneven spatial distribution in PCLM research. In the clinical aspect, the treatment of PCLM is mainly based on chemotherapy and immunotherapy, while diagnosis relies on methods such as prostate-specific membrane antigen positron emission

tomography/computed tomography. In the basic research aspect, the focus is on cell adhesion molecules and signal transducer and activator of transcription 3, among others. Traditional treatments, such as chemotherapy, remain the mainstay of PCLM treatment, while novel approaches such as immunotherapy have limited effectiveness in PCLM. This study reveals for the first time that pathways related to coronavirus disease 2019, cytokine-cytokine receptor interaction, and ribosome are closely associated with PCLM.

CONCLUSION

Future research should focus on exploring and enhancing mechanisms such as cytokine-cytokine receptor interaction and ribosome and improve existing mechanisms like cadherin binding and cell adhesion molecules.

Key Words: Prostate cancer; Lung metastasis; Chemotherapy; Immunotherapy; Bibliometric analysis; Enrichment analysis

Core Tip: Discovering new insights into prostate cancer with lung metastasis (PCLM), this study presents a systematic analysis of 280 high-quality papers and global datasets. The uneven distribution of PCLM research is highlighted. Notably, this study uncovers the association of PCLM with pathways related to coronavirus disease 2019, cytokine-cytokine receptor interaction, and ribosomes. While traditional treatments remain crucial, novel approaches like immunotherapy show limited effectiveness. Future research should prioritize exploring mechanisms such as cytokine-cytokine receptor interaction and ribosomes while enhancing existing mechanisms like cell adhesion molecules. This study's innovative findings contribute to the advancement of PCLM research, stimulating further exploration and potential improvements in diagnosis and treatment strategies.

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INTRODUCTION

Prostate cancer (PC) is the second most common cause of cancer-related fatalities[1]. Globally, there are more than 1.4 million new cases of PC and over 370000 deaths related to PC each year[2]. Due to the prostate's unique location and function in the male anatomy, the early diagnosis and treatment of PC face numerous challenges[3]. Consequently, many PC patients develop metastasis. Lung metastasis (LM) is a relatively common occurrence in PC, with over 10% of PC patients experiencing LM[4]. Patients with PC with LM (PCLM) often present symptoms such as difficulty breathing, persistent dry cough, chest tightness, hemoptysis, and pain, which significantly impact their overall health[5]. Moreover, PCLM often accompanies metastasis to other organs or tissues[6,7], which complicates the treatment process and increases patients' suffering, further reducing the chances of a cure. Currently, treatment options for PCLM such as radiation therapy, chemotherapy, and surgical resection impose significant physiological, psychological, and economic burdens on patients due to their complex treatment procedures and high-risk operations, and these treatment strategies have a limited ability to achieve a complete cure for PCLM[8-10]. Therefore, PCLM is a very harmful disease, regardless of the clinical characteristics of PCLM or the base number of patients.

Over the past 22 years, researchers have increasingly focused on the field of PCLM. With the development of PCLM, researchers have generated significant scientific output. However, as scientific output on PCLM has accumulated over the years, the knowledge structure of PCLM has become both disorganized and a hindrance to research efficiency[11,12]. Bibliometrics, a method that quantitatively analyzes and measures literature information using statistical methods and information technology has been widely applied in medical research with promising results[13-17]. Therefore, bibliometric analysis may provide a partial solution to the aforementioned challenges.

To comprehensively analyze and summarize the field of PCLM, this study retrieved relevant papers on PCLM from the Web of Science Core Collection (WOSCC) and conducted a bibliometric analysis of the citation references and keywords. Additionally, we conducted a preliminary exploration of potential biological behavior in the field of PCLM. This article aims to help researchers interested in the field of PCLM grasp the research trends in this field more accurately and quickly, and to deeply understand the related fields and technology development trends. We hope that this study can provide inspiration and assistance in the development and promotion of the research field of PCLM on a global scale.

MATERIALS AND METHODS

Collection of PCLM paper data

The data for PCLM papers were collected from the WOSCC (https://www.webofscience.com/). The search strategy used in this study was TS = (("prostat* cancer") OR ("prostat* carcinoma")) AND (("pulmonary metastas*") OR ("lung metastas*") OR ("metasta* tumor of lung") OR ("metasta* carcinoma of lung") OR ("metasta* lung carcinoma")).

The inclusion and exclusion criteria for PCLM papers in this study were as follows: (1) To avoid the impact of data fluctuation due to WOSCC updates and restrictions, only papers published between 2000 and 2022 were included; (2) To ensure analytical rigor, only research articles, review articles, and early access papers were included; (3) Due to restrictions of the relevant software, only English-language papers were included; and (4) Finally, after manual screening, papers that were not relevant to the topic were excluded. Therefore, 280 articles were included in this study (see Figure 1).

All the data for this study were downloaded from WOSCC in BibTeX format on May 2, 2023, with the recorded content being "full record" and "cited reference". The data collection work was conducted separately by two authors. Any discrepancies that arose between the two authors during this process were resolved through in-depth discussions involving both authors and other collaborators to reach a consensus.

Bibliometric analysis of PCLM paper data

We utilized R software (version 4.2.2) for advanced statistical calculations, visualization, and comprehensive bibliometric analysis. This included creating topic evolution maps and keyword temporal heat maps. Additionally, we employed VOSviewer software (version 1.6.18) to handle large amounts of data and create keyword clustering visualizations.

Exploration of molecular mechanisms in PCLM

We searched the Gene Expression Omnibus, the Cancer Genome Atlas, Sequence Read Archive, and ArrayExpress databases, to identify suitable human tissue datasets that included both PC and PCLM tissues. One dataset, GSE 74367, met our inclusion criteria, and we downloaded the corresponding data. Using R software, we extracted expression matrices from the dataset and identified differentially expressed genes (DEGs) specific to PCLM. The criteria for DEG selection were $|\log FC| > 1$ and P-value < 0.05. Subsequently, we performed gene ontology and Kyoto Encyclopedia of Genes and Genomes analyses of the selected DEGs to gain preliminary insights into the potential molecular mechanisms of PCLM. Furthermore, we utilized STING (version 11.5) and Cytoscape (version 3.9.1) to construct protein-protein interaction networks for further analysis of PCLM mechanisms.

RESULTS

Spatial and temporal distribution and changes in PCLM knowledge volume

From a spatial dimension, Figure 2A illustrates the overall increasing trend in the publication and citation count of PCLM papers since 2000. However, the annual publication trends appear to be less stable. This does not indicate that the PCLM field has not received enough attention, but may be related to some bottlenecks encountered in the PCLM field. The steady increase in citation counts over the years further supports this statement. In terms of spatial distribution, Figure 2B reveals that developed countries have made significant contributions to the PCLM field, including the United States (130 papers), Japan (41 papers), Germany (27 papers), and Canada (20 papers), among others. This reflects the imbalance in the development of PCLM research across different regions. Encouragingly, emerging economies such as China and India are gaining importance and playing an increasingly significant role in the field.

Transition of hot topics in the PCLM field

Major hot directions in the PCLM field: Figure 3 illustrates that "expression", "metastasis", and "E-cadherin" are popular keywords in the PCLM field. We conducted a co-occurrence analysis using VOSviewer to identify the main hot directions in the PCLM field and provide an in-depth understanding of its knowledge composition. We selected 111 keywords with a frequency of occurrence greater than four times from the PCLM papers to construct a co-occurrence network. Based on Figure 4 and Supplementary Table 1, the network can be primarily divided into four clusters. Cluster 1: Basic research on tumor metastasis mechanisms (red portion in Figure 4A) includes keywords such as epithelial-mesenchymal transition (EMT), E-cadherin, adhesion, and migration. Cluster 2: Clinical treatment and related research (green portion in Figure 4A) includes keywords such as therapy, surgery, radiotherapy, radical prostatectomy, gene therapy, immunotherapy, and chemotherapy. Cluster 3: Clinical diagnosis-related research [blue portion in Figure 4A includes keywords such as diagnosis, prostate-specific membrane antigen (PSMA), and positron emission tomography/computed tomography (PET/CT)]. Cluster 4: Other basic research on PCLM (yellow portion in Figure 4A) includes keywords such as signal transducer and activator of transcription 3 (STAT3), microenvironment, androgen receptor (AR), mouse model, and angiogenesis. Surprisingly, recent hot topics, such as immunotherapy, are not emerging trends in this field, while phrases related to chemotherapy and targeted therapy, such as abiraterone acetate, docetaxel, cabazitaxel, and enzalutamide, are emerging keywords in this field (Figure 4B).

Evolution of hot topics in the PCLM field: Figures 5 and 6 demonstrate the evolution of hot topics in the PCLM field. In recent years, themes such as interleukin (IL)-12, gene therapy, and ganciclovir therapy have experienced a significant

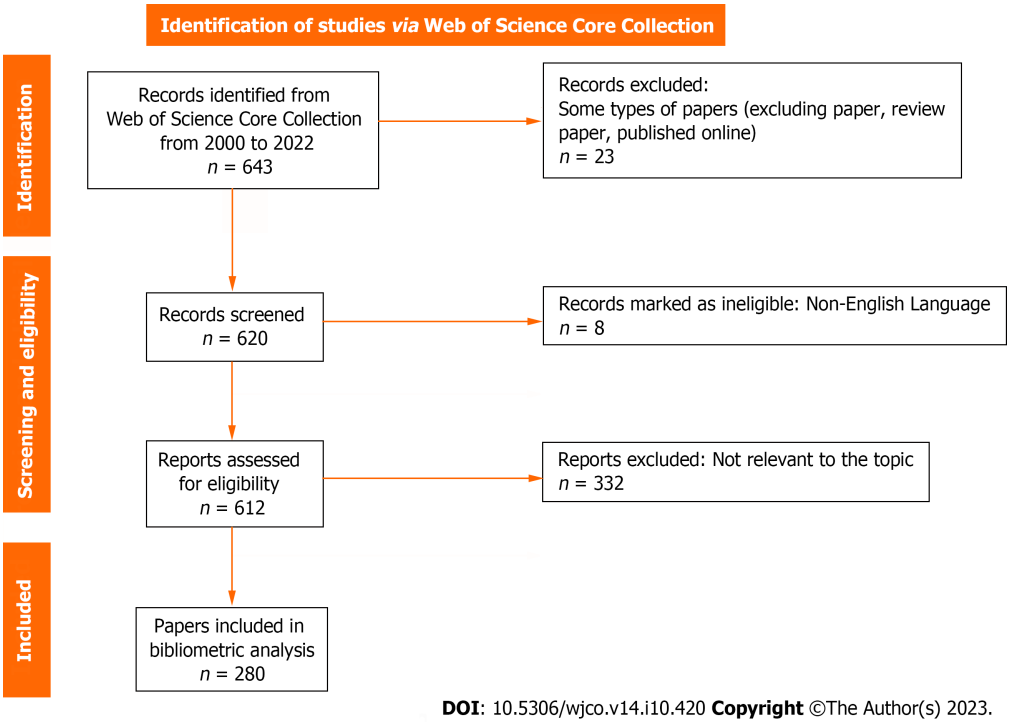

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Figure 1 Flowchart of data collection from papers on prostate cancer with lung metastasis.

decrease in attention. On the other hand, PET/CT and PSMA in the diagnostic domain, enzalutamide, abiraterone acetate, and cabazitaxel in the clinical treatment domain, and metabolism and BReast-CAncer susceptibility gene 2 (*BRCA2*) in the basic research domain have emerged as new hot topics. Meanwhile, immunohistochemistry, immunotherapy, radiotherapy, migration, and angiogenesis have remained long-standing hot topics in the PCLM field. Additionally, it is surprising that terms related to bone metastasis, such as bone, bone metastasis, and bone scintigraphy, have appeared with a relatively high frequency in the PCLM field.

Development status of major research topics in PCLM

We constructed a thematic strategic coordinate map based on Keyword Plus (ID) and Author Keywords (DE) in the PCLM literature to determine the development status of major research topics. Figure 7 reflects the following themes in the field of PCLM: Motor themes, including chemotherapy, docetaxel, migration, and mitoxantrone, which are important and well-developed topics; niche themes, including *Ga-68-PSMA*, *STAT3*, and tumor-associated macrophages, which currently have low impact but need further strengthening; emerging or declining themes, including cisplatin, immunotherapy, gene therapy, and *IL-12*; and basic themes, including PET/CT, radiotherapy, and radical prostatectomy, which are important but have not yet received significant development in the field.

Exploration of the biological behavior of PCLM

We collected 12729 DEGs from a global dataset and compared PC patients without LM (*i.e.*, locally metastatic) and PC patients with LM. Among these DEGs, 6138 genes were upregulated, and 6591 genes were downregulated. Figure 8A, which presents the gene ontology functional annotations, shows that, in the biological process category, there are pathways such as regulation of the immune effector process and lymphocyte proliferation. The molecular function category has pathways such as focal adhesion, while in the cellular component category, cytokine activity and cadherin binding are prominent (Supplementary Table 2). Figure 8B, representing the Kyoto Encyclopedia of Genes and Genomes' functional annotations, reveals pathways such as cell adhesion molecules, neuroactive ligand-receptor interaction, salmonella infection, cytokine-cytokine receptor interaction, and the cAMP signaling pathway (Supplementary Table 3). It is worth noting that the findings related to cadherin binding and cell adhesion molecules align with the previous discussions, further confirming their promotional role in the development of LM in PC patients. To further investigate and explore the relevant pathways of PCLM, we applied the maximal clique centrality method to identify the top 20 key proteins from the cadherin binding and cell adhesion molecule pathways and construct a protein-protein interaction network. We found that cell adhesion molecules are closely associated with the immunoglobulin superfamily, such as *CD8A*, *CD86*, and *ICAM1*, as well as integrin family proteins, including *ITGB1*, *ITGB2*, and *ITGAM* (Figure 9A and Supplementary Table 4). On the other hand, cadherin binding shows close correlations with calcium-binding proteins from the cadherin family, such as *CDH1*, *CDH5*, and *CDH11*, as well as with catenin family proteins, such as *CTNNA1* and *CTNNB1* (Figure 9B and Supplementary Table 5).

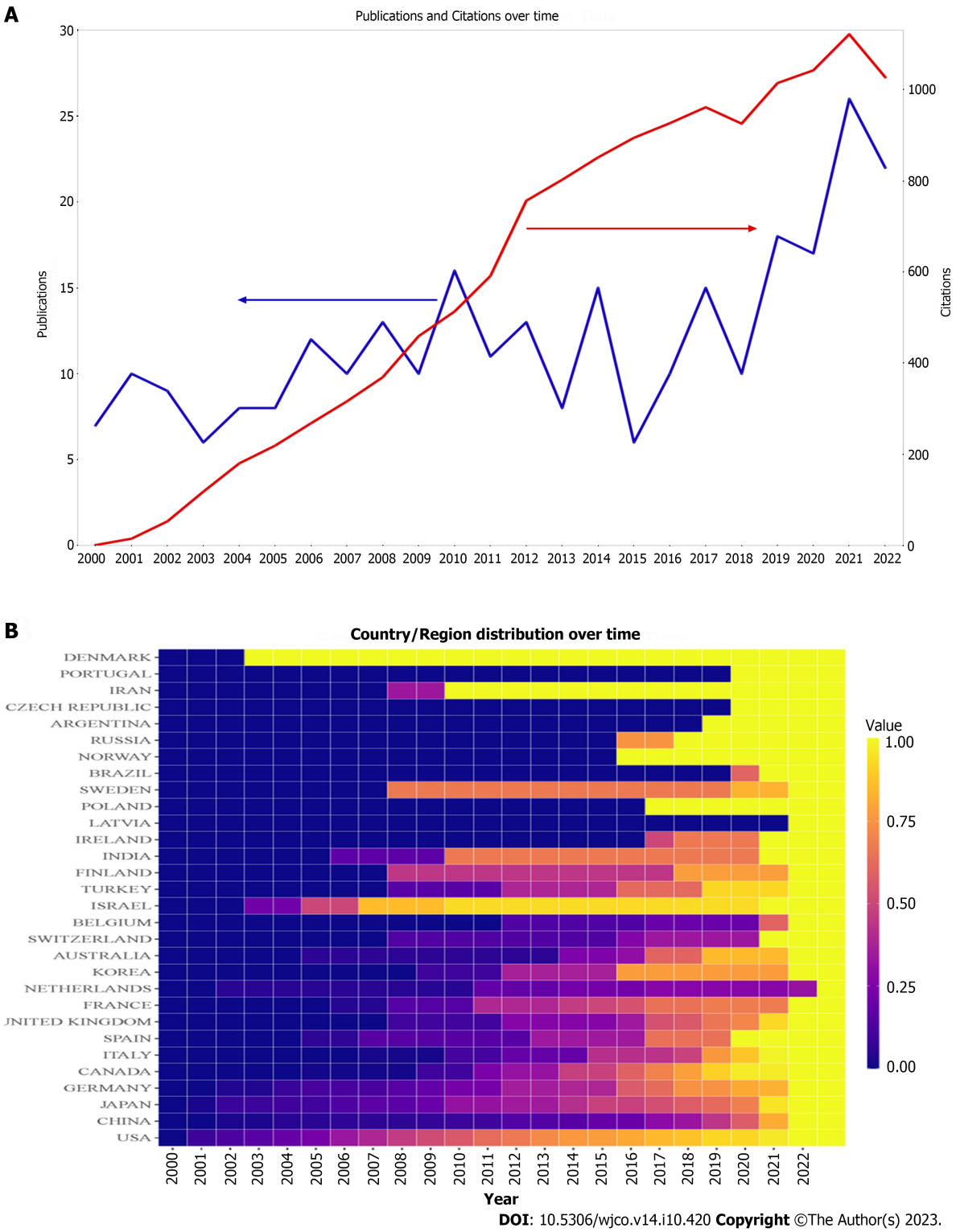

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Figure 2 Distribution and change in time and space of knowledge volume in the prostate cancer with lung metastasis field. A: Annual publications and citations of papers on prostate cancer with lung metastasis (2000-2022); B: Thermal diagram of the time distribution of national/regional papers. The *b* values represent the ratio of the total number of papers published in a country from 2000 to a certain year to the total number of papers published in a country.

DISCUSSION

PCLM is typically characterized by the presence of multiple nodules or areas of increased density in the lungs[18,19]. Metastatic lesions in the lungs can affect respiratory function and cause symptoms such as shortness of breath and chest tightness[5,20]. They can also exacerbate pre-existing lung diseases in patients, leading to poor prognoses. Extensive research efforts have been dedicated to understanding the biological behavior of PCLM, which has contributed to the continuous development of clinical treatment strategies. In recent years, the explosive growth and widespread adoption of bioinformatics, particularly next-generation sequencing technologies and single-cell sequencing, have enabled

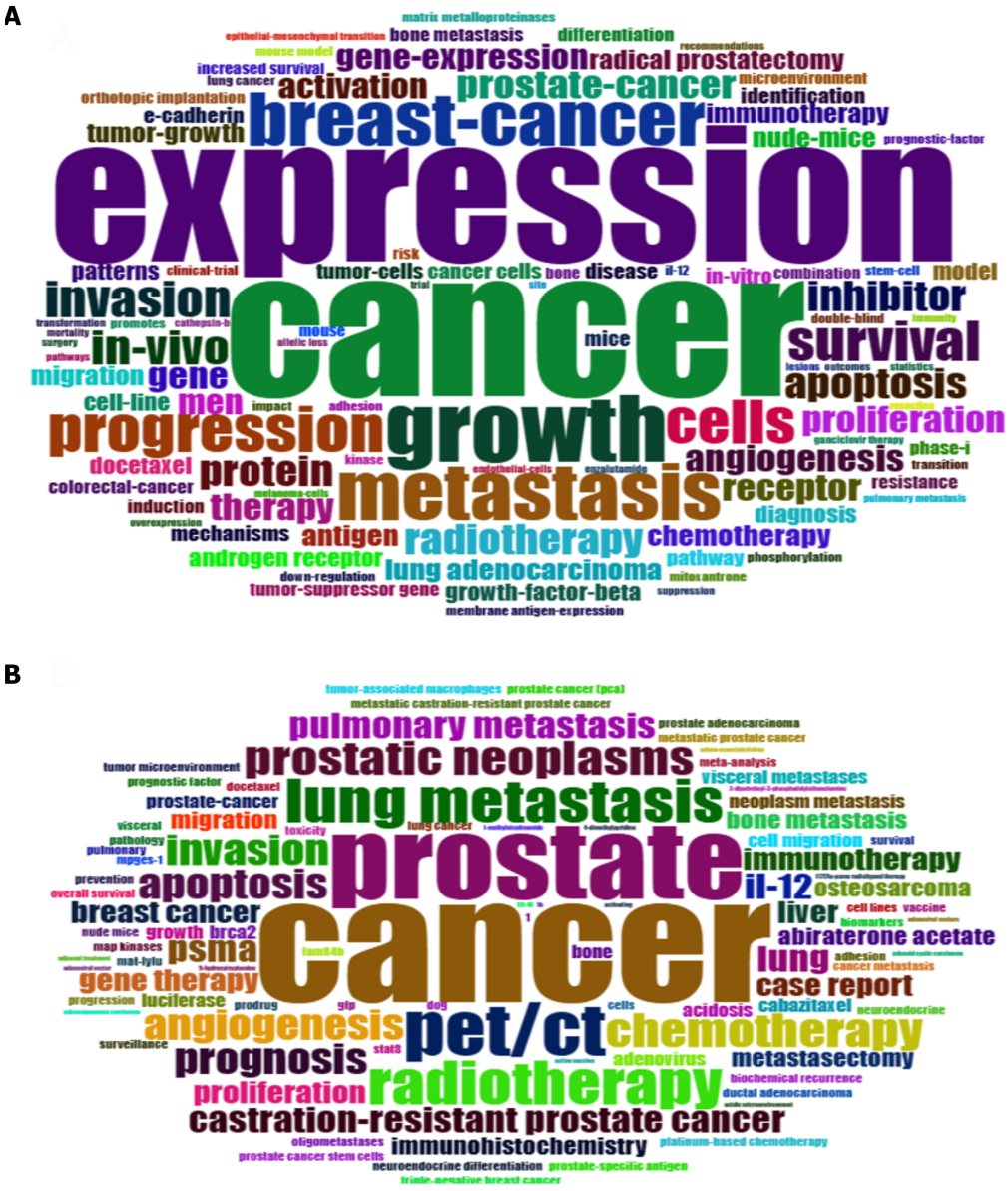

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Figure 3 Word clouds of high-frequency keywords in the papers on prostate cancer with lung metastasis. A: Keywords plus; B: Author's keywords.

researchers to delve into the molecular mechanisms of PCLM in depth, leading to unprecedented progress in the field. However, the accumulation of scientific output over the years has resulted in a chaotic knowledge landscape in the field of PCLM. Therefore, this study aimed to systematically reconstruct the global knowledge system of PCLM, providing a reference for the future development of PCLM.

Spatial and temporal distribution and changes in scientific output in the PCLM field

In recent years, more systematic and precise screening and treatment have significantly improved the prognosis of PC up to a point[21,22]. However, effective treatment of PCLM still faces significant challenges and requires further exploration and breakthroughs[23]. Moreover, the number of PCLM patients is very large globally[1,2,4], which is further driving the exploration of and research into PCLM by scholars worldwide. This is consistent with the expanding volume of PCLM knowledge over the years. However, the uneven distribution of scientific output in the field of PCLM across regions in the spatial dimension may be related to the social and scientific development capabilities of those regions[24]. This implies that the uneven country/region distribution of scientific output about PCLM in the spatial dimension may be related to two factors. First, developed countries and regions have invested more in healthcare resources and scientific research infrastructure. Second, they have a higher number of research institutes, laboratories, and researchers. In contrast, some developing countries or poor regions may face the challenges of limited funding and inadequate research conditions, resulting in a relative lag in scientific research. In this way, a contradiction has arisen between developing countries with limited medical technology but high PC morbidity and mortality and developed countries with advanced medical technology but reduced PC morbidity and mortality[25,26]. Therefore, developed countries should proactively

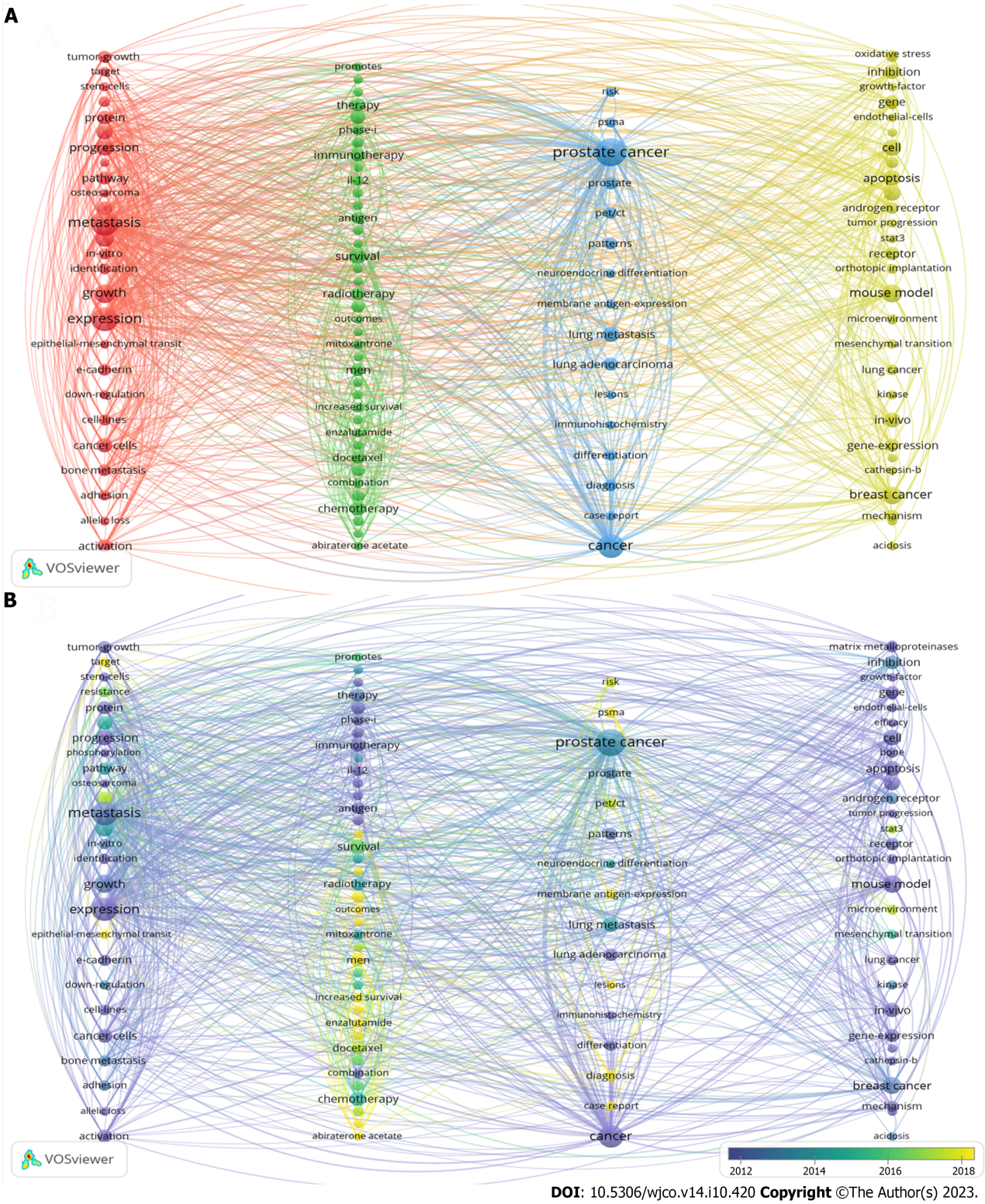

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Figure 4 Analysis of the co-occurrence of all keywords in the papers on prostate cancer with lung metastasis. A: Network visualization map; B: Overlay visualization map. The small circle represents the keyword. The area of the small circle represents the frequency of the keyword. The colors of the different areas represent their categories. The lines of the connecting circles represent keywords that appear in an article simultaneously.

conduct international exchanges and cooperation in the field of PCLM to promote the sharing of data, funds and equipment, technology and methods, and the establishment of international cooperation networks. Developing countries should increase their investment in PCLM-related research and actively seek transnational cooperation in the future. This will not only benefit the lives and health of the world's people but will also benefit the development of the field of PCLM by making full use of clinical resources and research due to the international cooperation network and the improvement of the technological level of developing countries. Additionally, it is exciting that, in recent years, some developing countries have been contributing more to research in the field of PCLM, which should further narrow the uneven spatial

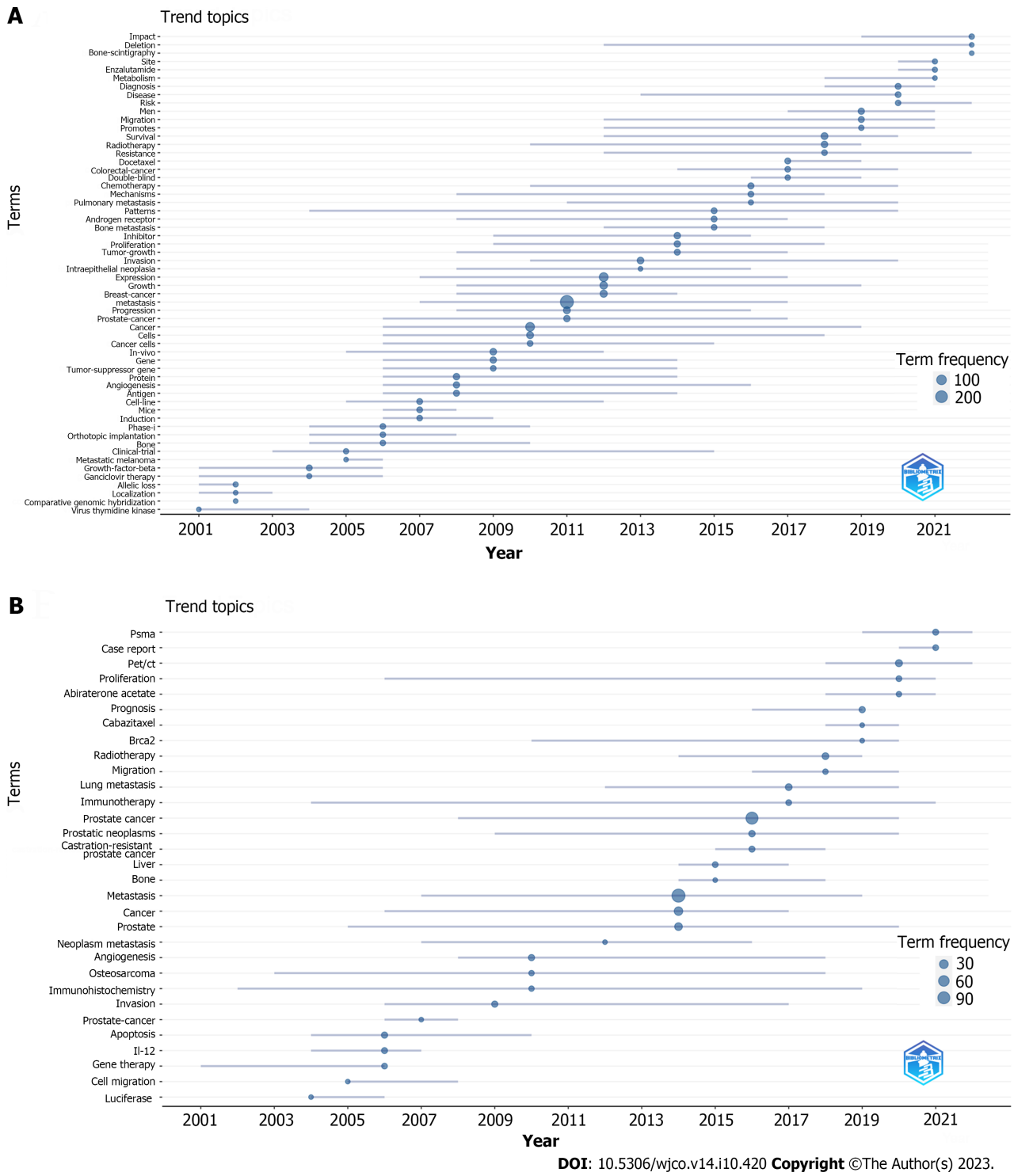

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Figure 5 Topic trend graph. A: Keywords plus; B: Author's keywords.

distribution of scientific output in PCLM.

Evaluation of hot research directions in the PCLM field

Researchers' continuous exploration and attention worldwide have propelled ongoing iterations and updates in the field of PCLM knowledge. These changes are primarily reflected in the aspects described next.

Clinical treatment directions in the PCLM field

In the early years, researchers such as Ren *et al*[27] utilized techniques like gene modification to enhance the expression of interferon-beta in mesenchymal stem cells in a mouse model of PCLM and found that tumor cell apoptosis increased and that natural killer cell activity, which is associated with anti-tumor activity, significantly increased. In addition, the invasion and metastasis suppressor gene *RhoGDI2* was identified by DNA microarray technology, and after the reconsti-

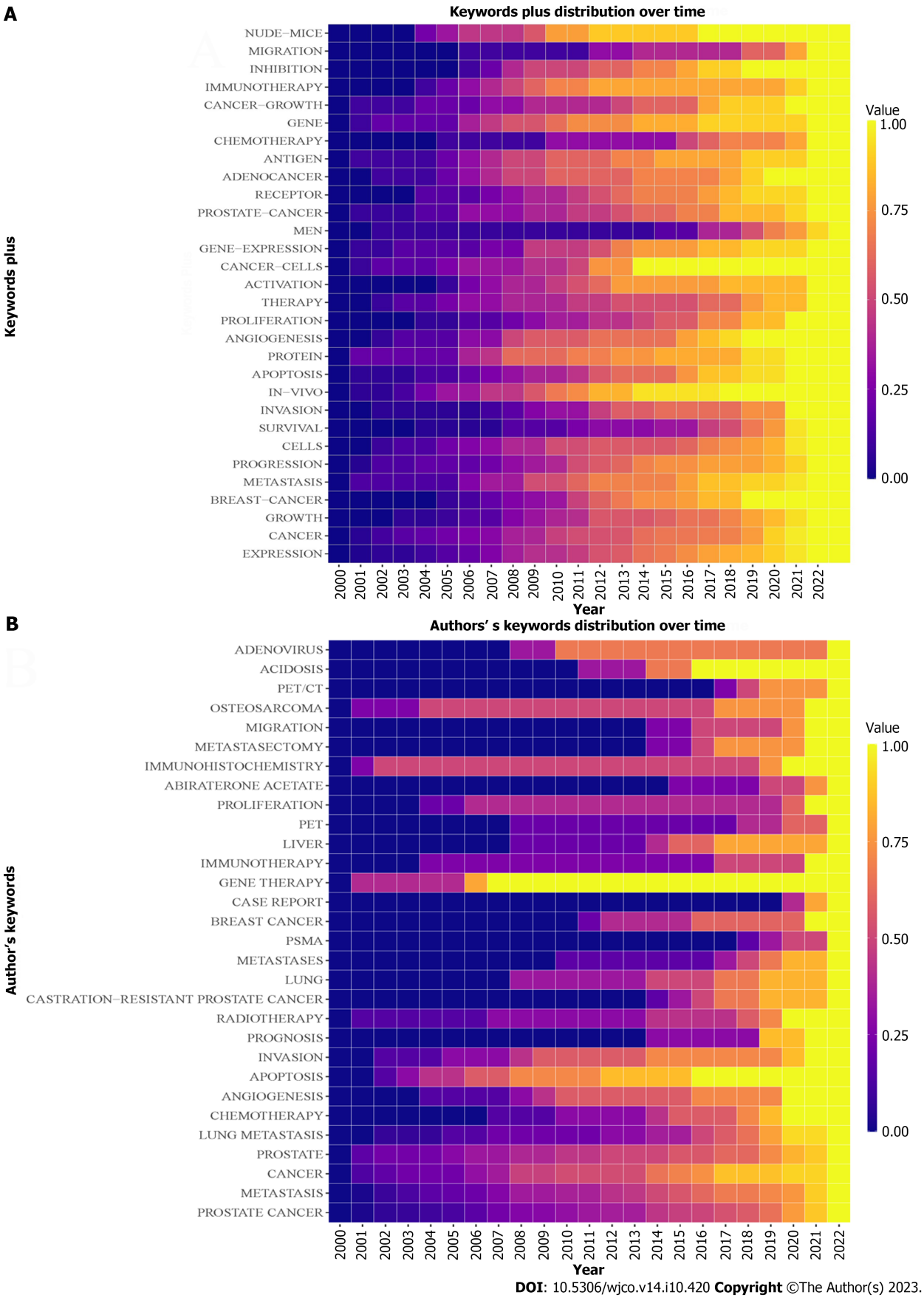

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Figure 6 Keyword time heat map. A: Keywords plus; B: Author's keywords. The values represent the ratio of the total frequency of the keyword from 2000 to a certain year to its total frequency; from top to bottom, the number of papers published by the country increased in turn.

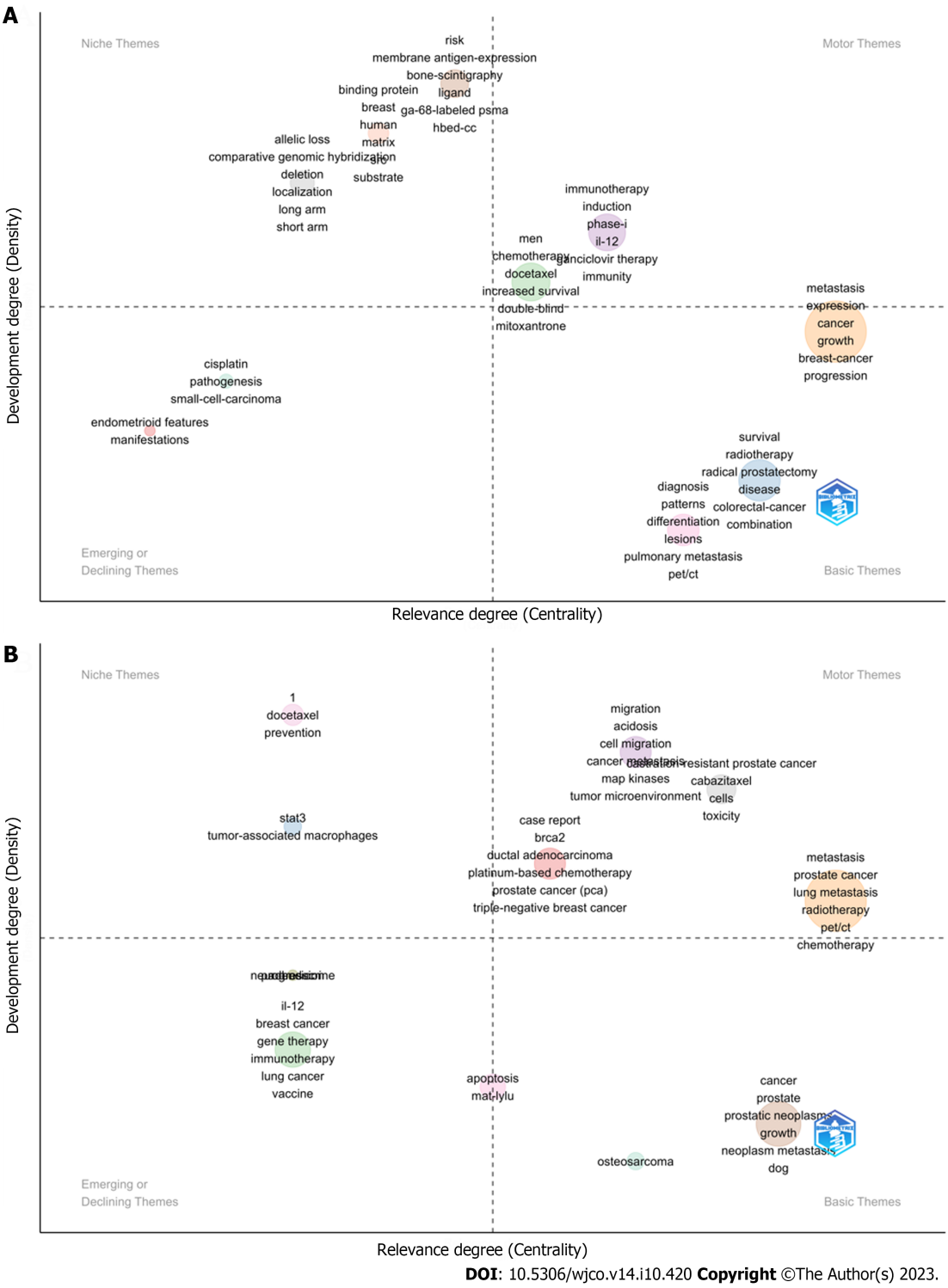

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Figure 7 Theme strategic coordinate map. A: Keywords plus; B: Author's keywords. PET/CT: Positron emission tomography/computed tomography; IL-12: Interleukin-12.

tution of *RhoGDI2* in metastatic cancer cells, it was found that LM was inhibited and the motility of cancer cells *in vitro* was reduced[28]. Therefore, interferon-beta and *RhoGDI2* are considered effective potential targets for gene therapy. Additionally, researchers combined *AdV-tk* gene therapy with radiotherapy and chemotherapy in a mouse model of PCLM and found a significant reduction in lung nodules and cancer cell colonization in the lungs[29]. However, studies have indicated that these gene therapies are challenging to deliver effectively to the tumor site, leading to inadequate gene delivery and resulting in various adverse outcomes[30]. Currently, no effective clinical trials have successfully addressed this challenge. As a result, gene therapy is currently considered a peripheral topic, and it is not surprising that its popularity in the PCLM field has declined significantly in recent years.

In this field, radiation therapy has always been an important and highly regarded topic. In recent years, some reports have shown promising results in the treatment of PCLM patients using 177Lu-PSMA radioligand therapy (Lu-PRLT)[31,

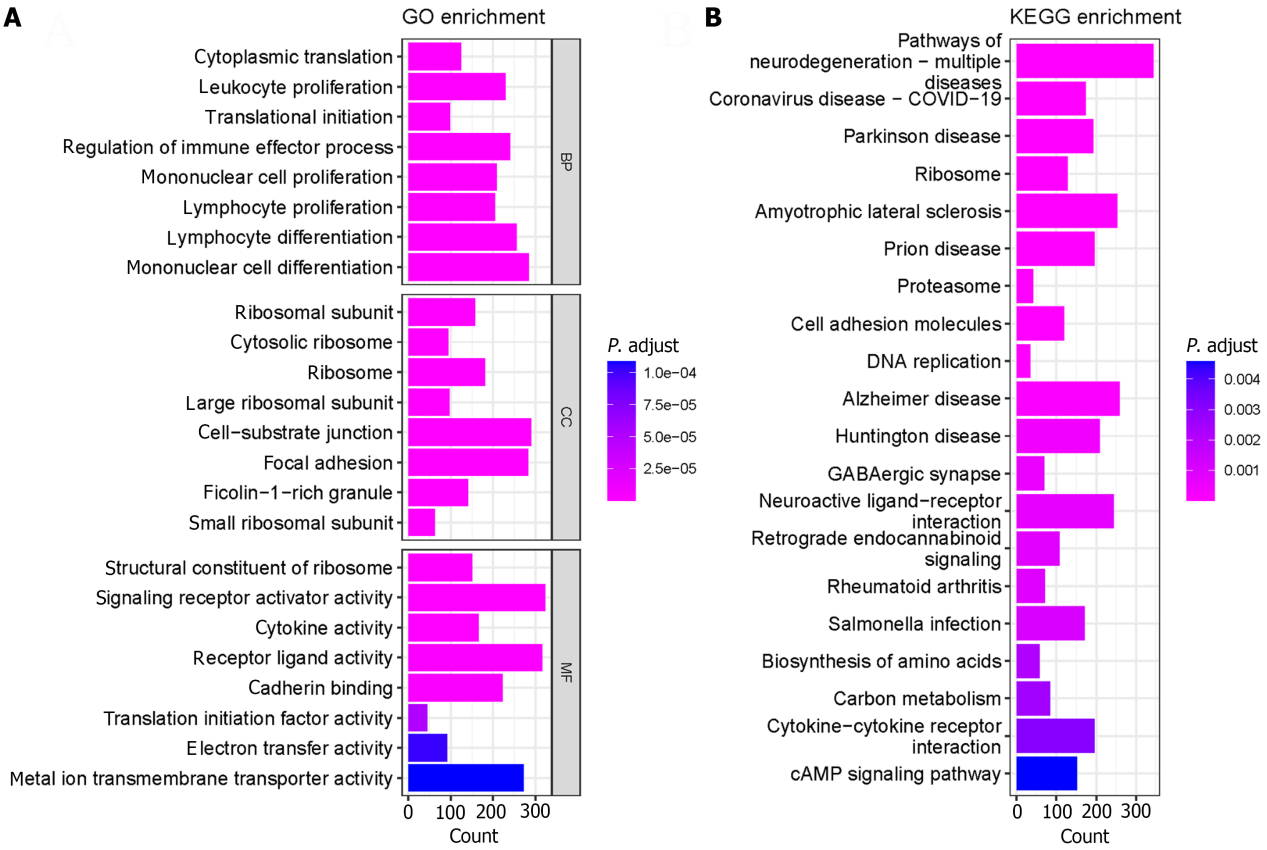

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Figure 8 Molecular pathway map of prostate cancer with lung metastasis. A: Bubble map of differentially expressed genes (DEGs) based on the gene ontology enrichment analysis; B: Bubble map of DEGs based on the Kyoto Encyclopedia of Genes and Genomes enrichment analysis. GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

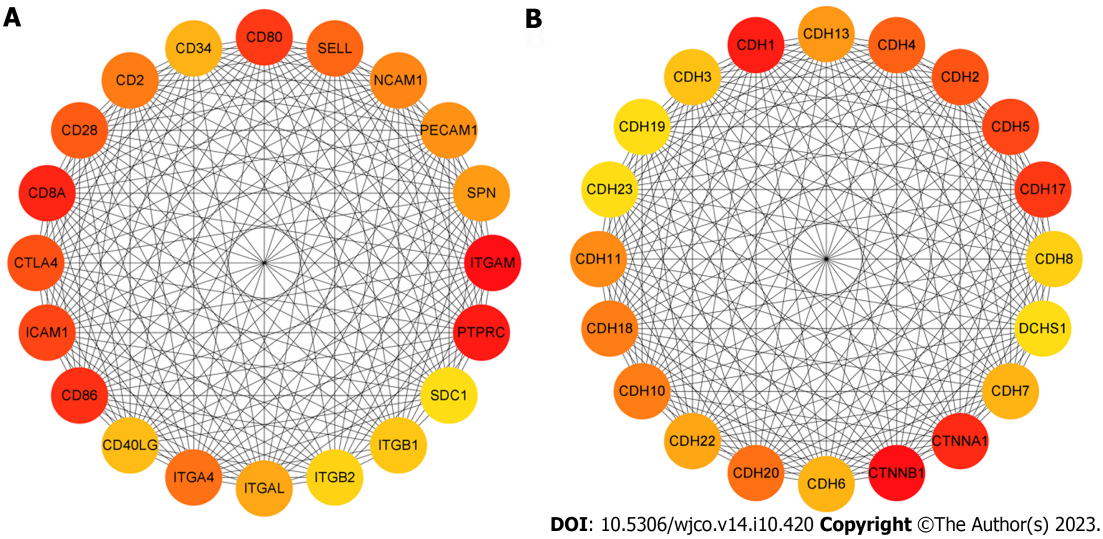

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Figure 9 Protein–protein interaction network graph of the top 20 key proteins ranked by the maximal clique centrality method. A: Cell adhesion molecules; B: Cadherin binding.

32]. However, PCLM patients who are PSMA(-)/fluorescein Di-β-D-galactopyranoside (FDG)(+) may not benefit from Lu-PRLT[33]. Therefore, some researchers have combined biologically guided radiation therapy with Lu-PRLT for PSMA(-)/FDG(+) PCLM patients and found this combination therapy to be beneficial[33]. Moreover, there have been case reports suggesting that the combination of stereotactic body radiation therapy and androgen deprivation therapy (ADT) can confer benefits in terms of biochemical response and disease-free survival in PCLM patients[34]. However, recurrences after radiation therapy in the treatment of PCLM frequently occur[35]. Furthermore, guidelines define radiation therapy for PCLM as palliative treatment and do not recommend it as part of curative approaches[35].

Therefore, although radiation therapy has broad prospects, it is considered a significant but underdeveloped topic in the PCLM field due to the many challenges it currently faces.

Radical surgery, as a traditional topic, has been widely applied in clinical practice for PC. However, there is currently insufficient evidence from evidence-based medicine and international guidelines to clearly define the role of radical surgery in PCLM patients[36]. Thus, radical surgery is a significant but underdeveloped topic in the PCLM field. Studies have shown that performing radical prostatectomy in animal models of PCLM can significantly reduce the number of lung metastases[37]. Furthermore, research reports have indicated that when the criteria for resection are met, LM resection as the preferred choice for PCLM patients can avoid or delay the use of ADT and its adverse effects, significantly improving patient prognosis[35]. This also demonstrates the promising development prospects of radical surgery in the PCLM field.

Immunotherapy, as an emerging direction, has been a topic of long-standing interest in this field. In the field of immunotherapy for PC, treatment plans have limitations. One example is sipuleucel-T, the only United States Food and Drug Administration-approved immunotherapeutic agent for metastatic desmoplasia-resistant PC, but it is indicated for asymptomatic or minimally symptomatic patients only[38]. Immune resistance poses another challenge in PC treatment. Factors like low tumor mutation loads and the presence of immunosuppressive cells can disrupt the immune system and create an immunosuppressive tumor microenvironment, leading to reduced therapeutic efficacy[39]. Additionally, there can be adverse effects associated with immunosuppressant therapy. For instance, patients may experience immune-related adverse events, such as ulceration of the lower lip[40]. Furthermore, the clinical utility of certain treatments has yet to be validated. For example, a study by Komaru *et al*[41] that is currently in the animal experimentation stage has a long way to go before its potential in clinical practice can be determined. In addition, there have been fewer studies on relatively well-established immunotherapies in the field of PCLM relative to other treatments. These may be due to the unclear mechanisms currently available, which do not provide a solid theoretical basis for the development of more effective immunotherapies. These are significant barriers to the widespread clinical application of immunotherapy in PCLM. However, in recent years, targeted and less toxic immunotherapies have shown better and sustained response rates compared to conventional therapies. Immunotherapy has the potential to cure malignant tumors, including metastatic melanoma, lung cancer, and others[42-45]. This also explains the broad prospects of immunotherapy, as it emerges as a relatively new and promising hotspot in the strategic landscape (Figure 7). For example, recent research reports have made clinical applications of oncolytic viruses, which can specifically replicate, proliferate, and destroy PCLM cells through the nanodrug packaging approach[46]. Additionally, researchers have designed a spatially drug-loaded M1 macrophage system in which M1 macrophage accumulates significantly in LM lesions, effectively enhancing the infiltration of cytotoxic T cells into lung metastases and boosting local anti-tumor immunity[47]. If these approaches could be widely implemented in clinical practice, a complete cure for PCLM might be within reach. In summary, the exploration of immunotherapy in this field has been long and challenging. However, breakthroughs in new technologies and a deeper understanding of molecular mechanisms in recent years have accelerated the progress of PCLM immunotherapy.

Contrary to immunotherapy, chemotherapy is a relatively new topic in the field of PCLM, despite being a traditional subject. Currently, there are several main directions for chemotherapy, including docetaxel, cabazitaxel, and combination therapy. Docetaxel is a well-established chemotherapy drug that has been proven to significantly prolong the survival of PCLM patients[48-50]. However, most PCLM patients develop resistance to docetaxel, leading to disease progression[50]. As for cabazitaxel, a phase 2 clinical trial has shown that it can significantly alleviate or stabilize the condition of metastatic castration-resistant PC and has the advantages of better tolerance and lower toxicity[51]. Furthermore, one study designed a cabazitaxel nanoparticle carrier that can be inhaled by M2 macrophage vesicles and that, in experimental models, was able to more effectively enter tumor tissue and inhibit over 93% of LM occurrences[52]. Additionally, combining chemotherapy with targeted therapy or immunotherapy has shown promising efficacy against LM[53-55]. Chemotherapy is utilized in PCLM treatment, but it has limitations and challenges. One issue is resistance, such as the enhancement of doxorubicin resistance in PC by the TrkB protein[50]. Additionally, PC cells display inherent and acquired resistance to cisplatin, making it ineffective as a first-line chemotherapeutic agent for PC[56]. Most PC patients who undergo ADT eventually develop castration-resistant disease[57]. Chemotherapy also has adverse effects. For instance, potentially life-threatening events like neutropenia and febrile neutropenia can occur in patients with metastatic PC who receive doxorubicin-related chemotherapy[58]. Furthermore, ADT for PC increases the risk of cardiovascular and metabolic syndrome, which can lead to fatal outcomes[59]. Despite these treatment efforts, chemotherapy alone cannot fully cure PCLM. However, in the context of the limitations of other non-traditional treatments, chemotherapy has been widely adopted in clinical practice, and its efficacy has been clearly demonstrated, whether applied alone or in combination with other therapeutic means. Hence, it is not surprising that chemotherapy is recognized as a mature and important topic in this field.

In addition, in the field of targeted therapy, enzalutamide, a next-generation AR inhibitor, has been proven to significantly prolong the survival of patients with metastatic PC, despite the inevitable resistance mediated by SPP1 through the phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (Akt) and extracellular regulated kinase 1/2 pathways or the reactivation and splice variants of the AR[60-62]. MiR-33b-3p inhibits metastasis by targeting DOCK4 in PC[63]. We could enhance miR-33b-3p expression to overcome the poor efficacy of proteasome inhibitors in metastatic PC in the future. It has also been reported that treatment with Lu-177-PSMA radioligand showed significant efficacy in PC patients and responded favorably to the treatment and regression of lung metastases after PSMA radioligand therapy (Lu-PRLT)[31]. High expression of C-C motif ligand 2 induced the production of carbon catabolite repression 4 (CCR4) in PC cells, which promotes migration and invasion of PC cells through enhanced Akt phosphorylation[64]. This study reveals CCR4 as a potential target for the treatment of PCLM. Putz *et al*[65] found that the cytokine signaling checkpoint CIS plays an important role in the occurrence of PC with LM and has a promising future in the treatment of PCLM.

Furthermore, in recent years, the emergence of abiraterone acetate has been confirmed by numerous studies to alleviate lung metastases and significantly prolong the survival of PCLM patients, and it has been regarded as a safe and effective treatment for many advanced PCLM patients[8,54,66,67].

In recent years, precision medicine has played an important role in a variety of diseases. In particular, tumors involve alterations in the biological behavior of multiple genes. The biological behaviors of various tumors are complex and diverse. Therefore, precision medicine with personalized treatment characteristics is a solution to the difficult problem of PCLM, which is hard to cure completely. One study reported that AR plays dual and opposite roles in vasculature encapsulating tumor clusters, emphasizing the complex function of AR and its importance in individualized cancer therapy[68]. This study provides new insights into the complex regulatory network of AR in metastatic tumors and lays the foundation for relevant precision medicine. It has also been reported that AuNSs@PDA-Ce6 nanoprobes significantly reduced tumor growth and inhibited LM, which has considerable potential for precise therapeutic diagnosis and metastasis inhibition[69]. In addition, Hlavac *et al*[70] revealed the characterization of prognostically distinct subgroups with precision medicine value by targeted sequencing of blood and archival samples from LM patients. However, regrettably, no mature precision medicine or personalized treatment for PCLM has been reported. In the future, precision medicine will also be an important endeavor in the field of PCLM.

Clinical diagnostic approaches in the field of PCLM

PSMA has been widely utilized in the PC screening. Many researchers have combined PSMA with PET/CT for clinical diagnosis. This includes the use of [99mTc]PSMA-T4 and 68Ga-PSMA-11, which have shown high efficacy in the diagnosis and detection of metastatic PC and recurrence, outperforming traditional imaging techniques[71-75]. However, there is still a notable false-negative rate in some patients[75]. Additionally, there are cases where lung metastases in PC patients are PSMA-negative, rendering PSMA-PET/CT unsuitable for detecting such patients[76]. Furthermore, 18F-fluorocholine PET/CT has shown higher specificity compared to traditional methods for staging PCLM patients, but its sensitivity still needs improvement[77]. Therefore, although this approach has some influence in the field of PCLM, several issues still need to be further addressed and developed in the future.

Exploratory mechanisms in the field of PCLM

In recent years, with the advancement and widespread application of bioinformatics, particularly the progress in second-generation DNA sequencing and single-cell sequencing technologies, researchers have been able to identify key molecules in PCLM more thoroughly and comprehensively, elucidating additional pathway mechanisms. This has led to the emergence of new research hotspots in basic research.

In terms of organism metabolism, studies have found that *Camkk2* not only mediates the metastasis and colonization of PC cells in the lungs, but also disrupts normal metabolism, such as glucose and lipids, leading to the occurrence of metabolic syndrome and other complications[78]. It has also been reported that the regulation of glutamine metabolism can upregulate *ARPC1A* in PC cells, resulting in changes in the PC cell cytoskeleton and the cells' migration and invasion of the lungs[79]. Furthermore, the regulatory role of the positive feedback loop between tryptophan hydroxylase 1 and β-catenin/ZBP-89 signaling, as well as the modulation of microribonucleic acids in acidosis mediated by the Warburg effect, can enhance the metastatic ability of PC cells[80,81]. These findings indicate a close relationship between organism metabolism and the metastatic behavior of PC cells. In recent years, numerous studies have shown that mutations in *BRCA2*, which possesses DNA repair functions, enhance the ability of PC cells to develop LM and other types of metastases[8,82,83]. However, these studies are based on sporadic cases, and it is necessary to conduct more comprehensive and systematic research for supplementary validation. Regarding *STAT3*, *CCL5* secreted by M2 macrophages enriched in the PC tissue microenvironment can promote *STAT3*-dependent EMT, enhancing the resistance and metastatic ability of PC cells toward the lungs[84]. In addition, immune checkpoints can inhibit T lymphocyte immune responses through the EGFR/JAK1/STAT3 pathway, promoting PC progression and the occurrence of LM[85,86]. Encouragingly, based on the related mechanisms of STAT3, research has found that the traditional Chinese medicine CFF-1 can effectively inhibit LM, prolong survival, and improve the quality of life for patients[85]. In terms of AR, PC cell growth is androgen-dependent *in vitro*, and the level of androgens in the body is positively correlated with tumor size *in vivo*[87]. Studies have also revealed that cell cycle proteins interact with AR, regulate the promoters of vascular endothelial-derived growth factor and matrix metalloproteinase 2, and enhance their expression, thereby promoting PC progression and increasing metastatic capacity[88]. These findings regarding AR indirectly provide theoretical evidence for the development and improvement of new-generation targeted drugs, such as enzalutamide, an AR inhibitor. These examples highlight the importance of translating basic research findings to clinical applications and improving PCLM treatment.

In addition, some mechanisms of PC metastasis have become independent clusters (Figure 4), indicating that this direction is relatively mature and independent as a hotspot. Studies have reported that the downregulation of E-cadherin, a result of certain inducing factors, promotes the migration and invasion of PC cells[89]. It has also been found that silencing *AKT1* downregulates epithelial-associated E-cadherin and upregulates mesenchymal-associated N-cadherin, promoting the occurrence of EMT closely related to PCLM[90]. Furthermore, some studies have indicated that decreased cell adhesion caused by C-terminal binding protein or metabolic acidosis-induced abnormal expression of microribonucleic acids enhances the metastatic ability of PC cells[81,91]. These findings suggest that abnormalities in cell-cell connections can enhance the likelihood of PC cell metastasis.

Finally, the presence of phrases such as "rats" suggests that many research results are still in the cellular, animal, and *in vitro* stages of experimentation and are still some distance from clinical translation. For example, the studies by Komaru *et al*[41], Pan *et al*[89], and Azhati *et al*[92] are still in the cellular, animal, and *in vitro* experimental stages and a long way from clinical practice. As mentioned above, PCLM scientific outputs represent countries/regions with a high

level of PCLM research but with fewer clinical case data due to the small number of PCLM patients, while countries/regions with high PCLM morbidity and mortality have a relatively weak level of research on PCLM. This may also be a major obstacle to the translation of basic research results into clinical practice. For this reason, international collaboration and knowledge sharing are particularly important. In addition, basic research often involves complex cellular, molecular, and biological processes, which may lead to problems of instability and reproducibility of results. One strategy to address this challenge is to increase the reliability and reproducibility of results through multicenter studies, validation experiments, and mutual evaluation. Clinical translation requires significant financial and resource support. However, research funding is often limited, and industry needs to consider commercial viability. Strategies to address this challenge include seeking support from public and private funding, building partnerships, and exploring new sustainable financing models. Thus, the translation of basic research findings into clinical applications is urgent in the context of the limited effectiveness of contemporary treatment options. In conclusion, basic research on PCLM is important but underdeveloped at the present time.

In addition, bone metastasis is a prominent point in the field of PCLM. This is mainly because LM often coexists with bone metastasis and other metastatic lesions, while isolated PCLM is less common, accounting for approximately 20.4% of all PCLM cases[6,7]. This highlights the complexity and refractoriness of PCLM. Therefore, further exploration of the relevant mechanisms is necessary.

Summary and exploration of mechanisms in PCLM

The global state-of-the-art PCLM pathway map we have constructed suggests that LM in PC patients is likely closely related to abnormalities in pathways, such as cadherin binding and cell adhesion molecules. This is in line with existing reports and the information discussed herein. However, most of these studies have only associated adhesion or cadherin abnormalities with PC cell migration and invasion, and there is still a lack of mature research revealing their specific roles in *in vivo* metastasis. Nevertheless, the specific interactions between the immunoglobulin superfamily and the integrin family, as well as the mechanisms leading to abnormal cell adhesion, have been elucidated in other tumors[93]. The mechanisms by which members of the cadherin gene family regulate EMT and promote breast cancer metastasis have also been identified[94]. Therefore, the interactions we have identified among the immunoglobulin superfamily and the integrin, cadherin, and calcium-binding protein families in the cadherin binding and cell adhesion molecule pathways in PCLM may be directions that merit further exploration in the field of PCLM.

Additionally, mutual interactions between coronavirus [coronavirus disease 2019 (COVID-19)] and LM of other tumors have been reported[95-97]. Cytokine-cytokine receptor interaction and cytokine activity have been shown to be closely associated with enhanced invasion in distant metastasis of thyroid cancer, lymph node metastasis of gastric adenocarcinoma, and liver metastasis of colon cancer[98-100]. Furthermore, the ribosomal protein S6 kinase, which is closely related to EMT, invasion, and metastasis of tumor cells, has been proven to be an effective target for anticancer therapy [101]. These findings indicate that COVID-19, cytokine-cytokine receptor interaction, and ribosomal pathways are closely related to tumor metastasis and have broad clinical application value. However, the detailed roles of these pathways in PCLM have not been reported. Therefore, these directions are also among worthy future explorations required in the field of PCLM.

Limitations of this study and future work plans

Several limitations of this study deserve attention. First, although most data in this study were analyzed using computer-based analysis methods that are objective, efficient, and relatively accurate, occasional errors that are difficult to avoid and detect may have occurred. In the future, we should strengthen manual interventions to address this issue. Second, due to the limitations of the analysis tools, our bibliometric analysis included only detailed data from English papers that are available globally. Some high-quality, non-English papers may have been overlooked. In the future, we should improve our analytical methods to further analyze these papers. Third, the paper data in this study came only from WOSCC. In future, we should analyze data from multiple databases to complement and validate our results. Fourth, due to the poor timeliness of the data, some emerging hotspots may have been overlooked. In the future, we should update the data in a timely manner and improve the analysis methods to better capture emerging hotspots. Fifth, while we have gained new insights into the pathways involved in the biological behaviors of PCLM, they still lack *in vivo* and *in vitro* experimental verification. In the future, we should conduct further experimental validations related to these pathways.

CONCLUSION

In conclusion, with the continuous advancement of scientific technology in recent years, PCLM has received widespread attention. In this study, we conducted a bibliometric analysis to summarize the global knowledge system of PCLM over the past 22 years. This included clinical aspects based on chemotherapy and immunotherapy, diagnostic aspects based on PSMA-PET/CT, and basic aspects based on cell adhesion molecules and STAT3. Although current treatment approaches can improve the prognosis of PCLM patients to some extent, resistance to traditional therapies and the limitations of novel therapies still prevent the complete cure of PCLM. Furthermore, we identified the close association of COVID-19, cytokine-cytokine receptor interaction, and ribosome-related pathways with PCLM for the first time. Therefore, future research in the field of PCLM should focus on exploring and enhancing mechanisms such as cytokine-cytokine receptor interaction and ribosome-related pathways, and further improving existing mechanisms such as cadherin binding and cell adhesion molecules. This study establishes a robust theoretical foundation for the advancement and enhancement of novel therapeutic approaches with the potential to facilitate the full remission of PCLM as soon as possible.

ARTICLE HIGHLIGHTS

Research background

Over the past 22 years, researchers have increasingly focused on prostate cancer (PC) with lung metastasis (LM), generating significant scientific output, but the accumulated knowledge has become disorganized and hindered research efficiency.

Research motivation

With the increase of researchers' research enthusiasm in the field of PCLM over the years, scientific output has continued to increase, but there is no complete PCLM knowledge structure system. The purpose of this article is to establish a complete structural knowledge system and future development direction.

Research objectives

In order to further clarify the future development direction of PCLM, we reconstruct the global knowledge system in the field of PCLM. This research aims to help researchers interested in the field of PCLM grasp the research trends in this field more accurately and quickly, and to deeply understand the related fields and technology development trends. This study can provide inspiration and assistance in the development and promotion of the research field of PCLM on a global scale.

Research methods

The research gathered data on PCLM papers from Web of Science Core Collection (https://www.webofscience.com/) using a specific search strategy, resulting in 280 high-quality articles published between 2000 and 2022. Data was downloaded on May 2, 2023. We conducted a bibliometric analysis of keywords, publication volume, and citation frequency. Additionally, we selected differentially expressed genes from global high-throughput datasets and performed enrichment analysis and protein-protein interaction analysis to further summarize and explore the mechanisms of PCLM.

Research results

Over the past 22 years, PCLM has gained attention, with uneven research distribution. Clinically, chemotherapy and immunotherapy are primary treatments, while diagnosis relies on prostate-specific membrane antigen and positron emission tomography/computed tomography. Basic research focuses on cell adhesion molecules and signal transducer and activator of transcription 3. Traditional treatments like chemotherapy dominate, but novel approaches like immunotherapy show limited effectiveness. This research unveils the coronavirus disease 2019 (COVID-19)-related pathway's newfound associations with PCLM.

Research conclusions

Recent scientific advancements have drawn attention to PCLM. This 22-year bibliometric analysis covered clinical diagnostic, and basic aspects. Current treatment improves prognosis, but resistance and limitations persist. The study identified novel associations with COVID-19 and pathways, suggesting future research should explore these mechanisms. This research provides a foundation for advancing novel PCLM therapies.

Research perspectives

Future research should prioritize enhancing cytokine-cytokine receptor interactions and ribosomal mechanisms while improving existing cadherin binding and cell adhesion molecules.

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FOOTNOTES

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CASE REPORT

Splenic lymphangioma masquerading as splenic abscess managed by laparoscopic splenectomy: A case report

Santosh Thorat, Febin Mohammed Shaji

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Abstract

BACKGROUND

Primary benign splenic tumours are unique and account for < 0.007% of all tumours identified during surgery and autopsy. Splenic lymphangiomas are rarely seen in adults. Splenic lymphangiomas may be asymptomatic, or may present with upper left abdominal pain, splenomegaly, hypersplenism, or splenic rupture with haemorrhagic shock. The clinical and radiological features of these lesions are not specific. This case report serves to remind the clinician to consider the rare but important differential diagnosis of splenic lymphangioma while treating splenic lesions.

CASE SUMMARY

We report a case of splenic lymphangioma in a 22-year-old woman who presented with left upper quadrant abdominal pain for three months. Initial investigations were unremarkable; however, computed tomography later revealed multiple splenic micro-abscesses. The patient underwent laparoscopic splenectomy, and histopathological examination revealed splenic lymphangioma. The patient was discharged on postoperative day three. One month after surgery, the abdominal pain resolved completely, with no new complaints. Splenic lymphangiomas present clinically as splenomegaly or left upper quadrant abdominal pain; prompt intervention is necessary for avoiding complications.

CONCLUSION

This case report concludes that splenic lymphangiomas should be considered in the differential diagnosis of splenomegaly or left upper quadrant pain, even in adults, because they are amenable to curative treatment. Delays in surgical intervention may lead to severe complications, such as infection, rupture, and hemorrhage. Such lesions can be safely managed with laparoscopy, involving less postoperative pain and early patient discharge with excellent cosmetic outcomes.

Key Words: Spleen; Lymphangioma; Oncology; Rare; Laparoscopic splenectomy; Hamartomatous process; Case report

Core Tip: We present the rare case report of splenic lymphangioma in an adult female. This is a very rare entity with only around 200 cases reported between 1970 to 2017. Isolated splenic lymphangioma is very rare and should be considered in the differential diagnosis of splenomegaly, for early intervention and prevention of potential complications.

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INTRODUCTION

Lymphangiomas are benign congenital malformations of the lymphatic vessels, commonly localised to the head, neck, and axillary regions. Intra-abdominal localisation is rare and occurs preferentially in the mesentery. Splenic lymphangiomas occur mainly in children and rarely in adults. Between 1939 and 2017, only 209 cases of splenic lymphangiomas in adults have been reported in the literature[1]. The rarity of lymphangiomas and their uncommon localisation pose a challenge for clinicians in making accurate preoperative diagnosis.

CASE PRESENTATION

Chief complaints

Complaints of intermittent left upper quadrant abdominal pain and fever for the three months.

History of present illness

Complaints of intermittent left upper quadrant abdominal pain and fever for the past three months, for which she had consulted multiple medical practitioners with no relief from her agony.

History of past illness

No history of any significant illness in the past.

Personal and family history

No significant personal or family history.

Physical examination

Physical examination results were unremarkable, except for mild tenderness on deep palpation in the left upper abdomen.

Laboratory examinations

Initial investigations, including blood tests, were unremarkable, except for a slight elevated white blood cell count.

The patient was investigated for possible sources of infection; blood cultures, urine cultures, sputum cultures were performed. Additionally, an infection panel screening was performed, and the result was negative. Cultures were also negative for the presence of any infection.

Imaging examinations

Abdominal ultrasonography revealed multiple splenic micro-abscesses. Further workup with contrast-enhanced computed tomography of the abdomen revealed splenomegaly and multiple hypodense lesions in the splenic parenchyma (the largest measuring 15 mm × 15 mm), suggestive of multiple splenic abscesses/cysts (Figure 1). The splenic vein and distal Superior Mesenteric Artery were dilated, indicating portal hypertension. Other findings included a right-sided hemorrhagic ovarian cyst. Upper gastrointestinal endoscopy was performed for portal hypertension, which showed mild gastritis and a Hill's grade 1 hiatal hernia.

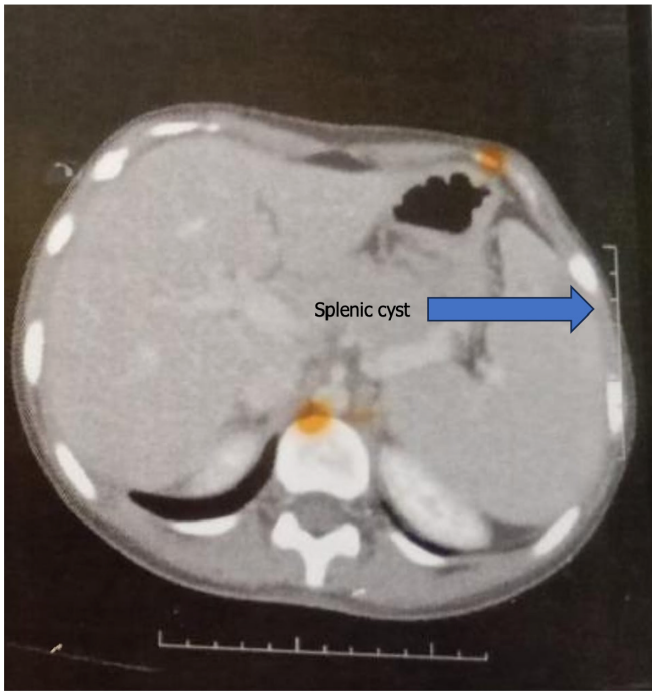

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Figure 1 Contrast-enhanced computed tomography film showing splenic cyst.

FINAL DIAGNOSIS

Splenic lymphangioma.

TREATMENT

Laparoscopic splenectomy.

OUTCOME AND FOLLOW-UP

The patient was admitted to our hospital and prepared for laparoscopic splenectomy (Figure 2). She was started on intravenous third generation cephalosporins and metronidazole to provide aerobic and anaerobic coverage, respectively, and administered vaccinations (including meningococcal, pneumococcal, and *H. Influenza* vaccines) 14 d before the surgery.

The patient underwent laparoscopic splenectomy. She was placed in the supine position, and the surgeon was positioned at the right lower side.

The spleen was removed using a Pfannenstiel incision, which was closed cosmetically with subcuticular sutures.

The total operative time was 160 min, with an estimated blood loss of 110 mL. Drains were placed at the postoperative site because the spleen formed adhesions with the pancreas. The drains were kept in place to check for any leakage, and they were removed on postoperative day (POD) two. The postoperative period was uneventful, and the patient was discharged on POD three.

The splenectomy specimen was sent for histopathological examination (Figure 3A). The spleen weighed 247 g. Histological examination findings revealed lymphangioma of the spleen, with areas of congestive splenomegaly (Figure 3B).

Postoperatively, all precautions were taken to prevent hospital acquired infections. The patient was advised to immediately present to the hospital in case fever develops after hospital discharge. One month after surgery, the abdominal pain resolved completely, with no new complaints.

DISCUSSION

Splenic lymphangioma is an uncommon malformation of the lymphatics of spleen mainly seen in children and rarely in

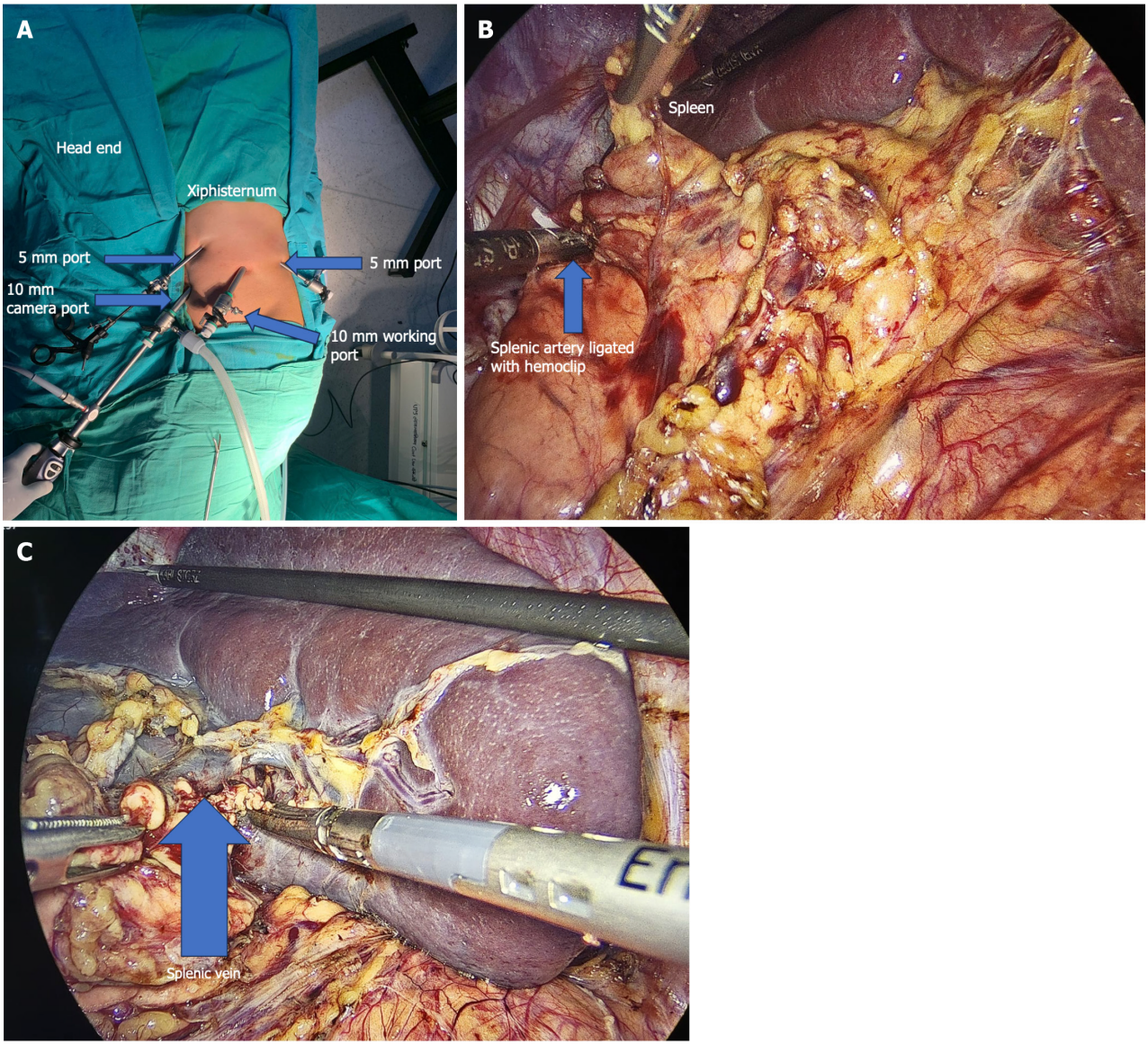

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Figure 2 Laparoscopic splenectomy. A: Port placement for laparoscopic splenectomy; B: Intraoperative view of the splenic artery ligated with a haemoclip at the upper border of the pancreas; C: Intraoperative view of the spleen.

adults. Although its aetiology is unclear, it is widely regarded as a hamartomatous change rather than a neoplasm[2]. Histologically, splenic lymphangiomas are characterised by cystic spaces lined by attenuated endothelial cells[3]. This condition may present involving only the spleen. However, in most cases, it is part of a systemic involvement of the lymphatic channels affecting multiple organs (systemic lymphangiomatosis)[4]. Most lesions are detected in imaging studies incidentally, whereas larger lesions can cause compression symptoms due to pressure on adjacent organs.

CONCLUSION

This case report concludes that in patients presenting with splenomegaly and left upper abdominal pain, splenic lymphangioma should be considered as an important differential diagnosis. Missed diagnosis and delayed treatment can lead to serious complications such as rupture and hemorrhage[5]. Such lesions can be safely managed with laparoscopy, involving less postoperative pain and early patient discharge with excellent cosmetic outcomes.

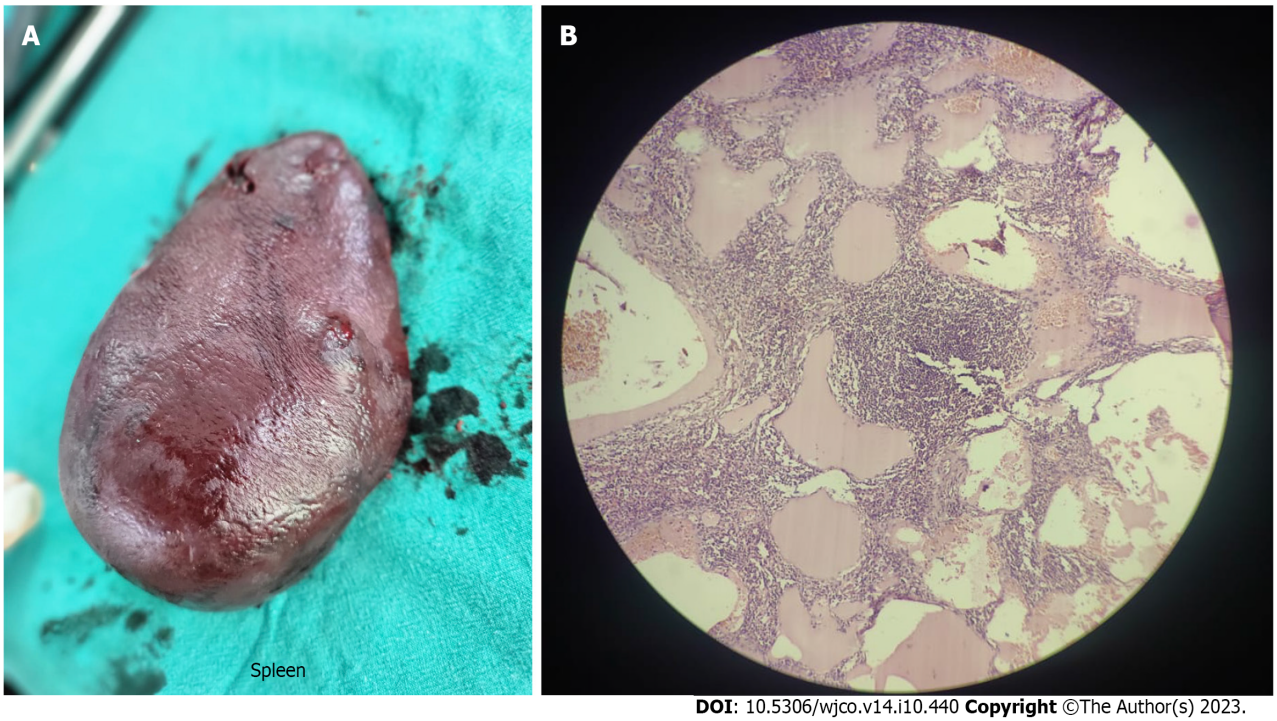

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Figure 3 Splenectomy specimen and histopathological slide. A: Splenectomy specimen; B: Histopathological slide showing multiple cystic spaces filled with eosinophilic proteinaceous material.

FOOTNOTES

Author contributions: Thorat S performed the surgery and was the chief consultant surgeon; Shaji FM assisted the case and compiled the information and created the manuscript.

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